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CONTENTS—PART 2

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

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CHRONOLOGICAL LIST OF SESSIONS

(see page xi for Thematic List of Sessions)

WEDNESDAY

THURSDAY

FRIDAY

SATURDAY

Thematic List of Sessions

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

Theme A: Development and Plasticity

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

Theme B: Cell Biology

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

Theme C: Excitable Membranes and Synaptic Transmission

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

Theme D: Neurotransmitters, Modulators, and Receptors

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

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

Theme E: Endocrine and Autonomic Regulation

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

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

Theme F: Sensory Systems

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

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

Theme G: Motor Systems and Sensorimotor Integration

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

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

Theme H: Structure and Function of the CNS

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

Theme I: Neural Basis of Behavior

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

206.13 SALT LOADING ENHANCES PRESSOR RESPONSE TO INTRAVENOUS ADMINISTRATION OF A DOPAMINE D2 RECEPTOR AGONIST, QUINPIROLE, IN THE SPONTANEOUSLY HYPERTENSIVE RAT. Y. Igarashi*, Y.F. Chen, S. Oparil (SPON: J. Brown). Hypertension Research Program, University of Alabama at Birmingham, Birmingham, AL 35294.

Previous studies in our laboratory demonstrated that intravenous (i.v.) administration of the selective dopamine (DA) D2 receptor agonist quinpirole (LY171555) induces a pressor response in conscious Sprague-Dawley rats and DOCA/NaCl hypertensive rats through a central DA mechanism. This pressor response was attenuated in the DOCA/NaCl rat. To test the hypotheses that 1) the pressor effect of quinpirole is blunted in a second hypertensive model, the spontaneously hypertensive rat of the Okamoto strain (SHR), and 2) pressor responsiveness to quinpirole is further altered by NaCl loading in SHR, quinpirole was administered i.v. (1 mg/kg) or intracerebroventricularly (i.c.v.) (0.1 mg/kg) to SHR and control WKY maintained on 8% or 1% NaCl diets for 3 wks beginning at age 7 wks. Results (mean ±SEM):

IV	NaCl	Basal MAP	Δ MAP post quinpirole 2 min	30 min	60 min
SHR	8% (10)	174±4++	29±2ΔΔ++	-11±3Δ	-9±3Δ
	1% (10)	155±4	18±2ΔΔ	-9±2Δ	-6±2Δ
WKY	8% (10)	112±3	20±1ΔΔ	1±2	0±1
	1% (10)	113±2	20±2ΔΔ	2±1	1±1
ICV			2 min	30 min	60 min
SHR	8% (10)	175±4++	24±3ΔΔ	7±2Δ	-3±2
	1% (10)	155±3	21±2ΔΔ	5±2Δ	-3±2
WKY	8% (10)	105±2	22±1ΔΔ	2±1	1±1
	1% (10)	103±1	22±1ΔΔ	3±1	1±1

Δp<0.05; ΔΔp<0.01, compared to basal value; ++p<0.01, compared to 1% NaCl group. MAP= mean arterial pressure.

The pressor response to i.v. quinpirole was significantly greater in the 8% NaCl SHR than in the 1% NaCl SHR or either WKY group; the quinpirole-induced pressor response was followed by a delayed depressor response in SHR but not WKY. The DA D2 antagonist flupentixol (0.3 mg/kg, i.v.) blocked the response to quinpirole in both SHR and WKY. Pretreatment with domperidone (2.5 mg/kg, i.v.), a peripheral D2 antagonist, enhanced the pressor response and blocked the depressor response to quinpirole in 8% NaCl SHR. These data do not indicate that the pressor response to quinpirole is blunted in SHR, nor do they suggest that dietary NaCl loading alters this centrally mediated pressor response in either SHR or WKY. Rather, these results suggest that peripheral mechanisms are responsible for the enhanced pressor response to quinpirole observed in NaCl loaded SHR.

206.14 URINARY CATECHOLAMINE EXCRETION IN SALT-SENSITIVE AND SALT-RESISTANT SHR AND WKY. M.J. Meldrum and R. Dawson, Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, FL 32610

Dietary sodium has been implicated in humans as well as several animal models as a risk factor in the pathogenesis of hypertension. Humans with salt-sensitive essential hypertension have also been shown to be unable to increase their urinary dopamine excretion when challenged with high salt diets. These studies were designed to evaluate the effects of elevated dietary sodium on salt-sensitive and salt-resistant forms of SHR and WKY. Groups of 9-10 week old salt-sensitive SHR and WKY rats were obtained from Taconic Farms (Germantown, NY) while salt-resistant SHR and WKY were obtained from Charles Rivers (Wilmington, MA). Animals were placed in metabolism cages and acclimated to the basal sodium diet for 7 days. High sodium diet (3.15% Na, Ralston Purina) was then given for 7 days after which the basal diet was again given. The following 24 hr parameters were measured: Food intake; water intake; urine volume; urinary Na+ and K+; and urinary catecholamines. Blood pressure (indirect tail cuff method) were measured at the beginning, and at 7 day intervals. Salt-sensitive SHR showed a significant increase in blood pressure after 7 days of salt treatment (172 ± 3.2 v. 199.0 ± 6.2 mmHg) while salt-resistant SHR showed no significant changes (174.2 ± 3.0 vs. 175.5 ± 2.3 mmHg) in blood pressure. Urinary Norepinephrine (NE) levels were significantly lower in both salt-sensitive and salt-resistant SHR compared to their WKY controls. Urinary NE levels in both sensitive and resistant SHR were significantly elevated by salt treatment compared to basal diet. Urinary dopamine (DA) levels were significantly lower in salt-sensitive SHR than their controls, while salt-resistant SHR were not different than their WKY controls. Exposure to high salt diets increased urinary DA levels in salt-sensitive SHR to levels seen in their WKY controls while salt exposure in salt-resistant SHR increased DA levels in both SHR and WKY to the same extent. These studies suggest that differences in salt handling occur in SHR, those SHR which respond to salt with increased blood pressure show differences in urinary DA excretion from their WKY controls, while salt-resistant SHR respond similarly to their WKY controls. This difference in renal DA handling may play a role in the salt-sensitivity effects on blood pressure.

Supported in part by a PMA Foundation Research Starter Grant and a Florida heart Association grant.

206.15 EFFECTS OF DOCA-SALT TREATMENT AND DEHYDRATION ON RAT ATRIAL NATRIURETIC FACTOR (ANF) CONTAINING CARDIOCYTES. B.H. Hwang, W.B. Severs and T. Inagami. Terre Haute Center for Medical Education, Indiana University School of Medicine, Terre Haute, IN 47809; Depts. of Anatomy & Pharmacology, The Pennsylvania State University, Hershey, PA 17033; and Dept. of Biochemistry, Vanderbilt University, Nashville, TN 37232.

The ANF is rich in the right atrium and has vasodilation, diuretic, and natriuretic effects. However, it is unknown as to whether/how ANF-containing cardiocytes are affected by deoxycorticosterone acetate (DOCA)-salt treatment and dehydration.

For the dehydration study, male Wistar rats were deprived of drinking water for 5 days and sacrificed for studying catecholaminergic (CA) terminals and ANF-containing cardiocytes in the right atrium. For the DOCA-salt study, animals were treated with DOCA and allowed to drink 1% NaCl solution. The CA terminals, ANF-containing cardiocytes and ANF-containing granules per cardiocyte were evaluated 2 weeks and 4 weeks after experiments. In addition, ANF contents in the plasma and right atrium were determined by radioimmunoassay.

Dehydration caused the increase of the number of ANF-containing cardiocytes. However, the number of ANF-containing granules/cardiocyte was not significantly different between the control and dehydrated groups. The CA terminal-cardiocyte distance was about 1.3 μ, not significantly different between the control and experimental groups.

The DOCA-salt treatment also caused the increase of ANF-containing cardiocytes. However, the number of ANF-containing granules/cardiocyte was reduced in the DOCA-salt treated rats at the 4-week stage. ANF content in the right atrium of DOCA-salt treated rats was also reduced at the 4-week stage, whereas the plasma ANF content was increased. The CA terminal-cardiocyte distance was not significantly different between the control and experimental animals. The results suggested that DOCA-salt treatment can stimulate both the increased ANF synthesis and the increased release of ANF. Such changes were not observed to be associated closely with CA neurons.

In conclusion, our preliminary data suggest that dehydration causes preservation of ANF in the cardiocytes, whereas DOCA-salt treatment induces the increased release of ANF. Although the right atrium is heavily innervated by CA terminals, effects of dehydration and DOCA-salt treatment mentioned above are not closely associated with the catecholaminergic nervous system (supported by American Heart Association grant 851334).

206.16 AMOUNTS OF ANTIGENICALLY ACTIVE ATRIAL NATRIURETIC PEPTIDE IN ATRIAL CELLS OF NORMAL AND SYMPATHECTOMIZED MALE AND FEMALE RATS. L.C. Zoller*, L. Wright and C. Waring* (SPON: E. Yetarian). Department of Anatomy, Boston University School of Medicine, Housman Research Center, Boston, Massachusetts 02118

In order to study the effects of sympathectomy on the control of atrial natriuretic peptide (ANP) in atrial cells, eight days following birth a group of male and female Sprague-Dawley rats received a bilateral transection of the cervical sympathetic trunk whereas littermates were incised and then closed without transection of the trunk. The trunk was avulsed proximally to insure that regeneration would not occur. On postnatal day 15 all animals were killed, the atria removed, immersion fixed in 4% paraformaldehyde in phosphate buffer (pH 7.4) for 2 hours, rinsed in buffer, dehydrated and embedded in paraffin. Five micron sections were cut, the paraffin removed and the tissue reacted with antibody to ANP (kindly provided by M. Cantin) using the Sternberger method and the antibody-antigen reaction visualized using 4-chloro-1-naphthol (4-Cl-1-N). The blue product was quantified using a scanning and integrating microdensitometer at 570nm, a wavelength specific for 4-Cl-1-N. Product was measured in cells from both atria. Amounts of product are given as integrated extinction which is the absolute absorbance as measured on the densitometer divided by a constant absorbance obtained through a neutral density filter. This enables results from various machines to be compared. For each animal examined, more than 10 sections were analyzed with reaction product being measured in over 100 cells per section. Differences in the mean of product amount between normal and sympathectomized males and females were considered significant at a probability level of 0.05 as analyzed by ANOVA and Tukey's test. The N represents the number of sections analyzed.

	Male		Female	
	Control	Sympathec	Control	Sympathec
N	34	34	34	34
Mean	0.097	0.152	0.149	0.123
Standard Error	0.011	0.012	0.008	0.008

Levels of ANP reactive product in atrial cells of control rats were significantly higher in females (54%) than in males. Following sympathectomy, levels in males significantly increased (57%) to identical levels found in control females. Levels in sympathectomized females, compared to controls showed a slight (19%) but significant decrease. It appears that sympathectomy has paradoxical effects on ANP content in atrial cells of male and female rats. Furthermore, normal female rats appear to have more atrial ANP than males. We are presently examining the implications of this study on the interaction between the autonomic nervous system and the heart and the role of ANP in the control of blood pressure and hypertension.

206.17 ROLE OF 5-HT_2 RECEPTORS IN THE PRESSOR AND RENIN RESPONSES TO QUIPAZINE. R. H. Alper and J. M. Snider*. Dept. Pharmacol., Tox. and Therap., Univ. Kansas Medical Center, Kansas City, KS 66103.

Studies have shown that the indirect serotonin (5-HT) agonist para-chloroamphetamine increases arterial pressure (AP) and plasma renin activity (PRA). Most of the hypertensive response is due to a peripheral effect and was neither 5-HT nor renin dependent. Quipazine is a direct 5-HT agonist which also increases PRA. It has been recently demonstrated that several other 5-HT agonists increase PRA through central 5-HT_2 receptors. It was the purpose of these studies to determine the role of 5-HT_2 receptors in mediating the effects of quipazine on AP and PRA.

AP, heart rate (HR), and PRA were determined in conscious, unrestrained rats 2 or more days after catheters were placed in the femoral artery and vein. Blood samples (0.5 ml) were withdrawn through the arterial catheter prior to and at various times after iv injections of quipazine, LY 53857 (a 5-HT_2 antagonist), or their vehicles. In a preliminary experiment rats were administered the 2 vehicles 5 min apart. AP, HR and PRA were measured prior to any treatment and 5, 15, 30 and 60 min after the second vehicle injection; all 3 parameters remained constant. Quipazine (0.3-3.0 mg/kg) caused dose- and time-dependent increases in AP and PRA with a negligible effect on HR. AP was elevated by all 3 doses; the peak response was approximately 55 mmHg 5 min after the highest dose. PRA was increased at 1 and 3 mg/kg at a maximum of approximately 10 fold. The peak pressor response to quipazine (3 mg/kg) was blocked by LY 53857 at doses as low as 0.03 mg/kg. A partial attenuation was noted at 0.01 mg/kg while 0.003 mg/kg was ineffective. LY 53857 was about 10 times more potent in blocking the renin response in these same rats. LY 53857 at 0.003 mg/kg totally abolished the quipazine-induced increase in PRA while at 0.001 mg/kg the 5-HT antagonist reduced the renin response by about 50%.

The data show that in conscious, unrestrained, normotensive rats, the non-selective 5-HT agonist quipazine produces marked increases in AP and PRA, although the duration of the pressor response was greater than the renin response. The selective 5-HT_2 antagonist LY 53857 inhibited both responses, but had no effect on AP or PRA per se. The difference in the potency of LY 53857 to block these effects suggest that quipazine may act at different sites or receptor subtypes to increase AP and PRA, or just that the pharmacokinetics are more favorable to antagonism of the renin response.

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REGENERATION: SPINAL CORD II

207.1 ADULT DORSAL ROOT GANGLION AXONS THAT CONTAIN CALCITONIN GENE RELATED PEPTIDE (CGRP) REGENERATE INTO TRANSPLANTS OF EMBRYONIC SPINAL CORD. B.T. Himes* and A. Tessler, Philadelphia VA Medical Center and Departments of Anatomy and Neurology, The Medical College of Pennsylvania, PA 19129, and J. Houle and P.Reier, Departments of Neurosurgery and Neuroscience, College of Medicine, University of Florida, Gainesville, FL 32160.

Dorsal roots transected in adulthood grow beyond the site of injury but fail to regenerate into spinal cord. We have previously used HRP-tracing methods to show that the central processes of adult DRG neurons regenerate into embryonic spinal cord transplanted into adult host spinal cord suggesting that transplants enhance the ability of dorsal roots to regenerate by (1) providing a more suitable terrain and/or (2) providing factors that augment the neurons' regenerative responses. It is unclear however whether regenerating neurons maintain their normal characteristics and also whether all DRG neurons share this regenerative capacity. DRG neurons can be classified into subsets based on several criteria including their content of CGRP. CGRP in normal adult spinal cord is found in primary afferent fibers and is abolished in the dorsal horn by dorsal rhizotomy, allowing it to serve as a marker for regenerating primary afferent axons. We have therefore studied the dorsal roots that regenerate into transplants using CGRP immunocytochemistry. Adult rats had one side of the L4-5 spinal cord replaced by a transplant of E14 or E15 spinal cord without its DRGs, and the cut dorsal roots were juxtaposed to the transplant. 2-9 months after transplantation CGRP was demonstrated in the grafts. CGRP immunoreactive fibers entered the transplant from the host dorsal root, arborized extensively, and displayed varicosities along their length. Most remained within 0.4mm of the site of entry, but occasional processes penetrated more deeply and some traversed the entire dorsal-ventral extent of the transplant (app. 1mm). Occasional immunoreactive neurons were seen in the transplant but only rarely in the region of these fibers. The results show that DRG neurons which regenerate into embryonic transplants maintain or reestablish their normal capacity to synthesise CGRP and that transplants enhance the capacity to regenerate of at least one subpopulation of DRG neurons.

Supported by the VA Medical Research Service, USAMRDC grant 51930002 and NIH grants NS22316 and NS24707.

207.2 EVIDENCE FOR RUBROSPINAL PLASTICITY IN THE DEVELOPING OPOSSUM. X.M. Xu*, R. Waltzer* and G.F. Martin. Department of Anatomy and Neuroscience Program, The Ohio State Univ., Coll. of Med., Columbus, Ohio 43210.

Rubral axons do not grow around or through spinal lesions in neonatal kittens, although cortical axons do so readily (Bregman and Goldberger, Science, 217:553-555). Rubral axons innervate the spinal cord well before cortical ones, however, (Cabana and Martin, Develop. Brain Res., 15:247-263) and they may simply have lost their potential for plasticity. This hypothesis would be difficult to test in placental mammals because rubrospinal development occurs prenatally, but should be testable in the North American opossum because rubrospinal development occurs after birth. The opossum is born 12 days after conception. We report here on the results of experiments designed to determine if rubrospinal axons are capable of plasticity in the North American opossum.

The rubrospinal tract was lesioned at cervical or thoracic levels in anesthetized adult and pouch-young opossums. Approximately 30 days later, one group of anesthetized animals was subjected to injections of Fast Blue or wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) several segments caudal to the lesion in an attempt to retrogradely label rubral neurons. In another group, the red nucleus contralateral to the lesion was injected with WGA-HRP to orthogradely label rubrospinal axons. In adult animals and older pouch-young, rubral neurons could not be labeled contralateral to the lesion and in the older pouch-young, there was evidence that many axotomized rubrospinal neurons underwent retrograde degeneration (the Gudden effect). In animals subjected to lesions at approximately postnatal day (PD) 20, however, rubral neurons were labeled contralateral to the lesion although evidence for some neuronal loss was still present. In the orthograde transport experiments at the same age, rubrospinal axons could be labeled caudal to the lesion and they seemed to take the most direct route around it. The position of rubrospinal axons caudal to the lesion varied and in one case they were present in the dorsal funiculus. Regardless of position, rubral axons appeared to innervate those areas of the grey matter supplied normally and in several cases they traversed the length of the cord. We have interpreted our results to suggest that rubrospinal axons are capable of plasticity at early stages of development. It is our working hypothesis that all descending spinal pathways are capable of plasticity at some stage of development although differences in critical period may exist. Differences in critical period may reflect differences in developmental history. (Supported by NS-10165).

207.3 REGENERATION OF BULBOSPINAL AXONS DURING REGNERATION OF THE LIZARD TAIL SPINAL CORD. B.M. Davis, M. T. Duffy* and S.B. Simpson, Jr. Dept. of Biological Sciences, Univ. of Illinois at Chicago, Chicago, Il, 60680.

Transection of the lizard (*Anolis carolinensis)* spinal cord at mid-back levels is followed by the infiltration of dense connnective tissue, formation of glial scar, cystic outgrowths of the ependyma, and degeneration of nerve axons separated from their perikarya. No nerve fibers regenerate across the site of injury as demonstrated by Boudain stained sections of the lesioned area. Functional losses are permanent. However, autotomy or transection of the lizard tail results in tail regeneration as well as concomitant CNS axon regeneration. The spinal cord at tail levels regenerates but consists of a simple ependymal tube surrounded by white matter. The radial processes of the ependyma cells form channels through which regenerating axons grow and form fascicles. Electron microscopic analysis reveal up to 2,000 regenerating central axons in tail spinal cord.

Using HRP labeling we have demonstrated that up to 450 bulbospinal axons are found in the normal tail spinal cord. Following regeneration of the tail, few (usually < 20) of the CNS axons that regenerate arise from bulbospinal neurons. In each regenerated animal examined, axons arising from different bulbospinal nuclei were identified indicating that many if not all of the bulbospinal regions are capable of growing regenerate axons. Application of HRP immediately rostral to the regenerating tail labeled a normal number of brainstem neurons. This suggests that the minimal regeneration of bulbospinal axons within the regenerate tail is not due to retraction of bulbospinal axons or to death of bulbospinal neurons. However, the majority of the regenerating axons have a local origin arising from primary sensory or spinal neurons.

These results demonstrate that although long descending bulbospinal axons can regenerate during tail regeneration they do not regenerate following cord transection at mid back level. These regenerating bulbospinal axons can be used as an unequivical criterion to assess CNS axon regeneration in experiments designed to induce spinal cord regeneration at higher cord levels. (MTD, BMD, SBS supported by NS24162 to SBS)

207.4 BULBOSPINAL AND PROPRIOSPINAL CONNECTIONS IN THE SALAMANDER REGENERATED SPINAL CORD M. T. Duffy*, B.M. Davis and S.B. Simpson, Jr. Dept. of Biological Sciences, Univ. of Illinois at Chicago, Chicago, Il, 60680.

Recently, a number of studies have shown that mammalian central nervous system axons can regrow following lesion if given the proper environment (Richardson, 1980; David and Aguayo, 1981; Bently and Aguayo, 1982; Silver and Ogawa, 1983; Goldberg et al., 1986, Bregman, 1986). These findings have renewed interest in regeneration of central nervous system neurons with much of the attention focused on inducing regeneration in species that under normal conditions exhibit little capacity for recovery of function, e.g., rats. While these experiments have produced dramatic and exciting results, they have not revealed the requirements for, or the mechanisms of, successful regeneration. The experiments described here are the first of a series of experiments designed to provide a detailed description of naturally occurring regeneration in an adult system, the salamander spinal cord.

Adult salamanders (*Triturus viridescens)* were completely transected rostral to the lumbar spinal cord. Animals recovered for at least 60 days at which time they swam and walked normally. Animals were then transected 1.0cm caudal to the first transection (mid-lumbar enlargement) and a pledget of HRP was placed between the cut ends of the spinal cord. In the 9 animals that have been examined between104 and 450 HRP labeled bulbospinal neurons were found. In normal animals 700-800 bulbospinal neurons are labeled by HRP application to the lumbar enlargement. The HRP labeled brain stem neurons were found in the hypothalamus, mesencephalon, and myelencephalon. Examination of regenerated axons projecting from the brachial enlargement to the lumbar enlargement showed that, in most cases, a normal number of descending cells reconnected the limb associated regions of the spinal cord.

These results indicate that all regions of the brainstem which normally project to the lumbar spinal cord are able to regenerate axons which grow at least 1.0cm past a spinal transection. The projection arising from the brachial enlargement is also reestablished following low thoracic transection. We conclude from these experiments that the ability to regenerate CNS axons is present in all salamander neurons. Histological examination of the center of the regenerated region revealed that recovery of function in a number of cases was accompanied by restoration of only a small percentage of the white matter found in normal animals. Taken together these results indicate that partial regeneration of descending axons is sufficient to restore locomotion and that rewiring of local spinal circuits may play an important role in recovery of function. (MTD, BMD, SBS supported by NS24162).

207.5 DEVELOPMENT OF CERVICAL SPINAL CORD DENDRITE BUNDLES IN THE RAT. J.B. Taylor*, W.J. Anderson, D.L. Bellinger. (Spon. E.P. Finnerty) Indiana University School of Medicine and Indiana State University, Terre Haute, IN 47809.

Previous studies from our laboratory have revealed three discrete dendrite bundles in the ventral horn of the 30 day old rat cervical spinal cord. A midline dendrite bundle (MDB) in the ventromedial gray matter, (C3-6), a central dendrite bundle (CDB) coursed the medial aspects of the ventral horn, (C3-5), and a lateral dendrite bundle (LDB) in the ventrolateral gray matter, (C2-4). These dendritic bundles were composed of longitudinally oriented dendrites in close apposition. Smaller transverse dendrite bundles radiated from the longitudinal dendrite bundles at right angles and appeared to interconnect the MDB, CDB and LDB. This study was performed to determine whether the cervical dendrite bundles develop postnatally, the degree of dendritic differentiation at birth and the possibility of catecholaminergic axon involvement in bundle formation. Long-Evans hooded rats were bred and at birth litters were reduced to eight pups and assigned an age group. Animals were sacrificed at days 1, 3, 5, 8, 10, 15, 20, 30 and 60. Cervical spinal cords wer dissected from the medullary obex to C8 and prepared for routine histology, Golgi-Cox method, and histofluorescence histochemistry. Our results differed considerably from work done with 30 day old rats. At birth and 5 days the motoneurons appeared to have minimal dendritic growth in all the bundles. Dendritic orientation appeared to be more transverse than longitudinal. A medial and a lateral column of dendritic bundles appeared to be present at the level of C1 and seemed to arise from a single column of motoneurons in the medullary region. At C1 there was a definitive transverse dendritic bundle interconnecting the MDB and LDB. The LDB was found to be present from C1-C7. The CDB was present from C3-C5 and the MDB appeared to be irregular and composed of separate cell groups rather than being continuous. At various cervical levels dendritic subunits appeared to interconnect obliquely the MDB, CDB and LDB. Transverse subunits radiated from the longitudinal bundles at right angles but did not appear as numerous as the oblique subunits. Catecholaminergic fibers were present at birth throughout the dendrite bundles. The CDB appears clearly to represent the phrenic nucleus while the MDB and LDB appear related to both cervical and accessory respiratory musculature. Recent studies indicate these motoneurons are related to cervical and accessory respiratory muscles. The presence of these dendrite bundles may permit the fine tuning of motoneuron activity for better coordination of movements involving synergistic and antagonistic muscle groups.

207.6 LAMININ DISTRIBUTION DURING CORTICOSPINAL TRACT DEVELOPMENT AND AFTER SPINAL CORD INJURY. A. Sosale*, J.A. Robson, and D.J. Stelzner (spon: G.H. Collins). Dept. of Anatomy and Cell Biology, S.U.N.Y. Health Science Center, Syracuse, NY 13210.

The corticospinal tract in the rat is a very useful model for studying development and regeneration in the central nervous system (CNS). The time course of CST development has been well delineated. It enters the spinal cord at birth and its development is completed by postnatal day 14 (P14). Growth of the CST after cutting its normal pathway is also age dependent. After postnatal day 6 (P6), the ability of the CST to grow around spinal injury is greatly diminished. Developing neurites and regenerating axons both require a specific microenvironment in which to grow. In vivo and in vitro studies show that adhesive molecules in the extracellular matrix are of primary importance in neurite outgrowth and elongation. One of the matrix molecules implicated to have a significant impact on neurite outgrowth is laminin.

This study examines the temporal and spatial distribution of laminin normally and after spinal injury during the period of CST elongation. Standard immunofluorescence techniques, staining frozen sections with anti-laminin polyclonal antibody were employed. Over 70 rats of different age groups (newborns, P3,6,9, 12,15 adults and embryos) were used. Particular attention was paid to the dorsal funiculus (dF) where the CST normally grows and the dorsal part of the lateral funiculus (dLF) - a pathway used mainly by the CST after spinal injury. Midthoracic spinal overhemisection lesions were performed on newborn and adult operates. This lesion is similar to injury where developing CST axons have been found to grow abnormally until P6. These animals were sacrificed 3,5,9 and 15 days after surgery and stained and compared together with normal controls. Adequate controls were maintained for each experiment.

Our results indicate that laminin is not present in the dF or the dLF postnatally when the CST is still growing down the spinal cord, though there is some diffuse overall reactivity seen at early embryonic stages. In the normal cord only the spinal roots, vasculature and meninges show laminin reactivity. Laminin is induced at the lesion site and around blood vessels near the lesion in newborn and adult operates at all the time periods examined. There is no increased laminin within the cord either rostral or caudal to the lesion site, ipsilaterally or contralaterally. From our studies it appears that during CST elongation laminin is not a requirement for growth. After injury laminin is localized to the lesion site and since it is induced equally in newborn and adult operates, it apparently is also by itself unrelated to the growth of the CST axons after spinal injury. (Supported by grants EY-03490 and NS14096.)

207.7 THE SWOLLEN NERVE FIBERS FOLLOWING MINOR SPINAL CORD INJURY: REGENERATING OR DEGENERATING ? Y.Naka*, K.Nakai, T.Itakura*, M.Ueno*, T.Okuno*, N.Komai*. Dept. of Neurol. Surg., Wakayama Med. Col., Wakayama 640 Japan. (SPON: T. Shirokawa)

We reported the appearance of swollen fibers immunoreactive to dopamine beta hydroxylase(DBH), vasoactive intestinal polypeptide(VIP) and substance P(SP)in the white matter near the primary lesion in 3 to 14 day-survived animals following the minor spinal cord injury (Naka et al. Soc.Neurosci.Abst. 11 :591, 1985).We regarded those swollen fibers as regenerative. In the present study we examined the alteration of each nerve fibers in later stages following the spinal cord injury and observed suggestive finding that they were regenerative nerve fibers.

Five adult female guinea pigs (250-300 g) were used. In 7 and 12 months after the minor spinal cord injury, the animals were perfused with 2 % paraformaldehyde and 0.25 % glutaraldehyde in cold phosphate buffer (PH 7.4). Parasagittal sections of the injured spinal cord were then processed for peroxidase antiperoxidase immunohistochemistry using antisera against DBH(gifted from Dr.J.Morrison), SP, and VIP. The cytochrome oxidase activity was also examined in the alternative sections.

The most prominent finding in the spinal cord of the long term (7,12 month) survival was the continued existence of similar swollen fibers as in the early stage with intense immunoreactivity against each of the antisera, although their size was larger and more lobulated. They were located in the white matter around the gliotic scar formation caused by the primary cord injury. The swollen axoplasm showed the elevated cytochrome oxidase activity in both early and late stages. Because the degenerating fibers are generally believed to retract and disappear in a relatively short period, it is speculated that the continued existence of swollen fibers with high metabolic activity in the later stages may not be degenerating but regenerating in nature, which is an interpretation consistent with that reported in the cat visual cortex(Nakai, Neurosci.Res. in press).

207.8 USE OF AN ELECTROMAGNETIC FIELD FOCUSSING PROBE TO GENERATE LESIONS IN THE SPINAL CORD OF THE RAT. S.H. Spillers, A.A. Patil*, W.S. Yamanashi*, and D.L. Hill*. Oral Roberts University School of Medicine. Tulsa, OK 74137

Recent reports have described the use of an electromagnetic field focussing (EFF) probe as a neurosurgical tool for cutting, coagulating, and vaporizing (Patil et al in press). The instrument functions as a resonance maximizing current sink and when applied to tissue in a near field of an electromagnetic field results in Eddy current convergence at the point of contact between tissue and probe tip. An insulated stainless steel needle serves as the probe. Hence the heat generated is both intense and focal; and because it is generated by convergent energy, there is very little spread to the surrounding tissues, limiting edema formation and tissue damage. This is in contrast to the laser which generates heat via penetrating energy resulting in more edema and tissue damage.

We are now using the EFF probe to transect the spinal cord of the rat in our ongoing regeneration studies. Because it functions via convergent current the probe can be used at higher wattages than a laser, resulting in focal vaporization of tissue producing a clean section without significant damage to adjacent tissue or edema formation; two factors thought to contribute to scar formation. Further, disruption and distortion of adjacent tissue encountered with a scalpel is avoided because significant pressure is not necessary for vaporization. Any vessels that may be severed during the procedure are coagulated, reducing blood loss. Finally, the size of the probe facilitates the severing of the cord while leaving the major dorsal artery intact, preserving the blood supply to the cord distal to the lesion.

We have begun to use the EFF probe to transect the spinal cord of the rat in regeneration studies. Preliminary results indicate that the EFF probe is superior to either the laser or scalpel, both in generating a clean cut and reducing subsequent scar formation.

Reference: Patil, A.A., et al: Electromagnetic Field Focussing Probe - A New Neurosurgical Tool. Acta Neurochurgica (In Press).

207.9 A TEST OF THE ABILITY OF LUMBOSACRAL DORSAL COLUMN AXONS TO GROW AFTER OVERHEMISECTION INJURY OF THE CERVICAL SPINAL CORD IN THE NEWBORN RAT. S.P. Lahr* and D.J. Stelzner. Dept. of Anat. and Cell Biol., SUNY Health Science Center, Syracuse, NY 13210.

The developing corticospinal tract (CST) of the neonatal rat grows around a high cervical or mid-thoracic spinal overhemisection lesion which interrupts its normal pathway in the dorsal funiculus. These axons maintain an aberrant position in the dorsal part of the lateral funiculus, but innervate appropriate areas of gray matter caudal to the lesion for many spinal segments (Bernstein and Stelzner J., Comp. Neurol. 221:382, Stelzner et al., Neurosci. Abst. 12:11). Ascending axons from dorsal root ganglion (DRG) cells are located in the dorsal funiculus adjacent to the CST. We are investigating whether DRG axons will also survive this same spinal lesion in the neonate and grow around it to innervate their normal target, the nucleus gracilis.

A spinal right overhemisection, which cuts the right half of the cord and the left dorsal funiculus, was performed at a high cervical level (C2) in neonatal rat pups within twenty-four hours of birth (n=5). Degeneration from such a lesion is cleared rapidly and is no longer apparent after 3 p.o. days (Gilbert and Stelzner J., Comp Neurol. 184:821). Three months after surgery, and in adult controls (n=2), the cauda equina was exposed and all the dorsal roots from L5 and caudal segments were cut bilaterally. Four days after the dorsal root lesion the animals were sacrificed and processed for Fink-Heimer staining of degenerating axons. Light microscopocic examination revealed degeneration argyrophilia within the dorsal funiculus and dorsal root projection zones of the lower lumbar and sacral segments, and within the postero-medial portion of the dorsal funiculus of thoracic and cervical cord to within 1-2 segments of the cervical overhemisection. Unlike controls, the projection thinned rostrally in thoracic and cervical regions as it approached the lesion and was apparent only along the postero-medial edge of the dorsal funiculus. No degeneration staining was seen in experimental animals rostral to the lesion in the cervical cord or in the nucleus gracilis, except for one animal which had an incomplete cervical lesion.

In this experiment ascending DRG axons, which lie adjacent to the CST, do not show the same ability to grow around an interruption of their normal pathway. We are currently repeating the experiment with a more sensitive transganglionic anterograde labelling technique using a cholera toxin-HRP conjugate. Previous experiments show that the DRG projection is more mature at birth than is the CST. We are presently determining if DRG axons from the caudal levels of the spinal cord have reached the nucleus gracilis at birth and whether these DRG cells survive axotomy by our lesion. Supported by NIH Grant NS 14096 (D.J.S.).

207.10 THE RESPONSE OF CORTICOSPINAL TRACT FIBERS FOLLOWING INJURY AND TRANSPLANTATION IN THE ADULT RAT SPINAL CORD. L. Jakeman and P.J. Reier. Departments of Neuroscience and Neurosurgery, University of Florida, Gainesville, FL, 32610.

Several laboratories have reported that developing corticospinal tract (CST) fibers can grow around lesion sites in neonatal rats. Also, when a graft of fetal spinal cord (FSC) tissue is placed into the immature spinal cord, CST axons will grow into and through the transplant. Together, these findings suggest that developing spinal cord tissue provides a suitable environment for CST elongation. Based upon these observations, we were interested in determining whether FSC grafts could stimulate the persistence or regeneration of adult CST axons which typically retract or die-back after injury. Partial spinal cord aspiration lesions, involving bilateral section of the CST, were made at the C6-C7 level in adult rats. One group received solid tissue transplants of E14 spinal cord tissue, while another group served as lesion controls. Six weeks later, CST fibers were labeled with bilateral application of combined HRP and WGA-HRP to the cortex. Histological examination of lesioned animals showed small cysts and/or areas of tissue necrosis interposed between the caudal border of the labeled CST and the margin of the lesion. The tract exhibited a tapered appearance, with some fibers terminating closer to the cut edge than others. No labeled axons were present directly at the edge of the lesion. A similar degeneration pattern was observed in most of the graft recipients, and consequently, direct apposition between the lesioned tracts and grafts was not routinely achieved. In a limited number of cases, however, fusion of host CST and graft tissues had occurred with no intervening cysts or necrosis. Under these more favorable conditions axons within the tract were observed at the edge of the interface, and in one example, a few axons had extended a short distance into the graft. Although limited, the results of this study suggest that under optimal conditions of host-graft apposition, FSC tissue may promote the persistence of injured CST fibers or stimulate their elongation. The graft recipients that did not differ from lesioned rats in their response to injury suggest that the timing of this apposition may be critical in the influence of embryonic transplants on injured spinal cord axons. Supported by NIH grants NS 22316 and MH 15737.

207.11 TRANSPLANTATION OF FETAL RAT SPINAL CORD INTO LONGSTANDIN CONTUSION INJURIES OF ADULT RAT SPINAL CORD. D. Winialski*, J. Houle', L. Jakeman, & P. Reier. Departments of Neurological Surgery and Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610.

Studies carried out in this laboratory have established that grafts of fetal rat spinal cord tissue will survive and establish some neuronal interactions with host tissue in both acute and longstanding aspirative hemisection cavities of adult rat spinal cord. The current study was carried out in order to evaluate the capacity of fetal tissue grafts to achieve anatomical integration with host tissue in contusion-induced cavitation lesions. Such lesions were made in female rats at the thoraco-lumbar region by a modified Allen weight-drop apparatus in which a 3.25 gram weight was dropped from a height of 20 cm onto an impounder placed on the dorsal spinal cord surface. At 2 to 14 months post-injury E14 fetal rat spinal cord tissue was grafted into the resulting cystic cavities. Transplant recipients were then sacrificed after 2 to 13 months for histological and immunocytochemical analysis.

Two months after contusion lesion, characteristic hemorrhagic necrosis with cavitation extending 5-6 mm in rostral-caudal extent was observed. Only a rim of white matter surrounding the cavity was visible. Greater than 90% of the grafts survived, growing to fill cavities up to 7mm in length. While the grafts appeared to be well interfaced with host tissue, glial fibrillary acidic protein [GFAP] immunocytochemistry revealed a dense matrix of astrocytic processes intervening between host and donor tissue. However, there were several zones of apposition displaying minimal scar formation, allowing direct fusion of host and graft tissue. Examination of 2mm plastic sections revealed regions of neuronal processes traversing the interface. Serotonin immunocytochemistry identified one specific axonal population crossing the rostral host-graft interface, extending into transplant tissue for distances of less than 2mm. This study documents the feasibility of using fetal spinal cord grafts to repair the severely contused spinal cord. Supported by NIH NS22316, PVA NBR 588-6, and APA TC 86-05

EXCITATORY AMINO ACIDS: MECHANISMS

208.1 EXCITATORY AMINO ACID SENSITIVITY OF NEURONAL CULTURES MAINTAINED IN MEDIA CONTAINING POTENT AGONISTS AND MODULATORS. K. Sugiyama and M.L. Mayer. Lab. Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

Neuronal cultures of mammalian spinal cord and hippocampus normally express receptors for three classes of glutamate receptor, classified by use of the selective agonists kainate, quisqualate and NMDA. Some recent reports have described culture preparations in which neuronal responses to NMDA were either absent or attenuated (Jahr & Jessell, J. Neurosci **5**, 2281; Lambert & Jensen, Neurosci. Lett **26**, S149). In these studies neurons were maintained in culture media containing either potent modulators of excitatory amino acid responses (DMEM: 400 μM glycine) or both modulators and excitatory amino acid agonists (Ham's F-12: 3 μM Zinc and 100 μM each of glycine, aspartic acid and glutamic acid).

We have examined the sensitivity of hippocampal neurons to kainate and NMDA. Sets of sister cultures were grown in media based on Eagles MEM with an insulin, transferrin nutrient supplement; 3 conditions were tested: 5% horse serum; 5% horse serum + 400 μM glycine; serum free + 400 μM glycine. These conditions were chosen to be similar to DMEM. A second set of cultures was grown in media based either on Ham's F-12 or MEM, to which the nutrient supplement and 5 % horse serum was added. Electrophysiological recording from coded cultures was performed without knowledge of growth conditions, using tight seal whole cell recording and CsCl filled pipettes for voltage clamp.

In the first set of experiments, using DMEM-like growth conditions, no difference in the amplitude of responses to kainate (100 μM) or NMDA (100 μM) was detected between the growth conditions. Responses were assayed on days 7,10 and 15 in culture, and showed a normal development in all media presumablely reflecting an increase in neuronal cell surface area (Mitchell and Westbrook, Soc. Neurosci Abstr **11**, 105).

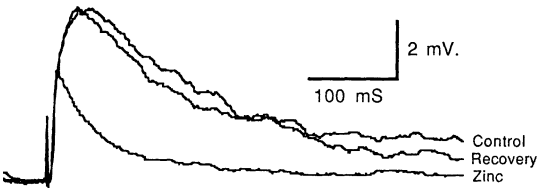
In the second set of experiments on day 7 in culture, no statistical difference was observed in the amplitude of responses to kainate or NMDA between cells grown in F12 medium and MEM medium. However, on days 10 and 15, responses to both kainate and NMDA recorded from neurons maintained in F12 medium became progressively larger than those in MEM medium. The average increase in amplitude compared to responses from cells grown in MEM was: kainate, 86 % on day 10 and 90 % on day 15 in culture; responses to NMDA was also increased by 41 % on day 10 and 60 % on day 15 in culture.

These results indicate that in hippocampal neurons, the sensitivity to kainate or NMDA is not decreased by chronic exposure to high concentrations of glycine. However the sensitivity to both excitatory amino acids increases following prolonged exposure to Ham's F-12 medium, containing both aspartic and glutamic acids. This was unexpected given O'Brien & Fischbach's suggestion (J. Neuroscience **11**, 3290) that L-glutamate down regulates the expression of excitatory amino acid receptors.

208.2 GLYCINE POTENTIATES AND ZINC BLOCKS A SLOW EPSP BETWEEN HIPPOCAMPAL NEURONS IN CULTURE. I.D. Forsythe, G.L. Westbrook and M.L.Mayer. Lab. of Dev. Neurobiology, NICHD, NIH, Bethesda MD. 20892.

Monosynaptic EPSPS mediated by excitatory amino acids can be separated into two kinetically distinct components: A fast conventional EPSP and a slow component which is voltage sensitive, calcium permeable and antagonised by 2-amino-5-phosphonovalerate, indicating that it is mediated by NMDA receptors (Forsythe & Westbrook, J. Physiol. submitted). Recent evidence suggests that the NMDA receptor channel is modulated by several endogenous substances. Sub-micromolar concentrations of glycine have been shown to potentiate NMDA responses by increasing the probability of channel opening via a strychnine insensitive mechanism (Johnson & Ascher, Nature,325:529, 1987). Zinc, on the other hand blocks NMDA channels by a non-competitive mechanism (Westbrook & Mayer, see adjacent abs.). Zinc is present in mossy fibre terminals and is released during synaptic stimulation, while glycine is present in the extracellular fluid. To examine the extent to which synaptic transmission can be modulated by these compounds; we have studied the effect of both glycine and zinc at an excitatory synapse in culture.

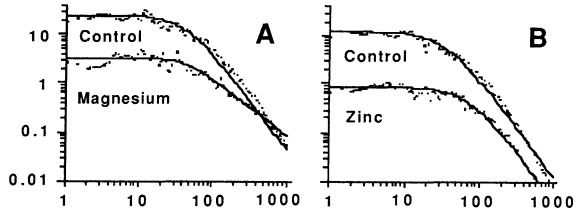
In low density cultures of hippocampal neurons monosynaptic EPSPs were recorded during perfusion of control medium and on the addition of glycine or zinc. The extracellular medium contained 1 mM Ca, no added Mg and 10 μM picrotoxin. Pairs of whole cell patch recordings were made with potassium methyl-sulphate filled electrodes at 25° C. Before perfusion the two components of the EPSP are unaffected by local perfusion of control medium from puffer pipettes; however perfusion of the whole bath by the same medium results in the abolition of the slow, NMDA receptor-mediated component. Switching the perfusion to control medium plus 1 μM glycine restores the slow component to control levels. Local application or perfusion of zinc (50 μM) also substantially reduces the slow component of the EPSP even in the presence of added glycine, as illustrated below. Each trace is the average of 16 sweeps at -67 mV. These results show that glycine and zinc, endogenous neuromodulators, have opposing actions on synaptically-activated NMDA-receptors, and that when the NMDA receptor is blocked by zinc it cannot be potentiated by glycine.



208.3 ZINC AND MAGNESIUM ACT AT SEPARATE SITES ON NMDA-RECEPTOR CHANNELS
G. Westbrook & M.Mayer. Lab.Dev.Neurobiol., NICHD, NIH, Bethesda, MD 20892.

Divalent cations in the extracellular fluid play a critical role in determining the impact of NMDA-receptor activation on neuronal function in that magnesium ions block the channel in a voltage-dependent manner, and calcium is highly permeable with an estimated relative permeability of PCa : PNa of 10.6 (Mayer & Westbrook, J. Physiol., in press). Although other divalent cations such as Ni and Mn can also interact with NMDA channels, this is of little physiological relevance since they are present only in trace quantities. An exception to this is Zn which is present in high concentrations in nerve terminals especially the hippocampal mossy fibers. Of particular interest to receptor function, Zn is released with stimulation with estimated concentrations in the synaptic cleft reaching several hundred micromolar. It is conceivable that synaptic release of Zn may affect synaptic transmission since we have recently found that Zn at micromolar concentrations blocks responses to NMDA, but not to kainate or quisqualate on cultured hippocampal neurons (Mayer & Westbrook, Biophys. J. 51:64a 1987). The effects of zinc are rapidly reversible and thus unlikely to result from reduction of sulfhydryl bonds. Complex formation between zinc and either glycine or agonists is also insufficient to explain the antagonism of responses to NMDA.

The antagonism by zinc is non-competitive, and unlike Mg, the block by Zn is not relieved by depolarization suggesting that Zn and Mg act at separate sites. We have used fluctuation analysis to examine the effects of Zn and Mg on the single channel properties of NMDA-evoked currents. NMDA (1-10 μM) was applied by a large bore flow pipe to evoke a steady inward current at -50 or -60 mV in the presence or absence of either 150 μM Mg or 30 μM Zn. Control spectra of agonist-evoked currents were well fit by a single Lorentzian. In Mg, two Lorentzians were required to adequately describe the power spectra (**A**), consistent with a channel blocking action of Mg. However, similar degrees of antagonism by Zn did not produce a distinct two Lorentzian spectra (**B**), but compared to the action of D-AP5 (5 μM) on the same neurons, Zn did cause a small but consistent reduction in the single channel lifetime and conductance.



208.4 ANALYSIS OF GLUTAMATE CURRENTS AND GLUTAMATE CURRENT NOISE OF CULTURED NEURONS DISSOCIATED FROM SEPTUM OF RAT.
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Membrane currents induced by pressure applications of L-glutamate (Glu), kainate (Ka), quisqualate (Qa) and NMDA were recorded from cultured neurons dissociated from septal area of rat under the whole cell clamp conditions. All neurons (70 cells) responded to Glu or the agonists. Current-voltage relations showed the Ka-current was larger than the Qa-current when Ka or Qa of 50 uM concentration was applied, however the Qa-current was larger than or equal to the Ka-current when the concentration was reduced to 20 uM or less. The currents induced by a puff of 100 uM NMDA were of similar magnitude to those by 20 uM Qa. Fluctuations of currents were analyzed to study the behavior of the whole glutamate channels of cells. The power spectrum density functions (PSDs) of the fluctuations had the following properties. In almost every case, the high frequency component of PSD had $1/f^{\alpha}$, $1 \leqq \alpha \leqq 2$ properties, however in rare cases α was -0.7(1 cell), 2.2(1 cell), 2.9(1 cell) at some holding potentials. The value of α changed when the holding potential was changed, depending on unknown conditions of cells. The mean of α's for holding potenitals between -20 and -120 mV was 1.7, except the cases of more than 100uM NMDA where α was alwatys 2.0. When the PSDs were fitted by a single Lorenzian or a sum of two Lorenzians (a sum of more than two Lorenzians did not improve the fitting), the cut-off frequencies of them were 22 and 70 msec. Effective conductance was culculated when PSD was fitted well to single Lorenzian which ranged from 1 to 6 pS for Ka, from 0.3 to 5 pS for Qa, from 9 to 35 pS for NMDA. Pressure applications of 10uM Glu and one of three agonists were conducted alternatively to the same cell to find the differences between PSDs for the drugs. These paired applications showed that PSDs by Glu and by an agonist for the same cell had strong correlations, that is, in 16 cells out of 17 cells both PSDs of the pair shifted upward or downward in parallel on the graph of PSD, by the change of the holding potential. It seems that the activation and the kinetics of channels strongly depend on the internal state of the cell.

208.5 NMDA RECEPTORS EXPRESSED IN XENOPUS OOCYTES ARE REGULATED BY Mg AND GLYCINE.
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The Xenopus oocyte translation system has been used recently to study the properties of mammalian amino acid receptors, and as a cloning tool for these receptors. RNA preparations that include mRNA encoding NMDA receptors have been notoriously difficult to obtain, perhaps due to the size and/or complexity of NMDA receptor mRNA. We have succeeded in this task. mRNA was isolated from primary cultures of rat brain by the guanidinium thiocyanate - CsCl method and oligo-dT cellulose chromatography. Each oocyte (stage V or VI) received 40-80 ng of mRNA by microinjection and was assayed by voltage clamp after 1-4 days of culture at 19°C in modified Barth's solution. For testing, oocytes were perfused with culture medium and voltage clamped with one or two microelectrodes; drugs were applied by perfusion.

Many mRNA preparations that induced the expression of receptors for kainate and AMPA did not induce NMDA responses in oocytes; only a few mRNA preparations encoded NMDA receptors. At a holding potential of -60 mV, NMDA evoked a smooth, nondesensitizing inward current that was readily reversible on washout. The EC50 of NMDA was approximately 40 μM. The NMDA receptor antagonist, D-2-amino-5-phosphonovaleric acid (D-APV, 10 μM), reduced the amplitude of the ionic current evoked by 100 μM NMDA by 83% (mean of 6 cells). Current-voltage curves of NMDA responses constructed in the presence of 1 mM Mg showed a nonlinear region between -80 and -20 mV, which was nearly eliminated when Mg was omitted from the perfusion fluid. The ratio of chord conductances at -60 mV and -30 mV was 0.13 in Mg but 0.91 without Mg (n=5), attesting to strong voltage dependent block by Mg. The maximum current evoked by NMDA was increased approximately 9-fold by 3 μM glycine, without a marked lateral shift of the NMDA concentration-response curve. Responses to ibotenate (100 μM), but not kainate (100 μM) or AMPA (50 μM) showed a similar voltage-dependence and potentiation by glycine.

These results demonstrate that both regulatory components of the mammalian NMDA receptor - its voltage-dependent block by Mg and potentiation by glycine - are preserved in frog oocytes injected with the appropriate mRNA. This preparation lends itself well to quantitative studies of the regulation of NMDA receptor activation, and may be useful for purification of mRNA(s) encoding these receptors. Supported by NS-17771, NS-22249, NS-23804 and a Pharmaceutical Manufacturers' Association Foundation Predoctoral Fellowship (TV).

208.6 MODULATION OF INTRACELLULAR FREE CALCIUM IN ISOLATED FOETAL BRAIN CELLS: MEASUREMENTS WITH FURA-2.
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Studies using fluorescence analysis have identified two types of calcium-permeable channels in mammalian prenatal brain cells. Brain slices from 12-14 day-old mouse embryos were used to prepare isolated cells in suspension (*Morris et al., Exp. Brain Res. 65: 520, 1987*). After treatment with trypsin/trypsin inhibitor, collagenase and histopaque, separated cells were incubated with 4 μm fura-2/AM at 37° for 90 min to achieve a final intracellular concentration of ≈ 0.1 mM fura-2, and then washed and resuspended in a Ringer solution containing 3 mM KCl, 10 mM glucose and 0.2 mM EGTA. Fluorescence cuvette measurements were made with 4 x 10^6 cells/ml and excitation and emission wavelengths of 339 (±4) and 499 (±8) nm. Limits of calcium binding of the ligand were determined by consecutive additions of ionomycin (1 μM) and Mn^{2+} (3 mM) in the presence of extracellular calcium ($[Ca^{2+}]_o$). Initial levels of intracellular calcium ($[Ca^{2+}]_i$) were 99 (SE ±3, n = 54) nM and increased to 197 (SE ±6) nM when $[Ca^{2+}]_o$ was raised to 1 mM. Subsequently, increasing extracellular potassium ($[K^+]_o$) (between 1-80 mM) evoked dose-dependent accumulations of $[Ca^{2+}]_i$ -- with a maximal increase of ≈ 50 nM at $[K^+]_o$ = 40 mM. These did not occur in the absence of $[Ca^{2+}]_o$ and were attenuated by TTX (200 nM) and nitrendipine (≤ 50 nM) and blocked by verapamil (2.5 μM). Small dose-dependent increases in $[Ca^{2+}]_i$ were seen with hexanol (2-20 mM), pentobarbital (5-200 μM) and ethanol (800 mM), but no changes with ketamine (5-200 μM). Although no changes were observed with either quisqualic acid or the kainate-like *Lathyrus* neurotoxin, L-3-oxalyl-2-aminopropionic acid (50-400 μM), N-methyl-D-aspartate (NMDA) (50-800 μM) evoked increases of 60-80 nM which could be blocked by Mg^{2+}, ketamine (≤ 20 μM) and 2-amino-phosphonovaleric acid (100 μM). A significant and dose-dependent potentiation of submaximal NMDA-evoked responses was produced by glycine (10-1000 nM); and a parallel shift of the NMDA concentration-response curve was apparent. The direct measurement of intracellular calcium in these experiments demonstrates (i) the existence in prenatal brain cells of both voltage-dependent calcium channels and NMDA receptor-activated channels which are permeable to Ca^{2+}, (ii) the ability to modulate influx and accumulation of $[Ca^{2+}]_i$ (by specific organic and inorganic blockers/antagonists, anaesthetic agents, glycine and changes in ion levels), and (iii) the development of a preparation which can be used to assess pharmacological blockade of anoxic/ischaemic brain damage.

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208.7 AMPA-ACTIVATED CHANNELS IN MAMMALIAN CEREBELLAR NEURONS. J.L. Christiansen and L.M. Nowak. Dept. of Pharmacology, College of Vet. Med., Cornell University, Ithaca, NY 14853.

It was reported recently that quisqualate, N-methyl-D-aspartate and kainate each activate ion channels with multiple conductance states (Cull-Candy & Usowicz, 1987; Jahr & Stevens, 1987; Ascher & Nowak, in press) and that each agonist activated some conductance states shared by other agonists. One hypothesis is that shared conductance states arise because agonists such as quisqualate and glutamate activate more than one receptor subtype and as mixed agonists they stimulate channel activity consistent with their affinity for the different receptor subtypes. This hypothesis assumes that each receptor is associated with a different channel protein each of which may have multiple conductances. We have approached this problem by studying the pharmacological profiles of quisqualate-activated ion channels in cultured neurons and quisqualate receptors in rat brain membranes by combining patch clamp electrophysiology and radioligand binding assays.

Microexplants of cerebella from one day old rat pups were grown in culture for at least 10 days before use in patch clamp recordings. Whole cell and outside-out patches were made with pipettes containing (in mM): 145 CsCl, 10 EGTA/1.0 $CaCl_2$ and Hepes-K (pH 7.2) from presumptive Purkinje neurons. The extracellular solution contained (in mM): 145 NaCl, 2.8 KCl, 1.0 $CaCl_2$, 10 Hepes-Na (pH 7.2) and 0.3 µM tetrodotoxin. Drugs were applied to cells by fast perfusion (U-tube method) and by bath exchange. Noise analysis was done on whole cell records to estimate channel open time (τ) and current-voltage plots were made from single channel data to measure channel conductance (γ). Radioligand binding assays were performed on well washed rat brain membranes and binding of ^{3}H-AMPA was done in the presence of 100 mM KSCN as described by Murphy et al. (1987). Displacement and saturation binding assays were done using the filtration method.

AMPA (5-10 µM) evoked large whole cell currents associated with a small increase in noise. Power spectra of AMPA noise were fitted by a single Lorenzian in some cells with a τ of near 10ms, or by two Lorenzians in other cells where there was a faster τ of about 1 ms. The single channel data showed predominantly small conductance channels similar to the 8-10 pS channels activated by quisqualate. This channel is also activated by L-glutamate (10-100 µM). Preliminary binding studies reveal that AMPA has a saturable, high affinity binding site with a 20-30 nM K_D on rat brain membranes which was displaceable by L-glutamate with an IC_{50} of roughly 0.3 µM. Further comparisons of the pharmacology of the binding sites and ion channel activation are in progress.

This research was supported by NIH grant #NS24467 to LMN and JLC was supported by a PMA predoctoral fellowship award.

208.8 ACTIONS OF EXCITATORY AMINO ACIDS ON MEMBRANE PROPERTIES OF DEVELOPING PURKINJE NEURONS IN CULTURE. M. Joels*, A.J. Yool, and D.L. Gruol, Div. Preclin. Neurosci. and Endocrin., Scripps Clinic and Res. Foundation, La Jolla, CA 92037.

In previous extracellular studies it has been shown that mature Purkinje neurons (PNs) in culture display a multiphasic response to glutamate (GLU). The response is characterized by an immediate increase in simple spike firing followed by a phase of bursting discharges, reduced activity and finally return to baseline activity (Franklin and Gruol, Neurosci. Abstr. 12, 1986). The effects of the excitatory amino acid agonist quisqualate (QUIS) were similar to those of GLU. Kainic acid (KA) on the other hand only induced an increase in simple spike firing. Immature PNs (7 days in culture) were excited by GLU, QUIS and KA but did not display the complex responses seen in the mature cells. With whole cell and single channel recording techniques we have investigated the changes in membrane properties and ion channel activity that may underlie the extracellularly observed responses. Recordings were made from neurons at two developmental stages; 7-9 days in culture, when PNs have only thin neurites and at 12-16 days in culture, when branching dendrites are present. GLU (10 µM), QUIS (1 µM) or KA (10 µM) were ejected from glass pipettes by pressure. In whole cell recordings from identified PNs (n=32) mean resting membrane potential was -56.7±1.2mV (±SEM). Mean input resistance (Rin) was 466±136MΩ for the immature PNs (7-9 days) and 260±37MΩ for the older PNs (~15 days). At both ages, GLU induced a depolarization (24.0±1.9mV) accompanied by a decrease in Rin. In approximately half of the cells the depolarization was followed by a small (3-4mV) hyperpolarization. In mature PNs the total duration of the responses (116±31sec) was longer than in immature cells (50±8.5sec). QUIS evoked prolonged depolarizations (22.1±2.5mV) accompanied by a decrease in Rin followed by a pronounced hyperpolarization (5.9±1.8mV). The GLU and QUIS depolarizations were characterized by an initial burst of spikes, a period of decreased spike-amplitude, gradual recovery of spike amplitude, then increased firing relative to control. During the subsequent repolarizing and hyperpolarizing phases, spike frequency was reduced. In contrast to GLU and QUIS, KA mainly induced a small (13±1.3mV) and short lasting (~25sec) depolarization and increase in spike frequency. These data indicate that PNs show differential membrane responses to the excitatory amino acid agonists, QUIS being the most potent. In preliminary single channel studies using the cell attached configuration, activation of a small conductance K+ channel was associated with the inhibitory phase of the QUIS response. Further studies of amino acid evoked changes in channel activity are in progress. (Supported by NIAAA Grant AA06665 to DLG and AA07456; C & C Huygens Grant H88-145 from the Dutch ZWO to MJ).

208.9 QUANTITATIVE STUDY OF THE USE DEPENDENT BLOCK OF EXCITATORY AMINO ACID CURRENTS BY KETAMINE AND PHENCYCLIDINE. J.F. MacDonald, P. Pahapill, I. Mody and P.S. Pennefather. Playfair Neurosci. Unit, The Toronto Hosp. & Dept. Physiology & Faculty of Pharmacy, Univ. Toronto, Toronto, Ont. M5T 2S8.

We have shown previously that ketamine will block the response to NMDA agonists in a use-dependent manner. Both the onset and offset of blockade is enhanced by repeated activation of NMDA receptors. This suggests that ketamine interacts selectively with the open channel state of the receptor and if the channel closes while blocked, the ability of ketamine to dissociate is greatly reduced. In effect, ketamine becomes trapped on the channel. The guarded receptor hypothesis proposed by Starmer et al. (Biophys. J. 49, 913, 1986) provides equations to describe this type of blockade. We have applied these equations to the use-dependent blockade of excitatory amino acid currents.

Currents evoked by brief pressure applications of L-aspartate were recorded in cultured murine hippocampal neurones with a patch electrode in the whole-cell configuration and a discontinuous voltage-clamp. Responses rose rapidly and decayed with a time constant of 1 to 2 s. The equations allow the rates of interaction during and between the response to be calculated from the fractional decrease per pulse of agonist, the steady state level of blockade and the frequency-dependence of the blockade. The analysis was simplified because the blockade by ketamine was independent of the frequency of agonist application, suggesting that the interaction was negligible between pulses.

At -60 mV, the calculated forward and reverse rates of interaction with the open channel for ketamine were 2×10^4 M^{-1} s^{-1} and 0.1 s^{-1}, respectively. These rate constants were independent of ketamine concentration and predict an affinity of 5 µM. Changing the pH from 7.4 to 9.0 reduced the fraction of ketamine that is positively charged (pK_a=7.5) and decreased the forward rate constant, implying that it is the charged form of ketamine that is most potent. With phencyclidine, the forward rate constant was similar to ketamine but the reverse rate constant was ten times slower; thus, accounting for the greater potency of this agent. The channel activated by L-aspartate has a mean open time of 5 ms and therefore we would predict that in the presence of a half-maximal concentration of ketamine an individual channel would have to open or close on average 2000 times before becoming blocked or unblocked.

The guarded receptor hypothesis provides a useful model for interpreting the actions of ketamine and phencyclidine on the NMDA-gated channel. Supported by MRC of Canada.

208.10 EFFECTS OF EXCITATORY AMINO ACIDS ON Ca^{2+}- AND K^+-CHANNELS IN RAT BRAIN SYNAPTOSOMES. M. Simonato*, R. Jope and L. Beani* (SPON: M. Koenig). Department of Pharmacology, University of Alabama at Birmingham, Birmingham, AL 35294 and Institute of Pharmacology, University of Ferrara, 44100 Ferrara, Italy.

Recent evidence indicates that excitatory amino acids play an important role in the normal activity of the mammalian CNS and in neuronal damage associated with a number of disorders. An association has been established between excitatory amino acid-induced cell death and elevated intracellular calcium. In this study we examined the effects of glutamate, kainate and N-methyl-D-aspartate (NMDA) on synaptosomal flux of calcium and rubidium (as an indicator of K^+ flux). This approach allows the measurement of direct effects on cation fluxes in nerve terminals, the site of presynaptic modulation of release and a possible site of action of excitatory amino acids.

Ca^{2+} influx mediated by fast channels (1 sec) has been studied in rat cortical and hippocampal synaptosomes by measuring influx in low K^+ and high K^+ media. The rate of Ca^{2+} influx in low K^+ and the net stimulation by high K^+ in cortical synaptosomes are 0.127 ± 0.015 and 0.607 ± 0.060 nmoles/mg prot, respectively, while in hippocampal synaptosomes they are 0.381 ± 0.057 and 0.397 ± 0.036, respectively.

Glutamate (5 mM) significantly increased the resting influx of $^{45}Ca^{++}$ in cortical synaptosomes (146 ± 8% of control), and decreased the net uptake after depolarization (76 ± 6%); glutamate did not have a significant effect in the hippocampus. Kainic acid (5 mM) did not alter cortical synaptosomal $^{45}Ca^{2+}$ flux, but decreased resting influx (73 ± 3%) and increased the net uptake (137 ± 18%) in the hippocampus. NMDA (5 mM) had no significant effect on any of these measures.

K^+ channels were studied by measuring $^{86}Rb^+$ efflux from synaptosomes purified from rat forebrain and preloaded with $^{86}Rb^+$ by the method of Bartschat and Blaustein (J. Physiol. 361, 419, 1985). The effects of glutamate, kainic acid and NMDA were measured at 1, 3 and 5 sec, in media containing low K^+, high K^+ without Ca^{2+} and high K^+ with Ca^{2+}. These results will be discussed with regard to the direct effects of excitatory amino acids on cation fluxes in nerve endings.
Supported by AG04719 and MH38752.

208.11 PHARMACOLOGICAL MANIPULATION OF GLUTAMATE-INDUCED CALCIUM INFLUX IN CULTURED NEURONS. A. W. Probert* and F. W. Marcoux (SPON: J. Marriott). Warner-Lambert/Parke-Davis, Pharmaceutical Research, Ann Arbor, MI 48105.

The excitatory amino acid, glutamate, exerts neurotoxic effects in vitro when present in high concentrations. These excitotoxic effects have been linked to the NMDA-type glutamate receptor and reported to involve calcium accumulation intracellularly. Whereas NMDA antagonists have been shown to prevent glutamate-induced excitotoxicity, less is known regarding their effects on calcium influx. We studied the effects of NMDA antagonists and other drugs on calcium influx induced by excitotoxic concentrations of glutamate in mammalian cultured neurons. Eighteen day old cortical neurons isolated from fetal rat brain were co-incubated in 100 μM L-glutamate and varying concentrations of test compounds. Calcium influx was assessed by measuring neuronal accumulation of $^{45}Ca^{++}$ from the culture medium.

Compounds examined included the competitive NMDA antagonists, 2-amino-7-phosphonoheptanoic acid (7-APH), 4-(3-phosphonopropyl)-2-piperazinecarboxylic acid (CPP) and gamma-D-glutamylglycine (DGG) and the non-competitive NMDA antagonists, ketamine (KET), phencyclidine (PCP) and (+)-10,11-dihydro-5-methyl-5H-dibenzo [a,d] cyclohepten-5,10 imine (MK-801).

CPP, DGG and 7-APH inhibited glutamate-induced $^{45}Ca^{++}$ influx with IC_{50}s of 28, 156 and 245 μM, respectively. MK-801, PCP and KET inhibited $^{45}Ca^{++}$ influx more potently with IC_{50}s of 0.10, 0.16 and 6.8 μM, respectively. These results with known NMDA antagonists parallel in rank order of potency other reports on the antiexcitotoxic actions of the compounds; this suggests that inhibition of Ca^{++} influx is involved in the antiexcitotoxic effects of NMDA antagonists.

208.12 ROLE OF NMDA-ACTIVATED AND VOLTAGE-DEPENDENT CALCIUM CHANNELS IN THE INDUCTION OF HIPPOCAMPAL LONG-TERM POTENTIATION. A.H. Ganong and T.H. Brown. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The induction of long-term synaptic potentiation in hippocampal CA1 pyramidal neurons requires conjuctive pre- and post-synaptic activity (Kelso, S.R., Ganong, A.H., and Brown, T. H., PNAS, 83: 5326-5330, 1986). Several lines of evidence suggest that one of the critical steps in the induction mechanism may involve increases in intracellular free calcium in the postsynaptic neuron. One formal model predicts that calcium entry through voltage-dependent calcium channels in the spine head can account for the spatiotemporal features of associative LTP (Gamble, E., and Koch, C., Science, in press). An alternative model suggests that calcium entry through the channel coupled to the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor is necessary for the induction of LTP. We have begun to test differential predictions of these models in the Schaffer-CA1 synapse.

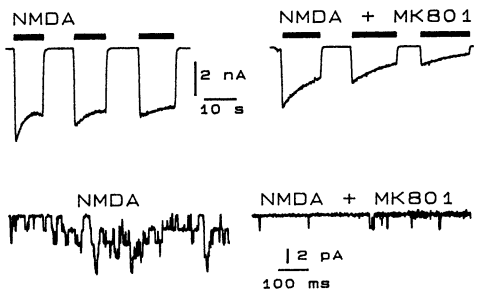
Intracellular recordings were from CA1 pyramidal neurons in rat hippocampal slices. The medium contained picrotoxin to reduce the synaptic inhibition that accompanies stimulation of the Schaffer excitatory input. The intracellular electrode contained 2 M CsCl and 50 mM QX-314 or QX-222 in order to suppress sodium spikes and help maintain postsynaptic potential control. Stimulus intensity was adjusted to elicit EPSPs of about 5 mV peak amplitude at a holding potential of -80 mV. As we have shown previously, high frequency presynaptic stimulation (100 Hz for 200 msec, 5 repetitions) during application of a voltage clamp to the postsynaptic neuron did not produce LTP. LTP was induced if the presynaptic stimulation was paired with an outward current step sufficient to elicit a QX-314-resistant spike, or a voltage-clamp step to -20 mV (which elicited a large inward current). LTP was not induced by either an outward current step alone (which elicited a QX-314-resistant spike), or a voltage-clamp step sufficient to induce a inward current.

Application of the NMDA receptor antagonist 2-amino-5-phosphonopentanoate (AP5) blocked the induction of LTP even if the intensity of the stimulation was sufficient to produce a QX-222-resistant spike during the depolarization caused by the high frequency afferent stimulation. The presence of AP5 prevented the induction of LTP even when strong afferent stimulation was paired with additional postsynaptic depolarization from the intracellular electrode.

These results are less easily explained by the Gamble-Koch model than the alternative. (Supported by NRSA NS08042-02, the McKnight Foundation, and AFOSR Contract F49620.)

208.13 NMDA-ACTIVATED CURRENTS IN RAT NEOCORTICAL NEURONS ARE BLOCKED BY THE ANTICONVULSANT MK-801. J.E. Huettner and B.P. Bean. Dept. of Neurobiology, Harvard Medical School, 25 Shattuck St., Boston, MA 02115

We have used whole-cell and single channel recording to study the action of MK-801 (Wong et al., PNAS 83:7104, 1986) on currents elicited by excitatory amino acids. Cortical neurons from young rats were maintained in cell culture for 1-3 weeks. Electrodes contained (in mM) Cs-methanesulfonate 120; HEPES 10 pH 7.4; EGTA 10; Mg 5; ATP 4 and GTP 0.3. Cells were clamped at -60 to -80 mV and drugs were applied at 1-50 uM (in NaCl 160; HEPES 10 pH 7.4; CaCl2 2) for 1-10 sec by local perfusion. NMDA (+1uM glycine), kainate and quisqualate (Quis) evoked inward currents in every cell. Application of 2 uM MK-801 +1 uM glycine for 10-20 sec had no effect on the response evoked by subsequent application of NMDA, kainate or Quis. When NMDA and MK-801 were applied simultaneously, the inward current elicited by NMDA decreased steadily with a time constant of several seconds. After washout with control solution, responses to NMDA recovered very slowly. Exposure to NMDA speeded the recovery. These results suggest that MK-801 blocks the channel activated by NMDA and that binding and unbinding of the drug are facilitated when the channel is open. MK-801 did not diminish currents elicited by kainate or Quis. Application of NMDA to outside-out patches activated channels of 45 pS. MK-801 reduced channel activity without changing unitary conductance.



208.14 EXCITATORY AMINO ACID RECEPTORS FROM RAT BRAIN EXPRESSED IN XENOPUS OOCTYES: SINGLE CHANNEL RECORDINGS. A.M.J. VanDongen(*), G. Frech(*), R. Joho(*) and A.M. Brown. Dept. Physiol. and Molec. Biophys., Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030.

Frog oocytes microinjected with mRNA can express exogeneous glutamate receptors (Gundersen et al, 1984, Proc.R.Soc.B211:127). We are using this powerful expression system to study the electrophysiological and molecular biological properties of excitatory amino acid receptors. At least three distinct types of glutamate receptors, characterized by the specific agonists NMDA, kainate and quisqualate have been proposed for the mammalian central nervous system. Reports by Jahr and Stevens (1987, Nature 325:522) and Cull-Candy and Usowicz (ibid p525) on single channel currents have shown that all agonists activate the same set of multiple conductances. However, each agonist predominantly activates one subset of conductances. Direct transitions between all the levels were observed. Taken together, the existence of a complex molecular entity, consisting of a set of receptors coupled (conjointly or separately) to an ionophore with multiple conductance states is suggested.

We micro-injected oocytes from Xenopus laevis with total mRNA isolated from whole rat brain or with size-fractionated mRNA. After 2-6 days at 19°C, whole cell two-electrode voltage clamp experiments showed inward currents following bath perfusion of kainate, glutamate, quisqualate and aspartate. Single channel current measurements were performed using outside-out patches excised from the oocyte membrane. Application of kainate activated multiple conductance levels with similar characteristics as those reported for native neuronal membranes. Preliminary data indicate that the response to glutamate may be different.

208.15 MECHANISMS BY WHICH QUISQUALATE SUPPRESSES KAINATE INDUCED ELEVATIONS OF CEREBELLAR CYCLIC GMP. P.P. McCaslin* and W.W. Morgan. Dept. of Cellular & Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

Primary cultures of cerebellar neurons from 8 day old rat pups were grown for 14 days in vitro in antibiotic-free media and then analysed for changes in both cyclic guanosine monophosphate (cGMP) and the influx of ^{45}Ca in response to several excitatory amino acids (EAA) or related antagonists. We have previously reported that kainic acid (KA), N-methyl-d-aspartate (NMDA) and quisqualic acid (QA; listed in order of potency) produce dose-, time- and calcium-dependent elevations of cGMP in these cultures. The effects of QA and NMDA on this parameter were additive as were the effects of KA and NMDA, however, 100 μM of QA completely prevented the KA-induced elevation of this cyclic nucleotide. Here, using Shield's analysis, we report that concentrations of QA as low as 2.5 μM (which has no effect on cGMP at this concentration) significantly reduced the elevations of cGMP induced by 100 μM KA by 25%. By contrast, 100 μM concentrations of the EAA antagonists, cis-2,3-piperidine dicarboxylic acid (PDA) and glutamylaminomethylsulphonic acid (GAMS), were required to reduce the effect of KA by an equivalent amount. Since binding studies have shown that KA has a 30-fold greater affinity for the KA-receptor than does QA, it is possible that the effect of QA on preventing the KA-induced elevation of cGMP is at some site removed from the KA receptor recognition site(s). Therefore, the EAA-stimulated influx of ^{45}Ca into these neuronal cultures was examined. Each of the three EAA agonists produced dose- and time-dependent increases in the influx of calcium and in the same order of potency as that seen with cGMP production. Additionally, QA prevented the KA-induced influx of ^{45}Ca in a similar manner to its antagonism of the KA-induced cGMP elevation. On the other hand, QA did not alter the stimulation of ^{45}Ca influx induced by NMDA. Collectively, these data suggest that QA selectively antagonizes the stimulatory effects of KA at least on cGMP formation in cultured cerebellar neurons and does so at least in part by suppressing KA-mediated calcium influx. Supported by DA 00083 and DA 00755 to WWM.

208.16 PERINATAL HYPOXIA-ISCHEMIA ENHANCES QUISQUALIC ACID(QA) STIMULATED PHOSPHOINOSITIDE(PPI) TURNOVER. C. K. Chen*, F. Silverstein*, S. K. Fisher, D. Statman* and M. V. Johnston, Neuroscience laboratories, Univ. of Michigan, Ann Arbor, MI 48104

Several lines of evidence indicate that enhanced release of the excitatory neurotransmitter glutamic acid contributes to the pathogenesis of hypoxic-ischemic neuronal damage. In neonatal animals, using in vitro autoradiography with ^{3}H-glutamate(GLU), we found that a focal cerebral hypoxic-ischemic insult leads to marked inhibition of GLU binding in target areas for irreversible ischemic damage(e.g., hippocampus and striatum) 24 hrs after injury(Silverstein F. et al., Dev. Br. Res., in press). At this developmental stage, Nicoletti et al(PNAS., 83:1931, 1986) showed that excitatory amino acid recognition sites are coupled to inositol phospholipid metabolism and that the GLU agonist quisqualic acid(QA) potently stimulates PPI hydrolysis. To assess the functional correlates of the acute reduction in GLU binding we observed, in this study, we assayed QA stimulated PPI turnover in hypoxic-ischemic tissue.

7 d.o. rat pups underwent right carotid artery ligation(RCL), followed by exposure to 8% oxygen for 2.5 hrs; this procedure predictably results in unilateral forebrain injury ipsilateral to ligation. Pups and litter-mate controls were sacrificed 24 hrs post-hypoxia, hippocampus(HIP) and striatum(STR) were dissected out and brain slices were prepared. For each anatomic region, tissue samples from the side of ligation, the contralateral hemisphere, and unoperated controls were prepared. In tissue preincubated with ^{3}H-myo-inositol, the accumulation of inositol phosphates(IPs) was measured, in the presence of Li, with addition of QA(10^{-4} or 10^{-6} M) or the cholinergic agonist carbamoylcholine(10^{-4} or 10^{-2} M).

We found that QA(10^{-4} M) preferentially stimulated PPI hydrolysis in HIP and STR ipsilateral to ligation. For each tissue sample, total IPs were expressed as % of basal. In HIP, agonist stimulated IPs accumulation was 1053±237% ipsilateral to RCL, 588±134% in the contralateral hemisphere and 631±147% in controls(N=9 assays, $p<0.005$, one-tailed paired t test); in STR, corresponding values were: 801±157%, 474±89%, and 506±115%($p<0.05$). In contrast, with the lower concentration of QA(10^{-6} M) and with both doses of carbamoylcholine there was no preferential stimulation of IP hydrolysis in ischemic tissue.

The data suggest that hypoxia-ischemia enhances coupling between the reduced number of glutamate binding sites remaining in the injured brain and PPI turnover. The changes in QA stimulated PPI turnover provide additional evidence for selective involvement of glutamate synapses in the evolution of neuronal damage. Enhanced PPI turnover may be related to altered neuronal excitability and/or regenerative responses which follow hypoxic-ischemic injury.

208.17 EFFECTS OF EXCITATORY AMINO ACID ANALOGUES ON PHOSPHOINOSITIDE HYDROLYSIS IN RAT BRAIN. F.T. Crews and M.R. McElhaney*. Department of Pharmacology, College of Medicine, Box J-267, JHMHC, University of Florida, Gainesville, FL 32610.

Glutamate stimulates the hydrolysis of phosphoinositides (PI) in the cerebral cortex, hippocampus and striatum. The glutamate analogues ibotenate, quisqualate, and N-methyl-D-aspartic acid (NMDA) were studied in all three brain regions to determine which glutamate receptors were involved. Quisqualate was the most potent compound in all three brain regions. The dose response curve in the cerebral cortex was sigmoidal with an ED_{50} of approximately 15nM. In the striatum and hippocampus the curve was biphasic with the first phase similar to the cerebral cortex curve and a second phase at higher concentrations. Ibotenate was more effective at stimulating PI hydrolysis than quisqualate or NMDA in the cerebral cortex. All three agents were equally effective in the hippocampus and NMDA was much less effective than either of the other two agents in the striatum. Ibotenate and NMDA were additive in all three brain regions, whereas neither ibotenate and quisqualate, nor NMDA and quisqualate were additive. Glutamate receptor subtype coupling to PI hydrolysis will be discussed. (Funded by AA06069)

209.1 IONIC DEPENDENCE DISTRIBUTION, AND PHARMACOLOGICAL PROPERTIES OF QUISQUALATE-SENSITIVE [^{3}H]GLUTAMATE BINDING SITES IN RAT BRAIN
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Quisqualic acid, an analog of glutamic acid, selectively interacts with a subclass of glutamate receptors. Quantitative autoradiographic techniques were used to study quisqualate-sensitive [^{3}H]glutamate receptors in rat brain. Adult rat brains were cut into 20µm sections and thaw-mounted onto gelatin-coated glass slides. Brain sections were then incubated with [^{3}H]glutamate (200nM) in the presence of varying concentrations of quisqualate or competitors. After rinsing with cold buffer and drying, glass slides were apposed to tritium-sensitive film and allowed to expose for 2-3 weeks. The resultant film images were analyzed via computer-assisted densitometry.

Quisqualate-sensitive [^{3}H]glutamate binding sites were found to be distributed throughout all parts of the rat brain. The relative distribution of quisqualate-sensitive [^{3}H]glutamate binding sites was: cerebellar molecular layer > stratum moleculare of the dentate gyrus = entorhinal cortex > stratum radiatum of CA$_1$ > cerebellar granule layer > striatum. No displacement of binding was observed in precentral cortex. Of these areas, the cerebellar molecular layer had the highest proportion (79%) of [^{3}H]glutamate binding sites displaceable by quisqualate followed by cerebellar granule layer (62%), striatum (34%), entorhinal cortex (31%) and stratum moleculare and stratum radiatum of the hippocampus (27%).

Binding of [^{3}H]glutamate was influenced by both calcium and chloride ions. Chloride ions (at concentrations up to 86mM) exerted a marked effect on quisqualate-sensitive [^{3}H]glutamate binding, with a maximal stimulation by chloride occurring at 40mM. Calcium (0.1uM to 100mM) also stimulated quisqualate-sensitive binding, but only in the presence of chloride. The maximal amount of stimulation by calcium occurred at 10mM for all concentrations of chloride tested. The stimulation of binding exerted by chloride did not depend on the presence of calcium. Calcium- and chloride-enhanced binding was completely quisqualate-sensitive: in the presence of 2.5µM quisqualate, no ionic-dependent increase in binding was observed.

[^{3}H]Glutamate binding in the cerebellar molecular layer was reduced 60% by 100µM L-serine-O-sulphate, 45% by 100µM N-acetylaspartyl-glutamate, 21% by AMPA and 16% by 100µM N-methyl-D-aspartate. There was no significant reduction of binding by kynurenate, GAMS, L-aspartate, or L-2-aminophosphonobutyrate (APB), all tested at 100µM. The calcium- and chloride-stimulated quisqualate-sensitive binding site thus does not appear to be an NMDA or APB receptor.

Supported by NIH NRSA 5T32 GM07863 and USPHS grant NS 19613.

209.2 QUANTITATIVE AUTORADIOGRAPHY OF GLUTAMATE RECEPTORS IN THE FELINE MOTOR THALAMUS.
G.R. Zuercher*, T. DeBoon* and K. Kultas-Ilinsky. (SPON: I. Ilinsky) Dept. of Anatomy, University of Iowa College of Medicine, Iowa City, IA 52242.

Though evidence points to glutamate (Glu) as the neurotransmitter in corticothalamic projections, specific Glu receptor subtypes associated with these pathways in individual thalamic nuclei remain unknown. In these studies we used quantitative receptor binding autoradiographic techniques with computer-assisted image analysis to determine the presence, binding parameters, and topography of Glu receptor subtypes in the ventral anterior (VA), ventral medial (VM), and ventral lateral (VL) thalamic nuclei. Binding assays were performed on serial coronal sections of the thalamus using an incubation medium consisting of 50 mM Tris-HCl buffer, pH 7.4, with 2.5 mM $CaCl_2$ containing varying concentrations of ^{3}H-Glu and one of the following displacers: quisqualate (QUIS), N-methyl-D-aspartate (NMDA), kainate (KA), homocysteine (HO), and amino-3-hydroxy-5-methyl-4 isoxazolepropionate (AMPA). In a separate series of experiments, ^{3}H-N-acetyl-aspartyl-glutamate (^{3}H-NAAG) was used as a ligand. The binding parameters (Bmax, K_D) were determined from the data obtained from the thalamic regions of interest of digitized images of autoradiographs. Autoradiographic tritium standards (Amersham) were used for construction of calibration curves.

The ^{3}H-Glu binding under the incubation conditions employed was saturable and a high density of binding sites was found throughout the thalamus without much variation between individual thalamic nuclei. A 40% inhibition by 5.0 µM QUIS of specific ^{3}H-Glu binding indicated that this receptor subtype was indeed present in the motor thalamus. Only 12% inhibition was observed with 10 µM HO, whereas addition of 30 µM KA or 100 µM NMDA induced enhancement of ^{3}H-Glu binding under the same conditions. We are now studying the effect of the same agents on ^{3}H-Glu binding with the use of Cl^- and Ca^{++} free incubation medium in an attempt to dissect the KA and NMDA sites from the total ^{3}H-Glu binding. Moreover, digital subtractions of images of ^{3}H-Glu binding with and without displacers are being performed to determine the distribution pattern of individual Glu receptor subtypes in the cat motor thalamus.

The ^{3}H-NAAG binding experiments demonstrated an extremely low density of ^{3}H-NAAG receptor sites in the thalamus. This was in contrast to a high concentration of these sites in the caudate present in the same sections. This suggests an insignificant role of the NAAG receptors in the cat motor thalamus. (Supported by NIH NS RO1 19280.)

209.3 EFFECTS OF SPIDER VENOM ON VERTEBRATE CNS GLUTAMATE BINDING.
Jean Hollis*, L.M. Kerr, J.K. Wamsley, T.N. Parks, and H. Jackson. Departments of Psychiatry and Anatomy, University of Utah School of Medicine, Salt Lake City, Utah 84132.

Certain spider venoms have been shown to suppress glutamate-mediated synaptic transmission in both insects and vertebrates. Particularly in the case of the vertebrate CNS, however, very little is known about the mechanism of these effects. In order to investigate the site of action of spider toxins and their possible applications as probes for the study of glutamate-mediated transmission, we have begun examining effects of spider venoms on [3H]-glutamate binding. Partially-purified fractions of venoms from several species have been tested against glutamate binding to sections of both rat and chick brain (after the methods of Halpain et al., J. Neurosci. 4:2247). In most experiments, non-radioactive glutamate was used as the displacer to determine specific binding, which ranged from about 65-75%. Results were similar using either rat or chick tissue.

We have previously shown that partially-purified material from the venom of the Hololena curta spider suppresses synaptic transmission mediated by non-NMDA receptors in chick brain. In the present study, we found that this material also produces dose-dependent reduction of specific glutamate binding to either chick or rat brain with a 50% reduction at a concentration of about 0.5%. To examine whether the toxin might be acting preferentially at particular classes of glutamate receptors, we tested its effects when combined with either NMDA, kainate, or quisqualate. The expectation was that we might observe a greater reduction in binding when the toxin was combined with a ligand specific for a class of receptor not affected by the toxin alone. Surprisingly, we found that glutamate binding increased to control levels in the presence of the spider toxin and any of these three ligands. When tested alone, kainate (1 mM) reduced glutamate binding by 94% and quisqualate (1 mM) reduced binding by 85%. NMDA (1 mM) alone had little effect on glutamate binding but nonetheless appeared capable of eliminating the effects of the spider toxin on binding. Pending further study, we suggest these findings may reflect a complex allosteric interaction between binding sites for these ligands. Such an interpretation is consistent with the recent hypothesis that the various classes of glutamate receptor-channel complexes may in fact reflect different functional states of a single structure (Jahr and Stevens, Nature 325:522).
Supported by grants NS 15132 and NS 17257

209.4 **L-GLUTAMATE BINDING SITE ON N18-RE-105 NEUROBLASTOMA HYBRID CELLS REPRESENTS A SEQUESTRATION PROCESS.**
L. M. Boland, B. Berry*, and R. Dingledine. Dept. of Pharmacology and Neurobiology Curriculum, Univ. North Carolina, Chapel Hill, N.C. 27514

An L-glutamate (L-glu) binding site was described on the neuroblastoma x retina hybrid cell line, N18-RE-105, (Malouf et. al., JBC, 259:12756, 1984), and evidence was presented that this site is an aminophosphonobutyric acid (APB)-sensitive, quisqualate-type of excitatory amino acid (EAA) receptor. We performed electrophysiological and radioligand binding studies on these cells in order to directly test the idea that the binding site discovered is an EAA receptor.

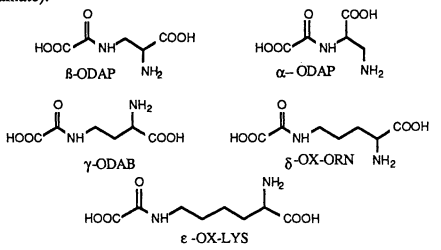
N18-RE-105 cells demonstrate many neuronal properties. These cells were strongly immunofluorescent for neurofilament but not for glial fibrillary acidic protein. In whole cell patch clamp experiments, cells had a resting potential up to -56 mV. They exhibited a number of voltage-dependent ionic currents, including a TTX-sensitive Na current, a 4-AP-sensitive K current, and a slow Ca current that could be blocked by cobalt. However, even when cells were grown in the absence of glutamine or in conditions that enhanced process growth (2% DMSO, 0-1% serum), perfusion with L-glu (3-300µM), L-glu plus glycine (3-20µM), or kainate (5-300µM) did not evoke the inward current expected of a glutamate receptor/channel complex.

Specific binding of [3H]-L-glu to washed membranes at 37°C demonstrated saturable binding sites with an apparent Kd of 0.64 µM and Bmax of 69 pmoles/mg protein. Binding was inhibited by quisqualate (Kd = 0.25 µM), DL-APB (Kd = 5.4 µM), and L-cystine (Kd = 0.17 µM), but not reduced by 1 mM kainate or N-methyl-DL-aspartate. Glutamate binding was subjected to similar tests described by Kessler et. al. (J. Neurochem., 48:1191, 1987) to distinguish binding from an exchange process. Binding of 100 nM [3H]-L-glu was stimulated by chloride (2-fold by 10 mM NaCl) but reduced by Na (half-maximal inhibition at 20 mM Na methylsulfate). Binding was also reduced 54% by 0.01% digitonin and 42% by hyperosmotic (400 mM glucose) assay medium. [3H]-L-glu bound to equilibrium at 37°C could be released from membranes much more effectively in the presence of a saturating concentration (100 µM) of unlabelled glutamate (53% released after 20 min.) than unlabelled quisqualate (30% released after 20 min.), but often there remained a non-releasable pool of [3H]-L-glu.

Although N18-RE-105 cells possess many neuronal properties, the results obtained are not those expected from reversible binding of L-glutamate to a channel/receptor complex, but are consistent with a chloride-activated sequestration or exchange process. Supported by NS22249 and NS23804.

209.5 **N-OXALYL-DIAMINO-CARBOXYLIC ACIDS ARE POTENT INHIBITORS OF GLUTAMATE RECEPTOR BINDING.** R.J. Bridges, M. Kadri*, D.T. Monaghan, P.B. Nunn*+, J.C. Watkins*++, and C.W. Cotman., Dept.of Psychobiology, University of California, Irvine, CA 92717, and +King's College London and ++University.of Bristol, England.

β-N-Oxalyl- α,β-diamino-propionic acid (β-ODAP) is an excitatory amino acid that has been identified as a major causative agent in the pathogenesis of human neurolathyrism (Chase et al., Neurosci. Lett., 55, 1985. This disease is associated with the excessive consumption of seeds containing the neurotoxin (e.g., *Lathyrus sativus, L. clymenum, and L. cicera*) and is characterized by a permanent spastic paralysis of the lower limbs. β-ODAP and several structurally related derivatives (see below) have been prepared and tested for their ability to inhibit the binding of radioligands to the three major excitatory amino acid receptor classes: NMDA (N-methyl-D-aspartate), QA (quisqualate) and KA (kainate).



QA, KA, and NMDA receptors in synaptic plasma membranes were assayed with specific ligands (^{3}H-AMPA, ^{3}H-KA and ^{3}H-L-Glutamate, respectively) as described (Monaghan et al.,PNAS, 83, 1986). Of the compounds listed above, including α-L-ODAP and β-D-ODAP, only β-L-ODAP was found to potently inhibit the binding of ^{3}H-AMPA (IC_{50} near 1 μM). Similarly, β-L-ODAP also selectively inhibited the binding of ^{3}H-KA (IC_{50} near 50 μM). In contrast, β-L-ODAP did not exhibit substantial inhibition of binding to the NMDA receptor. Binding to this receptor was most potently inhibited by L-and D-γ-ODAB (N-oxalyl-α,γ-diamino-butyric acid), δ-D-OX-ORN (δ-N-oxalyl-ornithine) and ε-D-OX-LYS (ε-N-oxalyl-lysine). Electrophysiological studies in the spinal cord neurons suggested the site of action of β-L-ODAP is at non-NMDA receptors (MacDonald and Morris, Expt. Brain Res., 57, 1984).. The findings reported here are consistent with that model and further suggest that at low concentrations the toxic action of β-L-ODAP may be a specifically attributable to the interaction with QA receptors. Structure-function studies with these derivatives should also provide insight into ligand specificity differences between KA and QA receptors.

209.6 IMMUNE LABELING AND PURIFICATION OF A 71 kDa GLUTAMATE BINDING PROTEIN IN BRAIN SYNAPTIC MEMBRANES. E.K. Michaelis, J.-W. Chen*, M.D. Cunningham and N. Galton*, Dept. of Biochemistry and Human Development and Center for Biomedical Research, Univ. of Kansas, Lawrence, KS 66045.

Immunoblot studies of synaptic membranes isolated from rat brain using antibodies raised against a previously purified glutamate binding protein (GBP) indicated labeling of an ~70 kDa protein band. Since the antibodies used were raised against a 14 kDa GBP, the present studies were undertaken to explore the possibility that the 14 kDa protein may have been a proteolytic fragment of a larger M_r protein in synaptic membranes. Protease activity during protein purification was prevented by introducing five protease inhibitors and a three-step purification procedure was developed that yielded a high degree of purification of glutamate binding proteins. The major protein enriched in the most highly purified fractions was a 71 kDa glycoprotein, but a 63 kDa protein was co-purified during most steps of the isolated procedure. The 71 kDa protein interacted very strongly with concanavalin A-biotin complex, whereas the 63 kDa bound this complex weakly. Antibodies raised against the 71 and 63 kDa proteins labeled most strongly a 71 kDa protein band in synaptic membranes. The 63 kDa protein appeared to be a proteolytic fragment of the 71 kDa glycoprotein. The isolated protein fractions did not have any glutamate-metabolizing activity and their glutamate-binding characteristics were very similar to those previously described for the 14 kDa GBP, including estimated dissociation constants for L-glutamate binding of 0.25 and 1 μM, inhibition of glutamate binding by azide and cyanide, and a selectivity of the ligand binding site for L-glutamate and L-aspartate. The neuroexcitatory analogs of L-glutamate and L-aspartate, ibotenate, quisqualate, and D-glutamate, inhibited L-[^{3}H] glutamate binding to the isolated proteins, as did the antagonist of L-glutamate-induced neuronal excitation, L-glutamate diethylester. The transport carrier inhibitor threo-hydroxyaspartic acid did not inhibit significantly the binding activity of the protein. Excitatory amino acid analogs of glutamate such as kainate, domoate, NMDA and AMPA produced weak inhibition of L-glutamate binding to these proteins. In terms of ligand selectivity the purified glutamate binding proteins resembled most closely the binding sites on cerebellar granule cells described by Drejer et al. [Life Sci. 38, 2077, 1986]. (Supported by grants DAAL03-86-K0086 from the ARO, AA04732 from the NIAAA, and KS-86-G-22 from the AHA-KS Affiliate).

209.7 PURIFICATION OF KAINIC ACID BINDING SITES FROM FROG BRAIN USING ION EXCHANGE AND LIGAND AFFINITY CHROMATOGRAPHY. D. R. Hampson and R. J. Wenthold. Laboratory of Neuro-otolaryngology, NINCDS, NIH, Bethesda, MD 20892

Binding sites for the neurotoxin, kainic acid, may represent a subset of receptors for the endogenous excitatory amino acids glutamate, aspartate, or a related compound. Previous studies have indicated that these binding sites are concentrated at synapses and have a unique distribution in the central nervous system.

We have established a protocol for solubilizing kainic acid binding sites from frog (and rat) brain using a combination of the detergents, Triton X-100 and digitonin in 0.5M phosphate buffer, pH 7.0. The solubilized binding sites from frog brain displayed a high affinity (K_D = 4.8) and low affinity (K_D = 39) component. Both the dissociation constants and the competition profiles are similar to those found in membranes and solubilized preparations from rat brain. Although the pharmacological properties of the solubilized binding sites from frog brain are similar to those observed in rat brain, the density of binding sites was 5-10 fold greater in the frog. The B_{max} values for the solubilized binding sites in frog brain were 3782 pmol/mg protein and 12756 pmol/mg protein for the high and low affinity sites respectively.

The solubilized binding sites were purified by ion exchange chromatography followed by ligand affinity chromatography using the high affinity kainic acid analog, domoic acid. Ion exchange chromatography on DEAE sepharose using a linear 0-0.2M NaCl gradient resulted in more than a 15-fold purification over the crude solubilized preparation. Pooled fractions from the ion exchange column containing binding activity were applied to the domoic acid affinity column; binding sites were eluted using kainic acid. The specific activity of the binding sites eluted from the domoic acid affinity column was several hundred fold greater than that of the crude solubilized preparation. Both the crude solubilized and the partially purified kainic acid binding sites from frog brain eluted from a Sepharose 6B gel filtration column in the presence of detergent, with molecular weights of 650,000 daltons. The calculated molecular weight of the crude solubilized binding site from rat was the same as that for the frog binding site.

The kainic acid binding sites from both rat and frog brain appear to be glycoproteins based on their binding to a variety of agarose-conjugated lectins, including wheat-germ agglutinin, Concanavalin A, and lentil lectin. The majority of the sites bound to wheat-germ agglutinin could be eluted with 0.35M N-acetyl-D-glucosamine, while none of the sites bound to Concanavalin A could be eluted using 0.35M α-methyl-D-mannoside.

209.8 NANOMOLAR AND SUBMICROMOLAR BINDING OF L-(H) PROLINE TO MOUSE BRAIN SYNAPTIC MEMBRANES. J.G. Ortiz, A.E. Negrón* and María S. Bruno*. Department of Pharmacology, Univ. of Puerto Rico School of Medicine, San Juan, Puerto Rico 00936.

L-proline has been shown to exert a variety of physiological and behavioral effects that are consistent with its possible role as either a neurotransmitter and or a neuromodulator. Some of these effects are: (a) inhibition of memory consolidation (Cherkin et al., 1981), (b) excitatory actions on hippocampal pyramidal neurons (Keller et al., 1981), (c) high-affinity synaptosomal uptake (Hauptmann et al., 1983), and (d) K^+-stimulated release (Nickolson, 1982). Recently, Greene et al., (1986) have described the micromolar binding of proline to hippocampal synaptic membranes.

In this study, the nanomolar and submicromolar binding of L-(^{3}H) proline to mouse brain synaptic membranes has been examined. We have been able to identify two additional binding sites to those described by Greene et al., 1986. Preliminary kinetic analysis reveals a binding site(s) with an apparent K_d of 2.9 nM and B_{max} of 2.5 fmole/mg protein. On the other hand, another binding site is also observed with an apparent K_d of 0.29 uM and B_{max} of 500 pmole/mg protein.

In contrast to the binding of pipecolic acid, (^{3}H) proline binding is inhibited by NaCl. The latter point suggests that these compounds are binding to different site(s). At 10 uM; L-proline, ornithine and pipecolic acid displaced approximately 50% of the bound proline; followed by glutamate, GABA and glycine (30-40%). Although there are many similarities between the binding of glutamate and proline, kainic acid (10 uM) was ineffective in displacing bound proline.

These results suggest the possibility that proline may bind to its own binding sites.

(Supported by NIH/MBRS 5-S06-RR08224-02)

209.9 EFFECT OF INSULIN-INDUCED HYPOGLYCEMIA ON EXCITATORY AMINO ACID RECEPTORS IN RAT NEOSTRIATUM

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Hypoglycemia, when severe enough to cause EEG isoelectricity, leads to delayed neuronal necrosis in the caudate nucleus. With increasing severity of the hypoglycemic insult, the damage to small and medium sized neurons first appear in the lateral part of the striatum, and spreads medially into the structure. Lesioning of the glutamatergic cortico-striatal pathway, as well as intrastriatal injection of an NMDA receptor antagonist (AP-7) protect against hypoglycemic damage. These results indicate that receptors for excitatory amino acids may play an important role in the development of the damage.

Autoradiographic methods were used in order to study possible changes in the properties of different glutamate receptors before onset of EEG isoelectricity (burst suppression EEG pattern), during EEG silence (10 and 30 min isoelectric EEG) as well as after different periods of glucose induced recovery (30 min isoelectric EEG with 1 h, 1 week or 4 weeks survival). NMDA displaceable 3H-glutamate, 3H-AMPA and 3H-kainic acid binding to 6 µm brain tissue sections was quantified by computer aided densitometry.

Male Wistar rats were injected with 9-22 IU/kg insulin and EEG silence was awaited (2-3 hours). Control rats were given an equal amount of insulin supplemented with glucose in order to maintain a normal plasma glucose level. The animals were sacrificed by *in situ* freezing of the brain with liquid nitrogen. The brains were removed and sectioned at -20°C. Paralell sections were used for *in vitro* binding of the tritiated ligands and for histological evaluation of neuronal damage.

Our results revealed a biphasic pattern in AMPA binding. Following 30 min of EEG silence, AMPA binding decreased to 50 % of control in both lateral and medial striatum. One hour following glucose administration, AMPA binding was no longer different from control binding, while prologation of the recovery period to 1 or 4 weeks resulted in a binding decrease compared with controls only in the more vulnerable lateral portion of the caudate.

NMDA displaceable glutamate binding was enhanced by approximately 30 % after 10 min of isoelectric EEG. The binding was thereafter normalized during the isoelectric and early recovery periods, while a reduction in binding was found 1 and 4 weeks following 30 min of severe hypoglycemia. No changes in kainic acid binding was found during EEG silence or in the early recovery phase. As the recovery period was extended to 1 or 4 weeks, a significant drop in binding was found in the lateral caudate.

We conclude that the early decrease in AMPA binding probably reflects a reversible desensitization of the quisqualate receptor due to increased extracellular concentrations of excitatory amino acids during EEG isoelectricity. The second decrease in AMPA binding as well as the loss of binding to NMDA and KA sites correlates with the distribution of damage and probably reflects cell death. The significance of the increase in binding to NMDA receptors at 10 min of isoelectricity is elusive, but is in accordance with an NMDA-receptor involvement in the mechanisms of hypoglycemia induced neuronal damage.

209.10 FUNCTIONAL STUDIES OF A NEW NON-NMDA ANTAGONIST (FG 9041) IN NEURONAL CELL CULTURE MODELS.

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We have recently developed a series of potent and competitive inhibitors of ^{3}H-AMPA and ^{3}H-kainate binding sites. (T. Honoré et al., this meeting). The pharmacological profiles of these substances including FG 9041 were evaluated using a newly developed model of excitatory amino acid (EAA) induced ^{3}H-GABA release from cultured mouse cortex interneurons (Drejer et al., J. Neurosci., in press).

The EC_{50} values for stimulation of ^{3}H-GABA release were for quisqualate 75 nM; NMDA 16 µM; kainate 29 µM and L-glutamate 12 µM. Responses to 20 µM NMDA were potently blocked by the NMDA selective antagonist D-APV (IC_{50} = 1.5 µM; and by the PCP/sigma opiate type of compounds (IC_{50} values were for PCP: 2 µM; MK 801: 3 µM; ketamine: 8 µM and cyclazocine: 11 µM). Responses induced by 500 nM quisqualate could be blocked effectively by the new EAA antagonist FG 9041 (IC_{50} = 3 µM) whereas other non-NMDA antagonists such as cis-2,3-PDA, GAMS, GDEE and kynurenic acid were very weak or ineffective (IC_{50} values higher than 500 µM). Schild analyses of FG 9041 inhibition of responses to quisqualate, kainate and NMDA indicated a competitive inhibition at all receptor subtypes with pA_2 values of 6.2, 5.9 and 5.4, respectively.

EAA neurotoxicity was studied in similar neuronal cultures using the blue formazan formed from MTT (a tetrazole compound) as a stain for surviving neurons. In this model FG 9041 (10 µM) effectively blocked quisqualate (50 µM) and kainate (50 µM) toxicity without protection against NMDA (50 µM) or L-glutamate (50 µM) toxicity.

FG 9041 was a potent inhibitor of quisqualate (10 µM) and kainate (25 µM) responses in a model of EAA induced ^{22}Na efflux from rat striatal slices (IC_{50} values were 4 µM and 2 µM, respectively) with weaker effects on NMDA responses (IC_{50} = 40 µM).

These results indicate that FG 9041 is a competitive EAA-antagonist with a selectivity for non-NMDA receptors and a potency several orders of magnitude higher than for known non-NMDA antagonists.

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209.11 DIFFERENTIAL PHARMACOLOGICAL RESPONSES TO THE CIS AND TRANS ISOMERS OF 1-AMINO-3-PHOSPHONOCYCLOPENTANECARBOXYLIC ACID (CYCLOPENTYL APB).

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The L-glutamate analogue L-2-amino-4-phosphonobutanoic acid (L-APB) antagonizes synaptic responses at micromolar concentrations for a very limited subgroup of putative glutamatergic pathways. It also acts as an excitant for ON-bipolar retinal cells and it becomes a potent excitant for hippocampal and prepyriform cortical neurons after they are exposed to quisqualate [The Quis effect: Robinson et al., Brain Res., 381 (1986) 187]. We previously synthesized two conformationally-restricted analogues of APB, cis- and trans-1-amino-3-phosphonocyclopentanecarboxylic acid (cis- and trans-cyclopentyl APB) and demonstrated that the trans isomer is more potent for inhibition of the APB-sensitive lateral perforant path in rat hippocampal slices [Crooks et al., J. Med. Chem., 29 (1986) 1988]. I wish to report differential pharmacological responses to these compounds for three other neural systems. The trans isomer is also a more potent inhibitor of the most APB-sensitive component of the extracellular synaptic field potential of rat prepyriform neurons activated by stimulating the lateral olfactory tract. Thus 10 µM L-APB or 300 µM trans-cyclopentyl APB inhibited this response 15-20%, whereas 300 µM cis-cyclopentyl APB inhibited less than 6%. [The synaptic responses which are not inhibited at these concentrations have very low sensitivity to higher concentrations of L-APB [Hearn et al., Brain Res., 379 (1986) 372]. In superfused retinal eyecup preparations from the red-eared turtle (Pseudymus elegans), 250 µM trans cyclopentyl APB inhibited the ON-bipolar extracellular response of the intraretinal electroretinogram more than 80% whereas the cis isomer inhibited 50% (data obtained by M.M. Slaughter). In contrast to these systems, the cis isomer of cyclopentyl APB was more potent for eliciting agonist-induced synaptic blockade in rat hippocampal CA1 pyramidal cells after exposure to 16 µM quisqualate (Quis effect; cis-cyclopentyl APB: IC_{50} = 120 µM; trans-cyclopentyl APB: IC_{50} = 1.8 mM; L-APB: IC_{50} = 70 µM). This is the first observation of differentiation of responses of L-APB-sensitive functions by conformationally-restricted APB analogues.

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209.12 INTERACTIONS OF AP4 WITH GLUTAMATE RECEPTOR AND UPTAKE SITES IN RETINA.

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Analysis of glutamate transmission in the vertebrate retina has been greatly facilitated by the use of compounds which bind with a high degree of specificity to distinct subclasses of glutamate receptors. AP4 (2-amino-4-phosphonobutyric acid, previously designated 2-APB) is a particularly useful compound which has been shown to block the activity of the ON channel presumably by acting as an agonist at glutamate receptors on ON bipolar cells (J. Neurosci., 1985, 5:224 - 233).

We have analyzed the binding properties of AP4 at glutamate receptors in rabbit retina and we find a good correlation between the pharmacological/kinetic characteristics of in vitro binding, and those reported for the electrophysiological effects of AP4. AP4 displaces ^{3}H-glutamate from a set of binding sites a) is sensitive to freeze-thaw procedures, b) requires chloride ions and c) is stereoselective for the L isomer.

However, recent reports from other CNS areas have suggested that AP4 may also interact with glutamate uptake sites. We have therefore examined the effect of AP4 on both the Na-dependent and the Cl-dependent glutamate uptake systems in rabbit retina synaptosomes. AP4 inhibits ^{3}H-glutamate uptake in both systems, thus suggesting that AP4 may have multiple effects on glutaminergic transmission in rabbit retina.

AP4-sensitive ^{3}H-glutamate binding to post-synaptic receptors cannot be differentiated from binding to uptake sites on the basis of in vitro binding assays. Both sites demonstrate temperature and chloride dependence and their pharmacological specificities are very similar.

AP4 preferentially blocks ^{3}H-glutamate uptake into the potassium-releasable pool of retinal synaptosomes. Thus, applications of AP4 in intact retina could alter intracellular and extracellular concentrations of endogenous glutamate. These findings raise the possibility that multiple sites may contribute to the functional responses seen with AP4.

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209.13 QUISQUALATE-INDUCED SENSITIVITY TO AP4 IN HIPPOCAMPAL SLICES IS ANTAGONIZED BY L-α-AMINO ADIPATE AND SERINE-O-SULFATE. E.W. Harris[1] and C.W. Cotman (Spon: W. Makous). Dept. Psychobiology., Univ. Calif., Irvine, CA 92717 & [1]Pharmacology Dept., Pharmaceut. Div., Pennwalt Corp., Rochester, NY 14623.

The functional role of the most-studied CNS glutamate binding site remains unclear. It is now being argued that this chloride-dependent binding site, sensitive to 2-amino-4-phosphonobutyrate (AP4) and quisqualate, may be a uptake site. We have suggested (Harris & Cotman, Brain Res., in press) that a recently reported interaction between quisqualate and AP4 (Robinson et al., Brain Res. 381: 187, 1986) may be mediated by this site, and provides evidence of a transport role for this site. To further test this hypothesis, we have examined the interaction between the quisqualate "priming effect" and other compounds that are potent ligands at the chloride-dependent binding site.

Compounds were applied at known concentrations to submerged hippocampal slices. Extracellular synaptic field potentials were recorded in CA1 stratum radiatum or stratum pyramidale after stimulation of stratum radiatum near CA2. Slices were "primed" by a single 2-4 minute application of 10-20 μM quisqualate. Priming is evidenced by 1) a lasting reduction in synaptic response amplitude apparent after quisqualate is washed out, and 2) sensitization to AP4 - a pronounced reduction of the synaptic potential during subsequent application of as little as 100 μM AP4; this is associated with CA1 pyramidal cell depolarization. Such sensitivity to AP4 is not seen in untreated slices.

We now report that quisqualate also sensitizes slices to the potent chloride-dependent site ligand L-α-Aminoadipate (LαAA), although this compound had effects different from AP4. In primed slices, 100 μM LαAA caused only a small decrease in synaptic potential that faded within 1-2 minutes, and a second application of LαAA had little effect on the synaptic response. LαAA also greatly reduced the sensitivity to application of AP4. Including 100 μM LαAA during quisqualate treatment blocked the priming effect although sensitivity to AP4 could be induced by subsequent application of quisqualate. L-serine-O-sulfate (LSOS) also antagonized the priming effect of quisqualate. In control slices, LSOS (up to 300 μM) had no pronounced effect on Schaffer responses, but application of LSOS to primed slices consistently reversed the persisting decrement in the synaptic response, and reduced the sensitivity to subsequent application of AP4.

These data provide further pharmacological evidence that the priming effect of quisqualate on hippocampal slices in mediated by the chloride-dependent glutamate binding site. But, most important, we have demonstrated antagonism by LαAA and LSOS of the priming effect of quisqualate, which will greatly facilitate the study of its underlying mechanism(s).

209.14 QUANTITATIVE STUDIES ON THE ANTAGONISM OF QUISQUALATE, KAINATE AND NMDA RESPONSES BY PENTOBARBITONE. J.A. Kemp*, S. Grimwood*, C. Wardell* and A.C. Foster, (Spon: L. Iversen). Merck Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex, UK.

Barbiturates have been suggested to block receptor-operated Na^+-K^+ conductances regardless of the receptor type involved (Barker, Brain Res., 92: 35, 1975). Thus, it is of interest that in mammalian CNS preparations, barbiturates are reported to antagonise quisqualate and kainate responses more potently than NMDA receptor mediated responses (Teichberg et al, Brain Res., 291: 285, 1984; Miljkovic and MacDonald, Brain Res., 376: 396, 1986). In order to study this antagonism of excitatory amino acids more quantitatively, we have examined the ability of pentobarbitone to antagonise quisqualate, kainate and NMDA responses using a rat cortical slice preparation.

Approximately 1mm wide cortical wedges (0.5mm thick) were mounted in a two compartment bath with the ventral margin of the cortical tissue traversing a greased slot, so that the cortical tissue lay almost entirely within one chamber and the white matter entirely within the other. The potential between the chambers was monitored using Ag/AgCl electrodes and continuously displayed on a chart recorder.

Pentobarbitone (30-300μM) produced concentration dependent shifts to the right of the quisqualate concentration-response curve (usually 3-30μM). Concentration-ratios estimated from the middle part of the control curve produced a Schild plot with a slope = 1.37 ± 0.26 (± 95% confidence limits) and a pA_2 = 4.7. At 300μM pentobarbitone, the quisqualate concentration-response curve appeared to be initially flattened but then steepened at concentrations above 300μM quisqualate. Addition of the competitive NMDA receptor antagonist, D-2-amino-5-phosphono valerate (D-AP5; 30μM), reduced the size of the responses to high concentrations of quisqualate. This was further investigated by examining the effect of pentobarbitone on full concentration-response curves to quisqualate in the presence of the selective, non-competitive NMDA antagonist, MK-801 (3μM), which completely blocked depolarising responses to NMDA. Under these conditions, pentobarbitone (300μM), reduced the slope of the mean concentration-response curve (n = 4 slices) from 0.91 to 0.58 and the maximum from 2.94 to 1.68 mV. In contrast, pentobarbitone (300μM) had little effect on kainate responses, producing a mean concentration-ratio of 2.99 ± 0.27 (n = 4) and had less effect on responses to NMDA, mean concentration ratio = 1.62 ± 0.21 (n = 12).

These results suggest that on rat cortical cells barbiturates preferentially block quisqualate receptor-mediated responses, in a non-competitive manner. They also indicate that at high concentrations quisqualate is not a selective agonist and activates NMDA, as well as quisqualate, receptors.

209.15 [^{3}H] MK801 BINDING TO THE EXCITATORY AMINO ACID RECEPTOR COMPLEX FROM RAT BRAIN IS ENHANCED BY GLYCINE. S.N. Murphy*, I.J. Reynolds, W.Hartwig* and R.J.Miller. (SPON. D.C. U'Prichard) Dept. Pharmacol. and Physiol. Sci., Univ. Chicago, Chicago IL 60637.

The excitatory amino acid glutamate (GLU) acts on at least three receptor types in the mammalian brain. These receptors are characterized by the actions of relatively selective agonists N-methyl-D-aspartate (NMDA), kainate and quisqualate. The novel anticonvulsant MK801 selectively inhibits the actions of NMDA by blocking the NMDA-operated calcium-selective ionophore. We have studied the binding of [^{3}H] MK801 to rat brain membranes to further investigate drug action at this site.

Binding assays employed membranes that had been washed a total of ten times in 20mM HEPES, pH 7.4, and had undergone 2 freeze-thaw cycles. [^{3}H] MK801 binding of this extensively washed membrane preparation had an affinity of 27nM and a density of 0.62pMol/mg protein of receptors. GLU and glycine (GLY) enhanced binding by increasing the affinity but and negligible effects on the density of receptors. Half maximal concentrations were 1.28 and 0.31μM respectively. GLU (100μM) and GLY (30μM) increased the affinity to 8.1 and 10.2nM. Maximal concentrations of the two were additive, and in combination the affinity was increased to 3.0nM. This indicates that GLU and GLY act at separate sites on the NMDA receptor complex.

The binding of [3H] MK801 was regulated by other drugs that are believed to interact with the NMDA receptor-ionophore complex. Thus, phencyclidine, CPP+ and Mg^{2+} inhibited binding with half maximal concentrations of 34.7nM, 540nM and 10mM respectively. By contrast, NMDA mimicked the effects of GLU by increasing binding in the concentration range 1-100μM.

Several other amino acids produced GLY-like effects in this assay. D-Serine was most effective, and D-alanine was also potent. L-amino acids were generally less effective. B-alanine and taurine had very little effect, and strychnine did not block the effects of GLY at concentrations of 10μM.

We have also studied the effects of amino acids on the increase of cytoplasmic free calcium produced by NMDA in single cultured mouse striatal neurons. Glycine increases the effects of threshold concentrations of NMDA, and increases the potency of NMDA in the range 0.01-10μM The amino acids D- and L-serine mimicked the effects of GLY, while taurine was ineffective.

We have demonstrated that GLY can potently interact with the NMDA receptor-ionophore complex, and that GLY can modulate the actions of agonists at this receptor. These results support the suggestion (Johnson & Ascher, Nature 325:529, 1987) that GLY may be an important modulator of the actions of GLU at the NMDA receptor in vivo.

209.16 **GLYCINE BINDING SITES RECIPROCALLY INTERACT WITH GLUTAMATE BINDING SITES AT THE NMDA RECEPTOR COMPLEX.** L. Nguyen*, D. T. Monaghan, and C.W. Cotman. (SPON: E. E. Mena) Dept. Psychobiology, Univ. California, Irvine, CA, 92717.

Agonist induced responses at the N-methyl-D-aspartate excitatory amino acid receptor have recently been shown to be potentiated by low concentrations of glycine. Using quantitative autoradiography, we have found that 0.1 to 10 μM concentrations of glycine enhance L-[^{3}H]glutamate binding (procedure: PNAS 83 (1986) 7532) at the NMDA receptor complex, while higher concentrations reduce the levels of binding. Likewise, D-serine was also active at increasing the levels of NMDA-sensitive L-[^{3}H]glutamate binding. These effects were neither mimicked nor blocked by 10 μM strychnine.

Conversely, L-glutamate alters [^{3}H]glycine binding. [^{3}H]Glycine has been reported to bind to a strychnine-insensitive binding site which has a distinct anatomical distribution similar to the NMDA receptor's and a high affinity for glycine and D-serine (Bristow et al., Eur. J. Pharmacol., 126:303 (1986). We have evaluated these binding sites using quantitative autoradiography (100 nM [^{3}H]glycine, NEN, incubated with thaw-mounted Sprague-Dawley rat brain tissue sections for 20 min. at 0-4 C in 50 mM Tris-citrate pH 7.2. followed by a 20 s wash) The anatomical distribution of these sites is very similar to that previously reported for NMDA-sensitive L-[^{3}H]glutamate binding sites. These sites appear to be closely associated with the L-glutamate binding site because L-glutamate and other NMDA agonists (L-aspartate, NMDA, and ibotenic acid) enhance binding at the [^{3}H]glycine binding site. In addition to being readily displaced by glycine and D-serine, binding at the [^{3}H]glycine site is also reduced by the NMDA antagonists 2-amino-5-phosphonopentanoate and 2-amino-7-phosphono-heptanoate (measured in the presence of 10 μM L-glutamate), but not by the inactive 4, 6, and 8 carbon analogues. Noncompetitive NMDA antagonists MK801 and ketamine also reduce the glutamate-stimulated [^{3}H]glycine binding.

NMDA antagonists, however, do not inhibit [^{3}H]glycine binding uniformly throughout the brain. Regions such as the striatum and septum appear to be more greatly affected than [^{3}H]glycine binding in the granule cell layer of the cerebellum. This regional variation may correspond to the differing classes of NMDA binding sites (see abstract by Monaghan et al., these proceedings). These results confirm that [^{3}H]glycine binds to a separate site on the NMDA receptor complex, and indicates that the glutamate and glycine sites reciprocally interact with each other in the NMDA receptor complex.

209.17 HIPPOCAMPAL NEURONAL LOCALIZATION OF NMDA AND TCP RECEPTORS. W.F. Maragos, J.B. Penney and A.B. Young, Dept. of Neurology and Neuroscience Program, University of Michigan, Ann Arbor, MI 48104.

Substantial evidence has accumulated that dissociative anesthetics exert some of their CNS effects by interacting with a site close to the ionic channel of the N-methyl-D-aspartate (NMDA) receptor. The NMDA receptor labeled with [3H]glutamate and the dissociative anesthetic receptor labeled with [3H]-N-1[(2-thienyl)-cyclohexyl]3,4-piperidine (TCP) have similar distributions in rat and human brain. The synaptic localizations of these sites, however, is unknown. We have investigated NMDA and TCP receptors in serial sections of rat hippocampus after lesions of the perforant pathway and of the dentate gyrus.

Eight Sprague-Dawley male rats (200 grams) were anesthetized, placed in a stereotaxic frame and a unilateral knife cut made through the angular bundle to sever the perforant path. Four additional animals were anesthetized and 2 injections of 2.5 ug colchicine (5 ug/µl) were made unilaterally into the dentate gyrus. After one week, the animals were decapitated, the brains rapidly removed and frozen on dry ice. Twenty micron sections were assayed for [3H]glutamate binding to NMDA receptors (Greenamyre et al., J. Pharm. Exp. Therap. 233:254, 1985) and [3H]TCP binding to dissociative anesthetic receptors (Maragos et al., Neurosci. Lett. 74:371, 1987). The sections were exposed to Ultrofilm 3H for 2-3 weeks, then developed and analyzed by computer assisted densitometry.

After perforant path lesions, NMDA binding in dentate gyrus was 4.0 ± 0.6 pmol/mg protein ipsilateral to the lesion as compared to 4.4 ± 0.6 pmol/mg protein on the control side. [3H]TCP binding was significantly decreased by 9% (0.35 ± 0.02 pmol/mg protein vs 0.32 ± 0.02) on the lesioned side. After dentate gyrus lesions, NMDA receptor binding was decreased by 84% (4.1 ± 0.23 vs 0.67 ± 0.21) and [3H]TCP binding by 92% (0.25 ± 0.02 vs 0.02 ± 0.01) on the lesioned side.

These data support the hypothesis that NMDA and TCP binding sites are closely linked anatomically since both receptors respond similarly to lesions. Furthermore, the results suggest that a very small percentage of NMDA/TCP receptors are presynaptic on perforant pathway terminals and that the majority of sites are postsynaptic on dentate neurons.

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209.18 COMPLEX INTERACTIONS BETWEEN A GLYCINE BINDING SITE AND NMDA RECEPTORS. M.Kessler, M.Baudry, T.Terramani* and G.Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine CA 92717.

Glycine has been reported to modify the electrophysiological characteristics of the NMDA receptor (Nature 325 (1987) 529-531). This prompted us to investigate the effect of glycine on glutamate binding to the NMDA site and to test for the presence of a glycine binding site associated with the NMDA receptor. Binding assays were done by incubating detergent-treated membranes isolated from rat telencephalon with radiolabeled ligands at 0°C for 30 min, followed by filtration.

In agreement with previous reports (J.Neurochem. 37 (1981) 1015-1024), 3H-glycine bound to a single strychnine-insensitive site with a K_d of 0.3 uM, a B_{max} of 4-8 pmoles/mg protein and a high affinity for serine (K_i: 0.8 uM). Glycine binding to this site was found to be highly sensitive to NMDA-receptor specific ligands; however, agonists and antagonists exerted opposite effects on glycine binding. Agonists like NMDA, glutamate and homocysteate increased glycine binding by 10-20%; the antagonists AP5, AP7 and CPP reduced glycine binding by up to 80%; in both cases, the change in glycine binding was primarily due to a change in B_{max}. The IC50 of the NMDA-receptor antagonists for inhibiting glycine binding corresponded closely with their K_i for the NMDA site; however, the maximum inhibition of glycine binding differed for each antagonist, reaching 80% for AP5, but only 30% for CPP. The inhibition of glycine binding by NMDA receptor antagonists was completely reversed by high concentrations of glutamate.

Glycine binding was also inhibited with high affinity by kynurenate ($K_i \approx 8$ uM). Inhibition by kynurenate differed from that produced by the NMDA-receptor antagonists in several aspects: (i) it was competitive, (ii) it was complete at saturating kynurenate concentrations, and (iii) it was not reversed by addition of high concentrations of glutamate. This suggests that kynurenate directly binds to the glycine site.

Glycine and serine increased binding of 3H-glutamate to the NMDA site. Conversely, kynurenate inhibited glutamate binding and the inhibition was, at least at kynurenate concentrations below 50 uM, reversed by high concentrations of glycine. Thus, kynurenate might act as an antagonist of the glycine site.

These results indicate that the NMDA and the glycine site are mutually interdependent. It is suggested that the glycine binding site exists in a low and a high affinity state and that NMDA receptor agonists and antagonists shift the equilibrium between these two states in opposite direction.

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209.19 **AUTORADIOGRAPHIC VISUALIZATION OF NMDA-TYPE RECEPTORS USING [3H]CPP: PHARMACOLOGICAL CHARACTERIZATION AND COMPARISON WITH [3H]TCP BINDING SITES IN RAT BRAIN.** Deborah E. Murphy, Michael F. Jarvis, Williams J. Brooks*, Matthew A. Sills and Michael Williams. Drug Discovery Division, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ 07901.

[3H]CPP (3-(±)-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid) selectively labels the N-methyl-D-aspartate (NMDA)-type excitatory amino acid (EAA) receptor with relatively high affinity in rat brain. [3H]CPP binding is highest in the hippocampus (CA-1) followed by cortex > thalamus ≥ granule cell layer of the cerebellum. The activity of a number of EAA agonists and antagonists as well as phencyclidine (PCP)-type compounds was examined in hippocampal CA-1 stratum radiatum, parietal cortex and thalamus using quantitative autoradiography. The most active compound examined was the newly described NMDA-antagonist, CGS 19755 (Lehmann et al., this meeting) with an IC-50 value of 73 nM in the stratum radiatum, being twice as active as CPP and AP5. The pharmacological activity of NMDA-type ligands, both agonists and antagonists, was highly correlated between the three brain regions ($r = 0.99$, $P < 0.01$). EAA ligands were most active in the parietal cortex with a rank order: CGS 19755 > L-glutamate ≥ CPP > DAP5 > L-aspartate ≥ AP7 > NMDA > L-homocysteic acid. Quisqualate had weak activity (IC-50 > 62 µM). AMPA and kainate, as well as the dissociative anesthetics PCP, dexoxadrol and tiletamine were without significant activity at 100 µM. The pharmacology of [3H]CPP binding to NMDA-receptors using autoradiography agrees well with data obtained from homogenate assays (Murphy et al., J. Pharmacol. Exp. Ther. 240: 778, 1987). The regional distribution of NMDA receptors labeled with [3H]CPP in general showed a good correlation ($r = 0.88$, $P < 0.01$) with the distribution of PCP receptors labeled with [3H]TCP. However, differences were observed in the percent of maximal binding in different brain areas. For example, a greater relative proportion of [3H]CPP binding was observed in the cerebral cortex and cerebellar granule cell layer than with [3H]TCP. These results suggest that there may not be a 1:1 ratio of NMDA and PCP sites in all brain regions and that the proposed NMDA/PCP receptor complex may be specific for discrete brain regions.

209.20 DEVELOPMENTAL CHANGES OF NMDA RECEPTORS IN CHICKEN CEREBELLUM. A.U. KLEIN[1]*, P. FREY[2]*, P.L. HERRLING[2], K.H. WINTERHALTER[3]*, M. CUENOD[1] and P. STREIT[1] (SPON: European Neuroscience Association). [1]Brain Res. Inst., Univ. of Zurich, CH-8029 Zurich, [2]Sandoz Res. Inst., CH-3001 Bern, [3]Lab. of Biochem I, Swiss Fed. Inst. of Technol., CH-8092 Zurich, Switzerland.

In adult rat, pigeon and chicken cerebellum, no signs for functional N-methyl-D-aspartate (NMDA) receptors can be found in [$^{22}Na^+$] efflux experiments. However, in chick cerebellar slices around hatching NMDA stimulated [$^{22}Na^+$] efflux was present at a considerable level, i.e. at about 50% of the value determined in adult rat hippocampus. NMDA stimulated [$^{22}Na^+$] efflux rapidly decreased during the first week to reach about 20% of the maximal level in 3 week old animals. - In extensively washed chick cerebellar homogenates, stored frozen at -20°C and thawed before use, NMDA displaceable L-[^{3}H]-glutamate binding could be measured only after incubation day 14, reached its maximum at hatching (430 fmol/mg protein) and decreased to about 30% of this level 3 weeks later. The time-course of this decrease was similar to that found in [$^{22}Na^+$] efflux experiments. The time-course of the increase, on the other hand, was the same as that observed for specific L-[^{3}H]-glutamate binding which also reached its maximum (440 fmol/mg) at hatching but did not decrease thereafter in frozen material. A higher maximal level of specific L-[^{3}H]-glutamate binding (920 fmol/mg) was reached already on incubation day 20 in experiments on freshly prepared homogenates and was maintained later. About 70% of this value were reached as early as on incubation day 14. - The freeze-labile component of specific L-[^{3}H]-glutamate binding may represent terminal uptake or APB binding sites. This site, thus would develop first and would be followed by NMDA receptors. The findings that NMDA receptors decrease after hatching and that the maximal levels of specific L-[^{3}H]-glutamate binding are maintained suggest that NMDA receptors would be replaced postnatally by other types of binding sites for excitatory amino acids. The role played by cerebellar NMDA receptors around hatching will have to be determined in the future.

210.1 SPECIFIC GLUTAMATE RECEPTORS IN THE BARORECEPTOR REFLEX ARC. W.T. Talman and P.A. Grieve*, Department of Neurology, V.A. Medical Center and University of Iowa, Iowa City, Iowa 52242.

In previous studies we and others have suggested that the excitatory amino acid glutamate is a neurotransmitter integral to the baroreceptor reflex arc in the nucleus tractus solitarius (NTS). We have shown that the baroreceptor reflex can be completely blocked by the microinjection of glutamate diethylester (GDEE) bilaterally into the NTS. However, the doses of GDEE required to block the baroreflex also inhibit other putative transmitters in the NTS. In this study we have sought to determine if the selective blockade of N-methyl-D-aspartate (NMDA) and kainic acid (KA) receptors by kynurenic acid (KYN) alters the baroreceptor reflex. Thirty-four adult male rats were anesthetized with pentobarbital (50 mg/kg) and instrumented for recording intra-arterial pressure and for administering intravenous drugs. Glass micropipettes filled with 0.9% saline vehicle, sodium L-glutamate (L-GLU), KA, NMDA, quisqualate (QUIS), acetylcholine (ACh), or KYN were stereotaxically placed unilaterally or bilaterally into the NTS after exposure of the dorsal medulla through an occipital craniotomy. Baroreflexes were tested by determining the reflex fall in heart rate to a 55 mm Hg rise in mean arterial pressure after the injection of phenylephrine intravenously. Microinjections (50 nl for agonists and 100 nl for KYN) were made into the NTS unilaterally or bilaterally. NMDA, QUIS, KA and L-GLU each produced a significant decrease in arterial pressure. After the microinjection of a 2 nmol dose of KYN the hypotensive response to NMDA was reduced from 39.2 ± 7.4 mm Hg to 6.6 ± 5.4 mm Hg (mean ± SEM) ($p < .01$), the hypotensive response to 3 pmol of KA was reduced from 43.7 ± 2.2 to 10.0 ± 5.6 mm Hg ($p < .01$), but the response to the microinjection of 10 pmol of QUIS was not changed (31.8 ± 4.2 mm Hg before KYN; 37.6 ± 5.3 mm Hg after KYN). The response to L-GLU was reduced by 60%. The bilateral injection of a 2 nmol dose of KYN into the NTS eliminated any significant baroreflex. Before KYN phenylephrine increased arterial pressure 60.8 ± 3.3 mm Hg with a decreased heart rate of 47.5 ± 16.1 bpm. After KYN blood pressure increased 54.5 ± 2.9 mm Hg and heart rate fell 8.8 ± 6.3 bpm. Microinjection of 20 nmol of KYN did not significantly alter the hypotensive response to the microinjection of ACh into the NTS. These studies confirm that the bilateral injection of kynurenic acid into the NTS blocks the cardiovagal limb of the baroreceptor reflex and suggests that NMDA and KA receptors, but perhaps not QUIS receptors, are integral to neural elements in the baroreceptor reflex. Supported by NIH RO1-HL32205 and VA Merit Review Tab 18. WTT was supported in part as an Established Investigator for the American Heart Association.

210.2 EXCITATORY AMINO ACIDS CONTRIBUTE TO SYNAPTIC EXCITATION IN RAT STRIATAL NEURONES IN VITRO

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The nature of the excitatory transmitter released after local stimulation in striatal slices is still uncertain. Previous studies suggested that the excitatory post synaptic potential (e.p.s.p.) evoked by intrastriatal stimulation is mediated either through the activation of a cholinergic nicotinic receptor (Misgeld, U. et al., Brain Res., 253, 317, 1982), or an excitatory amino acid receptor (Cordingley, G.E. and Weight, F.F., Br. J. Pharmac., 88, 847, 1986). Our experiments were aimed at further elucidating the nature of this excitatory transmitter in the in vitro slice preparation using intracellular recording techniques and a recently described broad spectrum and specific excitatory amino acid antagonists kynurenic acid (KYAC) and (D,L) 2-amino-7-phosphonoeptanoic acid (D,L)-AP-7.

In the presence of bicuculline (10-30 μM) intrastriatal stimulation evoked a pure e.p.s.p.. The relationship between e.p.s.p. and membrane potential was not linear. It decreased in amplitude and duration for values of membrane potential more negative that -80 mV and increased in amplitude and duration for values of membrane potential more positive than -50 mV. The mean reversal potential (with K+methylsulphate electrodes) was -9.2 ± 1.7 mV ($\bar{X}$ ± S.E.M., n = 4). Amplitude and duration of the e.p.s.p. were reduced in a dose dependent way by the endogenous excitatory amino acid antagonist KYAC (100-300 μM). However a residual depolarization remained even at high antagonist concentrations. In normal ACSF, the specific NMDA receptor antagonist (D,L)-AP-7 (30 μM) reduced the amplitude and duration of the e.p.s.p.s at depolarized membrane potentials (-50 mV or more)

but not at resting membrane potential. When Mg^{++} was removed from the bathing solution, the e.p.s.p.s. increased in amplitude and duration. Under these conditions, addition of (D,L)-AP-7 (30 μM) reduced the amplitude and duration of the e.p.s.p. even at resting potential. We conclude that the e.p.s.p. evoked by intrastriatal stimulation is mediated at least in part by the activation of excitatory amino acid receptors of the non-NMDA type in normal medium and resting membrane potential ; NMDA type receptors will be activated when the membrane is depolarized or in absence of Mg^{++}.

210.3 THE EFFECT OF EXCITATORY AMINO ACID AGONISTS ON RAT STRIATAL NEUROPEPTIDE-Y AND CHOLINERGIC NEURONS. R.J. Boegman and A. Parent. Department of Pharmacology and Toxicology, Queen's University, Kingston, Canada; and Laboratoire de Neurobiologie et Departement d'Anatomie, Universite Laval, Quebec, Canada.

The response of rat striatal neurons to excitatory amino acid agonists and an antagonist was studied by means of NPY immunocytochemistry, NADPH-diaphorase histochemistry, DFP histochemistry for acetylcholinesterase (AChE), and biochemical determination of choline acetyltransferase (ChAT) activity. Intrastriatal infusion of 0.5 μl of each drug dissolved in saline (pH 7.4) was carried out with the aid of a microinfusion pump attached to a cannula stereotaxically placed in the striatum. The injection coordinates were lateral 3.2 mm, anterior 0.12 mm and ventral 4.00 mm to bregma.

Striatal NPY neurons were more sensitive than cholinergic neurons to the neurotoxic action of kainic acid (KA, 6 nmoles/μl), quinolinic acid (QA, 120 nmoles/μl) or L-glutamic acid (GA, 2 μmoles/μl). All three compounds produced a marked loss of NPY neurons, but only a moderate decrease in the number of AChE neurons or ChAT activity. While KA resulted in a 45% reduction in striatal ChAT and QA in a 30% reduction, GA gave no significant change in striatal ChAT activity. Co-injection experiments indicated that the neurotoxicity of QA and GA, but not that of KA, could be antagonized by the specific NMDA receptor antagonist 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP). Removal of cortical afferents to the striatum, which are thought to be glutamatergic, by cerebral decortication protected striatal NPY and cholinergic neurons against the neurotoxicity of intrastriatal KA.

In the rat striatum the enzyme NADPH-diaphorase is considered a histochemical marker for neurons expressing NPY-like and somatostatin-like immunoreactivity. Following intrastriatal infusion of the excitatory amino acid agonists listed above, NADPH-diaphorase positive cells were found to be as sensitive to the neurotoxic effect of the infused drug as were the NPY immunoreactive neurons. Our experiments present the first morphological evidence that the NPY immunoreactive neurons in the striatum which belong to the medium-sized aspiny type I neurons receive prominent cortical amino acid afferents. Our data also supports previous reports that the AChE positive striatal neurons which correspond to the large aspiny type II neurons also receive glutamatergic cortical afferents. In addition, these neurons express excitatory amino acid receptors on their surface which mediate the neurotoxic response.

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210.4 RETARDATION OF AMYGDALOID KINDLING BY AND THE BEHAVIORAL EFFECTS OF KYNURENIC ACID, AN ANTAGONIST OF EXCITATORY AMINO ACIDS; A PRELIMINARY REPORT. Z. Dennison and D.P. Cain. Dept. of Psychology, U. of Western Ontario, London, Ontario, CANADA N6A 5C2.

Much can be discovered about the role of the dicaboxylic amino acids in epileptic phenomena by investigating the effects of antagonists of these compounds. Kynurenic acid, a tryptophan metabolite, has been shown to suppress epileptiform activity in an acute model (Ganong,Lanthorn, & Cotman, 1983). The present study was designed to examine the effects of kynurenic acid in a chronic model, amygdaloid kindling. In addition, the effects of an excitatory amino acid antagonist on motor behavior were quantified using a Digiscan activity monitor.

Male hooded rats were implanted with bipolar stimulating-recording electrodes aimed at the amygdala, and bilateral guide cannulae aimed at the cerebral ventricles. Between 1-2 weeks following surgery, an afterdischarge (AD) threshold was determined using 1 sec. trains of 60 Hz biphasic square waves of increasing intensity. Every 48 hours, KYNA rats received an icv injection of 63 ug kynurenic acid in 3.3 ul saline, using an infusion pump, and were then placed in the activity monitor for 30 minutes. Forty minutes following injection, the rats were stimulated at a frequency 50 microamps above AD threshold. Control animals received 3.3 ul of saline alone. Stimulation continued for 19 sessions,or until the animal reached a second stage 5 convulsion. Animals were tested in the activity monitor every 3rd stimulation session.

Saline animals reached a stage 5 convulsion after a mean of 10 stimulations, while KYNA animals reached a stage 5 after a mean of 16.6 stimulations [$t=8.57, p<.0005$]. These results indicate a significant retardation in rate of kindling development by pretreatment with kynurenic acid. In comparing movement time, as recorded by the activity monitor, the KYNA animals showed less spontaneous movement compared to controls. The strongest effect was seen in the first session, where the KYNA animals had a mean of 20.62 seconds of movement in a 5 minute block compared to a mean of 93.7 seconds of movement in a 5 minute block in controls. This effect decreased in strength and by the 10th session, the two groups were not distinguishable based on movement, as the mean movement time for KYNA animals was 82.3 seconds per 5 minute block and the mean movement time for controls was 86.5 seconds per 5 minute block. These results indicate that strong motor impairment is evident with the initial administration of kynurenic acid, but tolerance develops to this effect with repeated administration.

210.5 **ANTAGONISM OF NORMAL AND ABNORMAL ACTIVITY OF RELAY NEURONS IN THE RAT'S VENETROBASAL THALAMUS USING EXCITATORY AMINO ACID ANTAGONISTS.** D.T. Ross. Department of Clinical Neurosciences, Brown University, Providence, RI

Relay neurons in the thalamic ventrobasal complex (VB) axotomized by ablation of the primary somatosensory cortex (SI) exhibit abnormally high levels of spontaneous activity and an enhanced responsiveness to tactlile evoked stimulation in their peripheral receptive field (Angel and Clarke, 1975; Ross and Ebner, 1986). The observed hyperexcitability of axotomized relay neurons together with other anatomical and physiological alterations which occur within the thalamus suggest that thalamic retrograde degeneration following cortical injury may be an excitotoxic process. The present experiments were designed to examine the role of excitatory amino acid neurotransmission in the activity of normal and axotomized thalamic relay neurons.

Iontophoresis of the broad spectrum excitatory amino acid antagonist kynurenic acid (KYN) into the ventrobasal thalamus completely blocked the response of relay neurons to tactile stimulation in their peripheral receptive fields. Spontaneous activity was also somewhat decreased following KYN iontophoresis but bursting with spindle rythmicity (6-10 Hz) was not prevented. Stimulation of neurons in layer VI of the somatotopically corresponding region of the SI cortex by local iontophoresis of NMDA drove the firing of VB neurons. The response of the relay neurons to this corticothalamic stimulation was also antagonized by iontophoresis of KYN into the VB. These results are consistant with those of numerous other studies which suggest that both the corticothalamic and lemniscal synapses upon relay neurons in the ventrobasal thalamus may use an excitatory amino acid neurotransmitters.

Iontophoresis of KYN into the ventrobasal thalamus 6-8 hours after ablation of the SI cortex antagonized both the abnormal spontaneous activity and the abnormal response of the axotomized relay neurons to tactile stimulation in their peripheral receptive field. Iontophoresis of KYN onto identified VB units firing at spontaneous rates from 30-40 Hz totally abolished activity within 5 seconds of the onset of iontophoresis at 150-300 nA. Iontophoresis of 10-30 nA of L- glutamate reversed the blockade within 5-10 seconds. Spontaneous activity returned to 30-40 Hz within 5-10 minutes after cessation of KYN iontophoresis with 5 nA retaining current on the glutamate barrel. The response of the axotomized relay neurons to tactlie stimulation in their peripheral receptive fields was characterized by bursts of 4-12 very fast spikes which followed stimulation up to 10 Hz with very high fidelity . Within 20 seconds after the onset of KYN iontophoresis at 300nA these same units failed to follow the same tactile stimulation at any frequency. Responsiveness to tactile stimulation on the unit's peripheral receptive field returned within 5-10 minutes after the cessation of KYN iontophoresis or within 5-10 seconds after the onset of glutamate iontophoresis at 10-30 nA.

These results suggest that the axotomized relay neurons' abnormal levels of spontaneous acitvity and burst type response to stimulation are due to a derrangement in excitatory amino acid neurotransmission. Studies in progress will examine 1) the relative contribution of different excitatory amino acid receptor classes to the increased excitability of axotomized relay neurons, and 2) the efficacy of long term excitatory amino acid antagonist infusion on decreasing excitability and preventing retrograde degeneration of axotomized VB relay neurons. (Supported by NIH postdoctroal fellowship NS 07419-02).

210.6 OPIOID INHIBITION OF KAINIC ACID INDUCED-SCRATCHING: NALTREXONE SENSITIVE AND INSENSITIVE COMPONENTS R.C. Coghill,* H. Frenk,[1] D.F. Bossut, D.E. Kellstein, and D.J. Mayer. Department of Physiology, Medical College of Virginia, Richmond, VA 23298 and [1]Department of Psychology, Tel Aviv University, Ramat Aviv, Israel

Intrathecal (IT) injection of kainic acid to rats results in a behavioral syndrome characterized by vigorous scratching of the flanks with the hind paws and caudally directed biting and licking. Morphine inhibits scratching induced by IT kainic acid and strychnine, but not that induced by substance P. To further delineate the receptor type by which opioids inhibit scratching, we examined the effectiveness of mu, delta, kappa, and sigma agonists on reducing kainic acid-induced behavior.

Adult male rats (400-500g; Hilltop) were implanted with IT catheters terminating in the lumbosacral region of the spinal cord. After 5 days of recovery, animals were pretreated with morphine (90 nmol), levorphapol (30 and 90 nmol), dextrorphan (90 nmol), [D-ala^2, N-Met-Phe^4, Gly^5-ol]- enkephalin (DAGO, 0.4 and 1.1 nmol), [D-Pen^2-D-Pen^5]-enkephalin (PEN, 90 nmol), [D-ala^2, D-leu^5]-enkephalin (DADL, 10 and 30 nmol), dynorphin (DYN, 1.1 nmol), ethylketocyclazocine (EKC, 90 nmol), phencyclidine (PCP, 90 nmol), or saline ten minutes prior to injection of 0.468 nmol kainic acid. All drugs were administered IT in 10 microliters of saline except naltrexone (30 mg/kg) which was injected intraperitoneally.

The mu agonists morphine, DAGO, and levorphanol produced substantial reductions in kainic acid-induced scratching. The delta agonists DADL and PEN also reduced scratching elicited by kainic acid, while the kappa opioids EKC and DYN had no effect. Naltrexone, an antagonist known to reverse the behavioral effects of mu and delta agonists, partially reversed morphine-induced scratch reduction, but failed to alter scratch reductions produced by levorphanol. These findings indicate that opioid reduction of kainic acid-induced scratching is composed of naltrexone reversible and naltrexone irreversible components. The existence of a naltrexone insensitive mechanism is further supported by the ability of dextrorphan, the dextrorotary isomer of levorphanol, to reduce kainic acid-induced scratching. Such a naltrexone insensitive mechanism may be mediated through a sigma site, since both dextrorphan and levorphanol are known to bind to the sigma receptor. PCP, however, failed to reduce kainic acid-induced scratching, indicating that dextrorphan and levorphanol may elicit their effects via PCP-insensitive mechanism(s), such as the haloperidol/sigma receptor.

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210.7 AUTORECEPTOR REGULATION OF ASPARTATE AND GLUTAMATE RELEASE FROM THE SCHAFFER COLLATERAL-COMMISSURAL PROJECTION TO HIPPOCAMPAL AREA CA1. G. Bustos*, S. Bray* and J.V. Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Glutamate and/or aspartate is the probable transmitter released by synaptic terminals of Schaffer collateral, commissural and ipsilateral associational fibers in the hippocampal CA1 area. We have employed slices of the CA1 area to test the hypothesis that transmitter release in these pathways is regulated by feedback of the transmitters onto presynaptic excitatory amino acid receptors.

The CA1 area, excluding stratum lacunosum-moleculare, was dissected from 475 um-thick rat hippocampal slices. CA1 slices were transferred to small superfusion chambers, 5 slices per chamber, and superfused with artificial CSF at 32°C for 60 min. Transmitter release was provoked by increasing the K^+ concentration of the medium to 53.5 mM for 1 min at this point and then again 40 min later. Under the conditions of this study, aspartate and glutamate release is better than 90% Ca^{2+}-dependent and originates predominantly from the CA3-derived pathways. Samples of superfusate collected immediately prior to and during the K^+ pulses were analyzed for amino acid content by HPLC of the o-phthalaldehyde derivatives. Excitatory amino acid receptor ligands were added to the medium 5 min before the second K^+ pulse and their effect upon the ratio of amino acid released by the second pulse as compared to the first was determined.

Kainate (10-800 uM) and AMPA (20 uM) significantly reduced the K^+-evoked release of aspartate, but not of glutamate. The maximum reduction obtained (800 uM kainate) was about 50%. Neither kainate nor AMPA by itself evoked glutamate or aspartate release. N-Methyl-D-aspartate (NMDA; 100 uM) did not significantly affect excitatory amino acid release, even in the absence of Mg^{2+}. The relatively non-selective excitatory amino acid antagonist kynurenate (2 mM) enhanced the release of both aspartate and glutamate by about 25%. Conversely, the selective NMDA receptor antagonist CPP (20 uM) reduced aspartate release by about 23%, but only in the absence of Mg^{2+}.

These results suggest that the activation of NMDA and non-NMDA receptors has opposite effects on excitatory amino acid transmitter release in area CA1; NMDA receptor activation enhances release, whereas activation of non-NMDA receptors inhibits release. The actions of antagonists suggest that elevated K^+ releases enough endogenous amino acid to activate many of these receptors. Thus transmitter release at Schaffer collateral-commissural synapses may normally be regulated, in part, by the transmitters themselves. The transmitters may not only modulate the total quantity of amino acid released, but may also change the ratio of aspartate release to glutamate release. (Supported by NIH grant NS 16064.)

210.8 KAINIC ACID INHIBITS HIPPOCAMPAL CHOLECYSTOKININ RELEASE AND HAS DIFFERENT EFFECTS ON SYNAPTIC TRANSMISSION IN CA3 REGION OF RATS AND GUINEA PIGS. P.G. Aitken, G. Bustos*, P.Lee*, D.B. Jaffe*, J.-S. Hong, J.V. Nadler. Depts. of Physiology and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710 and Laboratory of Behavioral and Neurological Toxicology, N.I.E.H.S., Research Triangle Park, NC 27709.

We have previously suggested that the effects of kainic acid (KA) in the CA3 area may be mediated by disturbances in the endogenous cholecystokinin (CCK) system. To further address this possibility, the present research asked (1) Do the rat and guinea pig, which differ in the distribution of CCK in the hippocampal CA3 area, also differ in their response to KA?, and (3) Does KA alter CCK release from hippocampal slices?

For electrophysiology, hippocampal slices from Sprague-Dawley rats or Hartley guinea pigs were maintained at 35.5°C in an interface chamber. A stimulating electrode was positioned to activate the mossy fibers, and the postsynaptic extracellular field response was recorded in stratum pyramidale of area CA3b. Input/output (I/O) curves were generated by plotting the amplitude of the mossy fiber-evoked response against stimulus current over a range of stimulus intensities.

KA (50-200µM) was applied in the bath. Slices were monitored for spontaneous synchronous activity ("bursts"), and I/O curves were generated before, during, and after KA application. In both rat and guinea pig slices, KA caused bursting at frequencies from 26 to 64/min. In rat slices, KA increased the area under the I/O curve by 43%, indicating increased synaptic efficiency and/or postsynaptic excitability. In guinea pig slices, KA had no effect on I/O curves. All KA effects reversed upon washout. This difference in the effects of KA may be related to the presence of CCK in guinea pig, but not rat, mossy fibers.

For release experiments, slices of rat hippocampus were maintained in small chambers (4 slices/chamber, 32°C)and superfused with artificial CSF. CCK release was provoked by increasing the K^+ concentration of the superfusion medium to 53.5mM for two-1 minute intervals spaced 40 minutes apart. Samples were collected before and during each K^+ pulse and analyzed for CCK content by radio-immunoassay. In some experiments, KA was present during the second K^+ pulse; its effects on CCK release were assessed by comparing pulsel/pulse2 ratios from control and KA experiments.

In control chambers K^+ evoked the release of 92.8 ± 8.6 pg/min over baseline release of 17.7 ± 4.0 pg/min. KA, at 100µM and 1µM, reduced K^+-evoked CCK release by 64% and 22% respectively, but did not affect basal release. Because CCK appears to have a net inhibitory effect in area CA3 of rats, inhibition of CCK release may contribute to the epileptogenic and excitotoxic effects of KA in this region. (Supported by NIH grant 17771)

210.9 DIFFERENTIAL EFFECTS OF ACIDIC AMINO ACID ANTAGONISTS ON EPILEPTIFORM ACTIVITY IN THE IN VITRO HIPPOCAMPAL SLICE. G.B. Watson* R.K. Rader, and T.H. Lanthorn. Searle Research & Development, Chesterfield, MO. 63198

N-methyl-D-aspartate (NMDA) receptors are involved in some epileptiform burst firing in the hippocampus. Compounds which decrease the efficacy of NMDA receptors can inhibit epileptiform activity. Recently, we have begun to examine the effects of such compounds on epileptiform activity induced by different means in the hippocampal slice. Here we describe the abilities of D-2-amino-7-phosphonoheptanoate (D-AP7, 100μM), phencyclidine (PCP, 30μM) and kynurenate (KYN, 500μM) to alter epileptiform activity induced by penicillin (PEN, 3.4mM) and increased potassium (high K^+, 9.25mM).

Epileptiform bursts, recorded extracellularly in area CA1, were induced by stimulation of the Schaffer collateral/commissural fibers. All experiments were performed in a submersion chamber and all compounds were applied to the perfusate.

D-AP7, PCP, and KYN decreased the rate of spontaneous occurring potentials, decreased the duration of evoked bursts and decreased the number of population spikes per evoked burst in both PEN and high K^+ media. KYN decreased the amplitude of the remaining evoked population spikes in all slices examined. PCP either decreased or did not affect the amplitude of remaining evoked population spikes in both PEN and high K^+ media. Unexpectedly, D-AP7 increased the amplitude of the remaining evoked population spikes. This effect was more readily seen in PEN induced epileptiform activity (75% of slices examined) but was also seen in high K^+ medium (40% of slices examined; amplitude unaffected in remaining slices).

KYN was able to block all components of the epileptiform burst. This is consistent with its ability to block NMDA and non-NMDA acidic amino acid receptors and to block synaptic transmission at the Schaffer collateral/commissural-CA1 synapse. PCP and D-AP7 both selectively block NMDA responses. As expected, both compounds reduced the burst duration. However, D-AP7 was also able to increase the amplitude of the remaining population spikes, an effect not mimicked by PCP. This finding may suggest that PCP and D-AP7 can act on separate, as well as a common mechanisms.

210.10 EFFECT OF LOW GLUCOSE CONCENTRATIONS ON SYNAPTIC TRANSMISSION TO CA1 PYRAMIDAL NEURONS IN THE RAT HIPPOCAMPUS SLICE. J.C. Szerb, P. Fan* and P.A. O'Regan*. Dept. of Physiology and Biophysics, Dalhousie Univ. Halifax, N.S. B3H 4H7, Canada.

Severe hypoglycemia in vivo is known to slow down and then to abolish reversibly the EEG (Auer et al. *Diabetes* 33:1090, 1984). At the same time, there is a large increase in the extracellular concentration of potassium (Harris et al. J.Cerb.Bl.Flow Metab.4:187,1984) and of amino acids, especially that of aspartate (Sandberg et al. *J.Neurochem.* 47:178, 1986). In hippocampal slices a low glucose concentration (0.2 mM) increases the Ca-dependent, TTX sensitive evoked release of both aspartate and glutamate but that of aspartate is increased about three times more (Szerb and O'Regan, *Synapse*, in press). The purpose of these experiments was to reconcile electrophysiological and neurochemical observations on the effects of hypoglycemia on neuronal function by measuring synaptic transmission from Schaffer collaterals to CA1 pyramidal cells in the rat hippocampal slice in different glucose concentrations. Changing the glucose content of the superfusion fluid from 5 to 0.2 mM reversibly depressed the population spike and the dendritic focal EPSP, without affecting the size of the presynaptic volley. The maximal glucose content of the medium that resulted in a reduction of the population spike was about 1 mM, the same concentration of glucose that was just sufficient to increase the ratio of aspartate to glutamate released. The population spike of CA1 pyramidal cells stimulated antidromically from the alveus was depressed only slightly by low glucose. In contrast to low glucose, 5-10 μM ouabain or 10-12 mM K^+ intitially increased the size of the population spike, then depressed the presynaptic volley, along with the focal EPSP and population spike. These observations suggest that the loss of electrical activity in hypoglycemia is not due either to the failure of action potential conduction, or of transmitter release but to the blocking of the postsynaptic action of the released transmitter.

(Supported by the Medical Research Council of Canada.)

210.11 EFFECT OF PROLONGED GLUTAMATE (GLU) APPLICATION ON POSTSYNAPTIC RESPONSES TO GLU AGONISTS IN RAT HIPPOCAMPAL NEURONS. A.E. Cole, J.M.H. ffrench-Mullen and R.S. Fisher, Dept. of Neurology, Johns Hopkins Hospital, Baltimore, MD 21205.

Prolonged exposure (3-5 min) of rat hippocampal slices to perfusate containing 1-2 mM GLU induces a reversible and relatively selective blockade of excitatory transmission, with little effect on inhibitory pathways (Bernstein et al., Neurosci. Lett. 1985). This action is also seen when GLU is applied by ionophoresis into mid-stratum radiatum, the site of excitatory synaptic input from the Schaffer-collateral fibers (Cole et al., Neurosci. Abst. 1986). The present study examines the effect of prolonged application of GLU on postsynaptic responses to GLU agonists, at a time when the EPSP is depressed.

Multibarreled ionophoretic electrodes were positioned in stratum radiatum while recording intracellularly from CA1 neurons. Pulses of agonists (50 ms-1 sec) were ejected at 30 sec intervals with pipette concentrations of GLU (200 mM), quisqualate (QUIS,10 mM), N-methyl-d,l-aspartate (NMA,30 mM) and kainate (KA,10 mM). Ejection currents were selected to produce postsynaptic membrane depolarizations of equal amplitude. At a time when the EPSP was depressed to 37% of control (n=15;p<.001) by prolonged GLU application (115.6±10.9sec), the depolarization to a pulse of GLU was depressed to 43% of control (p<.005). The responses to pulses of QUIS(n=7), NMA(n=6) and KA(n=5) were also significantly depressed (p<.001 for QUIS and NMA; p=.02 for KA). An identical protocol was followed using prolonged ionophoresis of QUIS, NMA and KA. QUIS (n=7) selectively depressed the EPSP to 37% of control with little effect on the IPSP, and depressed the depolarizations to both NMA and KA (p<.05). In contrast, although prolonged NMA application selectively reduced the EPSP (n=9) to 52% of control, it had no significant effect on the brief depolarizations to QUIS or KA (p>0.1). Although KA (n=6) depressed the brief QUIS and NMA responses, this was always accompanied by a non-selective depression of the EPSP and IPSP.

These results show that prolonged QUIS and KA application decreased postsynaptic responses to GLU agonists,in addition to the EPSP. Conversely, NMA blocked the evoked EPSP but did not decrease postsynaptic responses to QUIS, KA or GLU. This suggests a primarily postsynaptic locus of action for QUIS and KA, and a presynaptic locus for NMA. The action of GLU, a mixed agonist, is likely to be a combination of pre- and postsynaptic actions. These findings support the presence of an intrinsic mechanism with both pre- and postsynaptic components that may regulate excessive excitatory transmission in the CNS.

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210.12 EFFECT OF GLUTAMATE AGONISTS AND ANTAGONISTS ON CALLING IN DOMESTIC CHICKS. L. Normansell, D. Zeisloft * and J. Panksepp. Department of Psychology, Bowling Green State Univ., Bowling Green, OH 43403.

Localized infusions of glutamate have been reported to induce vocalizations in both cats and squirrel monkeys, presumably by activating synaptic fields where vocalization pathways are relayed. We have investigated the effects of intracerebroventricular administration of glutamate, as well as several glutamate receptor agonists and antagonists, on calling in domestic chicks.

Glutamate, at doses from 25 to 500 μg/3μl, decreased calling in a dose-dependent manner. Administration of N-methyl-D-aspartate (NMDA), on the other hand, had no effect on vocalizations when the animals were tested in plain boxes, but doses of .5 and 1.0 μg induced dramatic increases in calling frequency (> 400 %) when the birds were placed into mirrored boxes, an environmental situation which has a quieting effect on control animals. Kainate (KA) had an effect similar to NMDA: No changes in vocalization frequency were detected in the plain boxes at any dose tested (.05, .1, .25, .5 μg), but dose-dependent increases occurred in the mirrored boxes. In contrast, quisqualate (QA) produced a dose-dependent decrease in the number of calls, except at the highest dose (1.0 μg) where calling frequency increased back to control levels.

The effects on vocalization of glutamate receptor antagonists have also been assessed. Both D-2-amino-5-phosphonovalerate (APV), a selective NMDA receptor antagonist, and gamma-D-glutamylglycine (DGG), a broad spectrum/QA-KA receptor antagonist, produced dose-dependent decreases in calling. Administration of either NMDA (1.0 μg) or KA (.25μg) completely reversed the suppression of calling that followed APV (.1μg) treatment. KA partially reversed the DGG-induced suppression of calling, whereas NMDA did not.

The finding, that NMDA and KA seem to effect vocalization frequency in one direction, whereas QA shifts the propensity to call in the other, may partially explain the vocalization reduction observed following glutamate administration. If an NMDA or KA system serves an activational role, while a QA system acts in a counteracting manner, the relative density of the receptor subtypes in the area near the injection site of the general agonist could result in either an increase or decrease in calling. The finding that all three agonists had behavioral effects suggests they are all present near the area of the 4th ventricle (the target site for the injection). Additionally, that the highest dose of QA produced an increase in vocalizations would seem to suggest that the specificity of agonists for a particular receptor occurs only within a limited concentration range.

210.13 LAMINAR DISTRIBUTION OF EXCITATORY AMINO ACID-INDUCED EXTRACELLULAR K^+ AND Ca^{2+} CHANGES IN THE ISOLATED TURTLE CEREBELLUM. M.E. Rice* and C. Nicholson. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

Excitatory amino acid (EAA) receptor subtypes are regionally distributed in the rat cerebellum (Greenamyre et al. JPET. 233: 254, 1985). Quisqualate (Quis) receptors are concentrated in the molecular layer, whereas N-methyl-D-aspartate (NMDA) receptors are mainly in the granular layer. Kainate (Kai) receptors are more evenly distributed, but are denser in the granular than molecular layer. Using ion-selective microelectrodes (ISMs) and iontophoresis, we have examined the correlation between these distributions and EAA-induced changes in $[K^+]_o$ and $[Ca^{2+}]_o$ in the isolated turtle cerebellum. Electrode assemblies consisted of a central double-barrelled pipette with two of five EAAs (200 mM, pH 7.6 glutamate (Glu), aspartate (Asp), Kai, NMDA, Quis) with a Ca^{2+}- and a K^+-ISM glued 50-60 µm away. Ion changes during EAA-iontophoresis were recorded at 100 µm steps through the cerebellar cortex. Kai was found to be the most potent EAA, producing large ion changes throughout the cerebellum, with the largest response in the granular layer. Quis and NMDA had more distinct response patterns. Quis produced larger changes in $[Ca^{+2}]_o$ and $[K^+]_o$ during iontophoresis in the molecular layer than in the granule cell layer, while NMDA induced ion changes in the granular layer, but had little effect in the molecular layer.

Glu and Asp were indistinguishable from each other and had laminar response patterns that mirrored the profile for Kai. The maximum increase in $[K^+]_o$ (4-6 mM) and decrease in $[Ca^{2+}]_o$ (0.3-0.4 mM) with Glu or Asp were seen in the granular layer, 200-300 µm from the ventral surface. The largest $[K^+]_o$ increase in the molecular layer (just above the Purkinje cell bodies) was 50% of the maximum granular layer response, while that in the Purkinje cell layer was 40%. To eliminate secondary effects from Ca^{2+} entry, 5 mM Mn^{2+} (1 mM Ca^{2+}) was included in the bath. Here, synaptic transmission was inhibited (indicated by elimination of the second component of peduncle-evoked fields) and $[Ca^{2+}]_o$ decrease was blocked, except in the granular layer at the site of maximum $[K^+]_o$ increase. Granular and molecular layer increases were reduced 20-40% and the Purkinje cell layer response was unaltered, however the largest increase remained in the granular layer. In summary, the functional locations of EAA receptors in the turtle cerebellum correspond well with those predicted by autoradigraphy. Moreover, Glu and Asp appear to act at Kai receptors, with little discrimination between Quis and NMDA sensitive sites. (NINCDS NS-13742 and NS-07745.)

210.14 REGIONAL SELECTIVITY OF EXCITATORY AMINO ACID ANTAGONISTS IN THE VERTEBRATE DORSAL HORN. R.L. Gannon and R.B. Leonard. Department of Pharmacology and Toxicology, Department of Physiology and Biophysics and the Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550,

Spinal cord physiology is being investigated in our lab using an in vitro spinal cord preparation from the Atlantic stingray, Dasyatis sabina, a vertebrate model for locomotion. Current research is directed to determining the role of putative excitatory amino acid neurotransmitters in the production of spinal reflexes. Stingrays are anesthetized and a length of spinal cord with spinal nerves attached to one side is removed, placed in a recording chamber, and superfused with a modified Ringer's solution. A laminectomy is performed to expose the dorsal surface and the side contralateral to the intact spinal nerves. Standard electrophysiological techniques using suction electrodes are employed to stimulate the sensory component of the spinal nerve and to record the volley on the dorsal root. Capillary glass electrodes (6-10 MΩ, 3M NaCl) are used to record field potentials in superficial and deeper areas of the dorsal horn, corresponding to small Aδ and larger Aα,β afferent fiber terminal projections, respectively. Dorsal horn field potentials produced by small Aδ fibers are inhibited by the non-selective excitatory amino acid antagonists kynurenate and DL-2-amino-4-phosphonobutyrate (APB), but not by D-α-aminoadipate (DAA). The inhibitory action of APB was further shown to reside in the L(+) isomer. The N-methyl-D-aspartate (NMDA) antagonist DL-2-amino-5-phosphonovalerate (APV) and its D(-) and L(+) isomers were found to have little affect. Phencyclidine and etoxadrol at high concentrations (300 µM) also inhibited the Aδ field potentials. Field potentials in deeper areas of the dorsal horn were also inhibited by kynurenate and etoxadrol at the same concentrations effective in superficial areas, but not by APB, APV, DAA, or phencyclidine. Dihydrokainate reduced the amplitude of field potentials produced by both fiber types. These results suggest that both Aδ and Aα,β primary afferent fiber types are releasing excitatory amino acid transmitters, but that presynaptic and/or postsynaptic receptor types may be different for the two afferent populations. Supported by NIH NS11255.

210.15 RESPONSE OF NEONATAL RAT LATERAL HORN CELLS TO GLUTAMATE AND N-METHYL-D-ASPARTATE IN VITRO. T. Miyazaki*, N. Mo* and N. J. Dun. Dept. of Pharmacol. Loyola Univ. Med. Ctr., Maywood, IL 60153

Intracellular recordings were made from lateral horn neurons including antidromically identified sympathetic preganglionic neurons situated in thin (500 µm) thoracolumbar spinal cord slices obtained from neonatal (10-20 days) rats. Glutamate (Glu) and N-methyl-D-aspartate (NMDA) were applied to lateral horn cells by pressure ejection. In normal Krebs solution containing 1.3 mM Mg, Glu and NMDA evoked a phasic depolarization with an amplitude of several to more than 10 mV. The responses were not appreciably affected by low Ca (0.25 mM) solution or Krebs solution containing tetrodotoxin (TTX, 0.1 µM). In a number of lateral horn cells, the Glu or NMDA-induced depolarization was accompanied by burst of small hyperpolarizing potentials resembling inhibitory postsynaptic potentials (IPSP's). The frequency of IPSP's induced by Glu or NMDA could be enhanced by changing to a Mg-free Krebs solution. As IPSP's were reversibly eliminated by superfusing the slices with a low Ca solution or solution containing TTX or strychnine (0.1-1 µM), they were probably due to the release of glycine from an interneuron(s) activated by Glu or NMDA. The Glu and NMDA-induced depolarizations were associated with a small decrease, increase or no apparent change of membrane input resistance in different cells studied. By changing to a Mg-free solution, the Glu and NMDA-induced depolarizations were consistently and markedly increased, often giving rise to intense cell discharge. In Mg-free solution, the NMDA-induced depolarization was associated with decrease of input resistance and the response was made larger on membrane hyperpolarization. The mean extrapolated reversal potential was about -20 mV. D-2-amino-5-phosphonovalerate (APV, 5-10 µM), DL-APV (50-100 µM) or ketamine (5-10 µM) reversibly blocked the NMDA-induced depolarizations in Mg-free solution. The Glu-induced depolarizations evoked in Mg-free solution on the other hand appeared to be less straightforward insofar as antagonists were concerned. For example, APV, DL-APV and ketamine in concentrations effective in blocking the NMDA-induced depolarizations only partially suppressed the Glu-induced responses. These findings suggest that rat lateral horn cells are endowed with NMDA receptors whose electrophysiological and pharmacological characteristics appear to be similar to those reported in other central neurons as well as non-NMDA excitatory amino acid receptors. While NMDA activates selectively the NMDA receptors, Glu appears to activate both the NMDA and non-NMDA receptors. In addition, inhibitory interneurons in the lateral horn seem to be endowed with NMDA and non-NMDA receptors the activation of which causes a release of glycine. (Supported by NS18710).

210.16 GLUTAMATE-SENSITIVE SITES CONTROLLING SLOW AND FAST MODULATIONS OF THE FREQUENCY OF ELECTRIC ORGAN DISCHARGES: LOCATION, MORPHOLOGY AND RESPONSE PROPERTIES OF PREPACEMAKER NEURONS. G. Rose, M. Kawasaki, L. Maler and W. Heiligenberg. Neurobiology unit, Scripps Institution of Oceanography, La Jolla, CA 92093, Dept. of Anatomy, Univ. of Ottawa, Ottawa, Ontario, K1H 8M5.

Certain types of South American electric fish produce rhythmic electrical discharges for the purpose of spatial orientation and social communication. These electric organ discharges (EODs) are controlled by a group of cells in the medulla, the pacemaker nucleus; each action potential of the pacemaker neuron triggers a single EOD cycle. The pacemaker maintains a rhythmic discharge even when isolated and placed in vitro. Modulations of discharge frequency of pacemaker neurons, and therefore of EODs, occur during social behaviors. Two forms of modulations can be distinguished: smooth frequency shifts with a time constant on the order of a second, and fast rises, or 'chirps', that may lead to a brief cessation of the pacemaker.

The objectives of the present study were: 1) Determine which neurons are afferent to the pacemaker N. by using retrograde tracing methods. 2) Identify sites that, when stimulated by iontophoretic application of L-glutamate, elicit the slow or fast modulations in the EOD frequency. 3) Record from single units in these glutamate-sensitive sites while presenting sensory stimuli that produce slow or fast EOD modulations. Experiments were conducted on two genera of electric fish, Eigenmannia and Apteronotus.

Modulations of the EOD frequency could be elicited by iontophoresis of L-glutamate at several sites in the midbrain tegmentum. One of these sites corresponds to the prepacemaker nucleus, a region where labelled cells are observed following WG-HRP injections into the pacemaker nucleus. The response properties of single units in the prepacemaker nucleus suggest that different types of neurons control smooth frequency shifts and chirps. Supported by grants NS 07261-02 to G.R. and BNS 82-05454 to W.H.

210.17 HOMOCYSTEATE AS A NEUROTRANSMITTER CANDIDATE IN THE BRAIN - PRESYNAPTIC AND POSTSYNAPTIC CHARACTERISTICS. C. Tsai*, P.L. Wood, and J. Lehmann(SPON: P. Etienne). Research Dept., Pharmaceuticals Division, CIBA-GEIGY Corp., Summit NJ 07901.

Homocysteic acid (HCA) has been proposed as an endogenous neurotransmitter acting at N-methyl-D-aspartate-preferring (NMDA-type) receptors, for two reasons. First, there is measurable depolarization-evoked release of HCA from brain tissue. Second, HCA produces the bistable depolarizing shifts characteristic of NMDA-type receptor activation when it is iontophoresed in the brain (K.Q. Do et al., J. Neurosci. 6:2226, 1986). We have pharmacologically characterized the actions of the stereoisomers of HCA at NMDA-type receptors on striatal cholinergic interneurons, and also examined the uptake system for [^{3}H]DL-HCA in crude synaptosomes.

Like NMDA, HCA evoked the release of [^{3}H]acetylcholine (ACh) formed from [^{3}H]choline in striatal slices. The concentration-response curve to L-HCA was virtually superimposable on that to NMDA, yielding equal EC_{50} values (18 uM) and maximal responses. However, D-HCA was weaker, with an EC_{50} value of 170 uM, and an apparently smaller maximal response. L-HCA evoked [^{3}H]ACh release was inhibited by the same categories of compounds which inhibit NMDA-evoked [^{3}H]ACh release: the divalent ion Mg (IC_{50}=14 uM), competitive NMDA antagonists DL-AP7 (IC_{50}=49 uM) and CPP (IC_{50}=17 uM), and dissociative anesthetics tiletamine (IC_{50}= 4.9 uM) and MK-801 (IC_{50}=0.09 uM).

L-HCA inhibited high affinity [3H]L-glutamate uptake with an IC_{50} of 2 mM, while D-HCA was inactive up to and including 10 mM.

[^{3}H]DL-HCA was accumulated by crude synaptosomal fractions from brain, but by a low velocity system. Whereas high tissue-to-medium ratios were obtained for the uptake of [^{3}H]L-glutamate (116), that for [^{3}H]DL-HCA was only 3.5 under optimal conditions, which was closer to the values obtained for [^{3}H]dopamine (10.5), and [^{3}H]choline (9.6). The regional distribution of [^{3}H]DL-HCA uptake did not parallel that of [^{3}H]L-glutamate uptake. It is questionable if the major route of inactivation of synaptic HCA would involve a high affinity uptake system. Rather, there may exist specific enzymes to inactivate HCA.

210.18 PARTIAL PURIFICATION OF N-ACETYLATED-ALPHA-LINKED ACIDIC DIPEPTIDASE (NAALADASE): A QUISQUALATE-SENSITIVE PEPTIDASE THAT CLEAVES N-ACETYL-ASPARTYL-GLUTAMATE TO N-ACETYL-ASPARTATE AND GLUTAMATE *IN VITRO*. M. B. Robinson, B. L. Stauch*, and J.T. Coyle. Dept. of Neuroscience, The Johns Hopkins School of Medicine, Baltimore, MD, 21205

The heterogeneous regional distribution of N-acetyl-aspartyl-glutamate (NAAG) and its ability to depolarize specific neurons which receive proposed glutamate afferents suggest that NAAG may act, in part, as an excitatory neurotransmitter at a subpopulation of putative glutamatergic synapses.

Metabolic studies using rat forebrain synaptosomes suggest a potentially rapid mechanism of inactivation whereby the peptide is degraded to N-acetylaspartate (NAA) and glutamate with subsequent glutamate reuptake (Blakely et al. J. Neurochem. 47 (1986) 1013-1019). Similiarly, striatal injections demonstrate a parallel route of NAAG catabolism *in vivo*. We have recently identified and characterized a quisqualate-sensitive, chloride-dependent, membrane bound, metallopeptidase activity which demonstrates a high apparent affinity for NAAG with a K_m = 540 nM and a V_{max} = 180 nM/mg protein per min (Robinson et al. Eur. J. Pharm. 130 (1986) 345-347). This activity demonstrates specificity for **N**-Acetylated **A**lpha-**L**inked **A**cidic **D**ipeptides, and therefore has been named "**NAALADase**".

NAALADase was partially purified from lysed synaptosomal membranes prepared from frozen rat forebrains and solubilized with Triton X-100 (0.5 %) followed by sequential column chromatography in Tris-Cl buffer (10 mM, pH 7.8) with 0.05 % Triton. NAALADase activity was assayed by quantitating the liberation of [^{3}H] Glu from [^{3}H] NAAG in a Tris-Cl buffer, followed by resolution of product and substrate by anion exchange chromatography.

The accompanying table summarizes the results of these sequential purification steps. Maximal selective solubilization of NAALADase activity was achieved at a Triton X-100 : protein ratio of 10:1. The peptidase eluted in the void volume of the anion exchange column and at 0.25 M NaCl from the cation exchange column suggesting that NAALADase has an unusually high isoelectric point.

	Purification Factor	% Yield
Crude Homogenate	1	100
Lysed Synaptosomal Mem.	4.1	69
Triton X-100 Extract (0.5 %)	9.7	73
DEAE-Sepharose	96	77
CM-Sepharose	950	32

Preliminary data from a Sepharose size exclusion column suggest a molecular weight of 180 kDa. Silver stained SDS gels of fractions surrounding the NAALADase peak of activity from a CM column and material from each purification step show enrichment of a 90 kDa band.

Under these chromatographic conditions only one peak of activity was observed; futhermore, this activity was quisqualate-sensitive, as was found in lysed synaptosomal membranes. These data support the hypothesis that this quisqualate-senstive activity is responsible for NAAG catabolism *in vivo* and may function to inactivate neurotransmitter pools of NAAG. [Supported by USPHS # NS 13584 and a postdoctoral fellowship (# 07870) to MBR]

210.19 MECHANISM OF ANTICONVULSANT AND CEREBROPROTECTIVE ACTIVITY OF U-50488H and U-54494A. M.Camacho Ochoa, T.A. Jackson*, C. S. Aaron* and P.F. Von Voigtlander. The Upjohn Company, Kalamazoo, MI 49001.

Recent investigations have linked excitatory amino acids to the etiology of epilepsy and other neurodegenerative diseases. We have previously showed that U-50488H blocks (H3)Kainic acid ((H3)KA) binding in the presence of Ca++ and Cl- ions.

We have used 45Ca++ uptake, 2-Deoxyglucose (2-DG) uptake, (H3)KA binding and KA-induced lesions in the mouse hippocampus to study the mechanisms underlying the anticonvulsant and cerebroprotective activity of U-50488H and U-54494A, a diamine analogue of U-50488H. The results revealed that the calcium uptake in K+ depolarized synaptosomes was significantly reduced by these two compounds only at concentrations of 10^{-4} - 10^{-6}M. (H3)KA binding was significantly decreased in the presence of Ca++ and Cl- ions in a mouse synaptic membrane preparation. Both the total specific and the high affinity binding were decreased when similar concentrations of these two compounds were used (10^{-4} to 10^{-5}M). The KA-induced 2-DG uptake increase in various regions of mouse brain was minimally affected by U54494A and U-50488H. Cerebroprotective activity was measured by image analysis as the decrease of area of vacuolization in the CA3 region of the mouse hippocampus lesioned with intracerebroventricular KA. Both compounds afforded a high degree of protection.

Hence, although the biochemical data on calcium uptake, (H3)KA binding and 2-DG uptake do not provide us with a precise cellular mechanism by which these compounds protect the brain, the morphological data clearly suggest that a cytoprotective mechanism is at play. This may be related to the anticonvulsant activity of U-50488H and U-54494A.

210.20 NETWORK BURSTS TRIGGERED BY SINGLE CELL STIMULATION IN THE RAT HIPPOCAMPUS. R.S. Neuman, E. Cherubini and Y. Ben-Ari. INSERM U.29, 123 Bd. de Port-Royal, 75014 Paris, France.

In the present experiments we examined the action of N-methyl-D-aspartate (NMDA) and low Mg2+ on burst generation in CA3 neurones.

Transverse hippocampal slices (500 μM) from 100-150 gram rats were used. Standard intracellular and extracellular recordings from CA3 pyramidal neurones were obtained from submerged slices.

Superfusion of slices with Mg2+ free medium or NMDA (5-10 μM) first induced endogenous (pacemaker) bursts followed by network driven bursts. Endogenous and network bursts were readily distinguished as the former were not accompanied by burst in the extracellular recordings. Furthermore, hyperpolarization abolished endogenous but not network bursts.

As the endogenous bursts always preceeded the network bursts we wondered if the former triggered the later. More specifically, did any CA3 neurone, impaled at random, possess the appropriate follower circuitry to trigger a population response. To test this hypothesis, bursts of action potentials were produced by current pulses applied to the intracellular electrode. As expected, in control medium, this single cell stimulation did not induce network bursts. However, in Mg2+ free medium activating a single neurone could trigger network bursts which were evident both in the intracellular and extracellular recordings. CsCl filled electrodes were more effective in triggering bursts, as Cs+ blocked the K+ conductance and thereby prolonged the directly evoked spike discharge.

From our observations we conclude that there is no requirement for a special group of neurones to act as "pacemakers" for burst production. Instead, any neurone (at least in CA3) can trigger a network burst providing the excitability of the tissue is such that the appropriate follower circuitry is activated.

R.S.N. was the recicipent of a Canadian MRC Traveling Scientist Award.

210.21 **EXCITATORY AMINO ACIDS IN THE RETINO-TECTAL SYSTEM OF XENOPUS LAEVIS.** B.E.S. Fox and S.E. Fraser. Dept. of Physiol. Biophys., Univ. of Calif, Irvine, CA 92717.

Glutamate (GLUT) has been proposed to be the major excitatory transmitter in the CNS of mammalian and nonmammalian species. Two classes of receptors have been characterized by the artificial agonists, either kainic acid (KA) or n-methyl-d-aspartate (NMDA), which selectively bind them. In some cases, KA is such a potent agonist that neurotoxicity results from excessive membrane depolarization (Olney, Rhee & Ho, 1974, Brain Res., 77, 507).

We have investigated whether GLUT acts as a transmitter in the Xenopus retino-tectal system in either young adults or stage 47-50 tadpoles. Laminar field potentials were recorded with a saline filled micropipet in the optic tectum and retina in response to a flash of white light (200ms duration). Evoked potentials 75-90% greater than noise level were recorded from each animal prior to drug administration. GLUT, KA, NMDA, and the NMDA specific antagonist, amino-phosphono-valeric acid (APV) were administered by squirting small volumes (0.002-1ml) of the drugs onto the exposed tectal surface of the adults or bath applying it to the tadpoles.

The agonists and antagonists both blocked the evoked tectal laminar field potentials. This was the result of disruption of local membrane potential shifts by either continuous depolarizations caused by the agonists or elimination of membrane potential shifts by the antagonists. GLUT (5mM,) and KA (10um) completely blocked the evoked field potentials in the adults in 1-5min and 10-15 min respectively. APV (5mM) however, blocked only 60% of the response in 15-20 min. In tadpoles, 300uM APV blocked 55-70% of the response in 15-30min. 100uM APV blocked 40-70% of the response in 10-20min, and 10uM APV blocked 50-70% of the response in 35-40min. 100uM NMDA completely blocked the response within 2 min, while 10uM NMDA had no effect. 100uM KA completely blocked the response within 5 min. 10 uM NMDA and 10uM KA, when microinjected into the cranial cavity, blocked the reponse within 1 min. Retinal field potentials were maintained in all animals up to 1 hr following drug administration.

The effects of KA are reversible. Retinal and tectal potentials could be evoked in tadpoles which received 10uM KA injections 5-24 hrs earlier. An 80uM KA injection also failed to block the response when tested 24 hrs. later. The LD-50 for this drug, when injected into the cranium, was determined to be 50uM.

We suggest, based on the actions of the excitatory amino acids tested, that GLUT is the primary transmitter of the Xenopus retino-tectal system. In light of the proposed role of GLUT/NMDA receptors in longterm changes in synaptic strength and its presence in this system, we suggest that GLUT/NMDA receptors may be involved in activity dependent retinotectal patterning. (Supported by NSF.)

OPIATES, ENDORPHINS AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS II

211.1 MORPHINE EFFECTS ON LOCUS COERULEUS NEURONS ARE DEPENDENT ON THE STATE OF AROUSAL AND AVAILABILITY OF EXTERNAL STIMULI: STUDIES IN ANESTHETIZED AND UNANESTHETIZED RATS.
Rita J. Valentino and Richard Wehby*. Department of Pharmacology, George Washington University, Washington, D.C. 20037

Since the physiological characteristics of noradrenergic locus coeruleus (LC) neurons differ in anesthetized and unanesthetized rats, the effects of morphine (MOR) on LC activity recorded in both conditions were compared. Intracerebroventricular (i.c.v.) administration of MOR inhibited spontaneous LC discharge in both halothane-anesthetized and un-anesthetized rats. However, MOR was at least 10 times more potent in anesthetized rats suggesting that the state of arousal can affect the potency of opiates on LC activity. LC discharge in anesthetized rats was also evoked by repeated presentation of sciatic nerve stimulation (footshock). This stimulation resulted in a consistent pattern of LC discharge characterized by a brief period of excitation (80 - 100 msec duration) occurring shortly after the stimulus (10-20 msec), followed by a longer duration postactivation pause. The excitatory component of this response was insensitive to doses of MOR (0.03 or 0.1 ug) that inhibited spontaneous discharge recorded during the presentation of stimuli. Only the highest dose of MOR (0.3 ug) which completely inhibited tonic activity significantly decreased evoked discharge. In parallel experiments the presentation of auditory stimuli to unanesthetized rats evoked a pattern of LC discharge similar to that evoked by sciatic nerve stimulation in anesthetized rats. While MOR (1.0 and 3.0 ug) decreased both tonic and evoked discharge in these rats, tonic LC discharge was more sensitive to MOR as was observed with anesthetized rats. For example 3.0 ug MOR significantly decreased tonic LC discharge but not discharge evoked by auditory stimuli. In some LC neurons recorded in both anesthetized and unanesthetized rats spontaneous discharge which was completely inhibited by MOR could still be evoked by presentation of sensory stimuli. Quantitative analyses of these effects indicated that MOR tends to alter the temporal pattern of LC discharge to sensory stimuli such that the signal-to-noise ratio (ratio of evoked:tonic activity during stimulus presentation) is increased. MOR effects were reversed by naltrexone, 1.0 mg/kg, s.c., in anesthetized rats, and 1.0 ug i.c.v., in unanesthetized rats. The present results indicate that the degree of arousal and the availability of environmental stimuli are important determinants of opiate effects on LC cells and can antagonize the effects of opiates on these neurons. This work was supported by PHS Grants DA03695 and MH40008.

211.2 INTRACELLULAR GTPγS RESTORES MORPHINE HYPERPOLARIZATION OF RAT LOCUS COERULEUS (LC) NEURONS AFTER BLOCKADE BY PERTUSSIS TOXIN.
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Pertussis toxin, an inactivator of certain G proteins, is widely used as a tool to study the link between receptors and the class of G proteins which are substrates for this toxin (e.g., G_i, G_o, G_t). A previous study on noradrenergic neurons of the LC has shown that pertussis toxin blocks the hyperpolarizing effect of the opiate agonist morphine (Aghajanian and Wang, Brain Res., 371:390, 1986). In the present study, we examined whether the hyperpolarizing effect of morphine on LC neurons could be restored in brain slices from pertussis toxin-pretreated rats by intracellular guanosine 5'-O-(3-thiotriphosphate) (GTPγS), a hydrolysis resistant analog of GTP.

Male albino rats were injected in vivo with pertussis toxin (1 μg in 5 μl) or vehicle in a lateral cerebral ventricle; LC slices were obtained for recording 1-5 days later. Slices were perfused with artificial CSF composed of (in mM): NaCl, 126; KCl, 5.0; Na_2HPO_4, 1.25; D-glucose, 10; $NaHCO_3$, 26; $CaCl_2$, 2.5; $MgSO_4$, 2.0 (95% O_2/5% CO_2). Intracellular recordings were performed using an Axoclamp-2 and low resistance electrodes (15-20 MΩ) filled with 2M KCl, or 2M KCl with 2 mM GTPγS or 2 mM GTP.

As previously reported, when electrodes containing 2 M KCl were used, the hyperpolarizing effect of morphine on LC neurons was blocked in slices from pertussis toxin-pretreated rats; only a partial suppression of firing rate could be seen. In the same slices, when electrodes containing 2 mM GTPγS were used, the ability of morphine to hyperpolarize LC neurons was restored and responses were similar in magnitude to those in control slices using 2 M KCl electrodes. Morphine responses obtained with GTPγS containing electrodes were not reversible by drug washout. GTPγS (2 mM) alone also caused a hyperpolarization of LC neurons in slices from pertussis toxin-treated rats, but the response developed much more slowly than in the presence of morphine. GTP (2 mM), unlike GTPγS, did not restore the effect of morphine in slices from pertussis toxin-treated rats, nor did it alone have any effect on the LC neurons from either pertussis toxin-treated or control rats.

As the hyperpolarizing effect of morphine on LC neurons was restored by intracellular GTPγS but not GTP, we conclude pertussis toxin impairs receptor-mediated activation of G protein(s) by GTP but not GTPγS. This would suggest that coupling between opiate receptors and G protein(s) can still occur even after pertussis toxin treatment.

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211.3 μ, δ, AND κ OPIOID ACTIONS IN THE RAT HIPPOCAMPUS. J. F. Neumaier and C. Chavkin. Dept. of Pharmacology, University of Washington, Seattle, WA 98195.

The purpose of this study was to characterize the electrophysiological effects of opioids on dentate granule cells, to determine which opioid receptor types mediate these actions, and to determine whether the mechanism of opioid action in the dentate gyrus is similar to that observed in the CA1 region. Extracellular recordings were made from rat hippocampal slices; dentate population spikes were evoked by medial perforant path stimulation and CA1 population spikes were evoked by Schaffer collateral stimulation. Selective opioid agonists were applied either by bath superfusion or pressure micropipette, and opioid antagonists (naloxone or ICI-174,864) were applied by superfusion. Agonist dose-response curves were constructed and antagonist K_e values were determined by Schild analysis.

Opioids had similar actions in the CA1 and dentate regions, although some differences were apparent. Biphasic stimulus-response (S-R) curves were detected in the dentate gyrus; we interpret these to have been due to the recruitment of inhibitory inputs as stimulus intensity was increased. Opioids induced a change from a biphasic to a sigmoidal S-R curve in a reversible and naloxone-sensitive manner. Unlike the case in CA1, opioids did not shift the S-R curve to the left in dentate recordings. In the dentate region, μ, δ, and κ agonists all increased cell excitability in a manner which resembled the action of bicuculline; therefore, opioids appear to act via disinhibition in the dentate gyrus, as they do in CA1. Opioids also induced secondary afterpotentials in both CA1 and dentate recordings; this is also consistent with a disinhibitory mechanism.

The potencies of bath applied selective agonists suggested that μ, δ, and κ receptors were activated in the dentate gyrus, but only μ and δ receptor mediated opioid actions were observed in CA1. The μ agonist PL017 induced a greater response than the δ agonist DPLPE in both the CA1 and dentate regions. DSLET induced a complete response in both regions; however, the dose-response curves were shallow, suggesting that both δ and μ receptors were activated. The δ selective antagonist ICI-174,864 blocked DPLPE actions with a K_e in the low nanomolar range, but did not antagonize PL017. Dynorphin-A(1-17) (DYN) also had a shallow dose response curve in the dentate gyrus; this result is consistent with activation of both κ and μ receptors by DYN. In CA1, DYN had only low potency actions via μ receptors with a dose-response curve of normal steepness and a naloxone K_e of 1 nM. U-50,488H and tifluodom had low potency in both dentate and CA1 regions and sometimes produced depressions of the primary spike. We interpret these data to suggest that κ receptors can be activated in the dentate gyrus, but that maximal activation of the κ receptors induced a lesser response than μ receptor activation.

Preliminary evidence suggests that naloxone may intensify the biphasic nature of dentate S-R curves; this would suggest that endogenous opioids play a role in modulating the inhibition induced at moderately high stimulus intensities. The presence of functional μ, δ, and κ receptors in the rat dentate gyrus and the demonstration of a naloxone sensitive component of perforant path transmission suggests that enkephalin and dynorphin peptides may act as neurotransmitters in the dentate gyrus. This work was supported by NS23483, GM07266, and the Poncin Fund.

211.4 **EFFECTS OF DYNORPHIN ON EXCITABILITY OF CA1 AND CA3 PYRAMIDAL CELLS IN RAT HIPPOCAMPAL SLICES.** S.B. Ryan* and H.C. Moises. Dept. of Physiology, University of Michigan, Ann Arbor, MI 48109.

In keeping with the immunohistochemical localization of dynorphin peptides within the mossy fiber projection from dentate granule neurons to hippocampal pyramidal cells (HPCs) in CA3 is the recent demonstration of calcium dependent release of endogneous dynorphin from rat hippocampal slices. Electrophysiological studies, however, have failed to establish a clear role for these peptides in hippocampal synaptic transmission. To address this issue we sought to determine the actions of dynorphin on the resting membrane properties and excitability of HPCs in vitro.

Intracellular recordings were obtained from pyramidal cells in the CA1 and CA3 cellular fields of rat hippocampal slices maintained under submersion. Dynorphin A1-17 (DYN) was applied to the slice by addition to the perfusate (concentrations of .1nM to 1μM) or by pressure ejection through a micropipette (containing 1 to 100 μM DYN) positioned immediately above the stratum radiatum. Application of DYN had no consistent effect on resting membrane properties (membrane potential, input resistance, rheobase) in the majority of cells tested. In the cells which did respond to exogenous DYN application, the predominant action of the peptide was manifested by a change in both spontaneous and current evoked action potential discharges. These effects were characterized by: 1) an elicitation of more spikes in response to depolarizing current injection through the electrode, and 2) a shift in spontaneous firing from single spikes to bursts, accompanied by the appearance of pronounced after-hyperpolarizations (AHPs) which sometimes gave way to depolarizing afterpotentials.

To test whether DYN might directly enhance neuronal burst generating properties, we examined calcium action potentials (recorded during superfusion of media containing 1μM TTX and 5 mM TEA) in the absence and presence of the peptide. TTX-resistant calcium spikes were elicited in 12 HPCs by injecting depolarizing current (400 msec, .5-2 nA) through the recording electrode. DYN (100nM) appeared to potentiate calcium spikes in 3 of the 12 neurons as evidenced by decreases in spike latency, increases in spike amplitude, and enhancement of spike AHPs. These changes in calcium spike and AHP morpology were not associated with any consistent changes in resting membrane potential.

The present data suggest that DYN might exert subtle effects on neuronal excitability which could be important in regulating the efficacy of mossy fiber input to CA3 pyramidal cells. (Supported by NIDA grant DA03365 and a career development award to H.M. from the Chicago Community Trust/Searle Scholars Program).

211.5 A MU-SPECIFIC OPIATE AGONIST, D-ala^2-mePhe^4-gly-ol^5, INCREASES EXCITABILITY OF HIPPOCAMPAL PYRAMIDAL NEURONS. A.M. Moudy and M.B. Laskowski. Dept. of Physiology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

The rat hippocampus contains all major subtypes of opiate receptors, mu, delta and kappa. Previous studies have demonstrated that delta-selective ligands, such as [D-Ala^2, D-Leu^5] enkephalin, (DADL), and generally mu-specific ligands, such as morphine and morphiceptin increase the excitability of hippocampal pyramidal cells. Here we report the effects of a highly mu-specific opiate peptide D-ala^2-mePhe^4-gly-ol^5 (DAGO), on the electrical activity of pyramidal cells in the CA1 region of the hippocampus. Slices of the hippocampus were perfused with artificial cerebrospinal fluid while stimulating afferents in the Schaffer collaterals and while recording population spikes in stratum pyramidale and EPSPs in stratum radiatum. Superfusion of slices with DAGO produced a concentration - dependent increase in the amplitude of population spikes recorded in stratum pyramidale. No change was observed in the corresponding EPSP slope recorded simultaneously in stratum radiatum. This selective increase in population spike amplitude leads to a left - shift in the derived input - output curve. At a concentration of 10-6M, DAGO - perfused slices showed an average population spike output 20% greater than control slices in response to the same EPSP input. In addition, DAGO superfusion led to extra spiking at higher stimulus intensities. Naltrexone reversed the DAGO - induced increase in excitability, as well as prevented additional spikes. This supports the conclusion that opiate receptors mediate the observed response. The results also suggest that the excitatory response to mu receptor - specific opiate peptides in the hippocampus is not dependent upon extra - hippocampal input but is a result of changes in activity within intrinsic neuronal circuitry. Moreover, it suggests that the increased population spike is independent of enhanced transmitter release by afferents to pyramidal cells. (Supported in part by NSF, NIH and the American Cancer Society)

211.6 ENKEPHALIN EFFECTS IN THE CA3 REGION OF THE RAT HIPPOCAMPUS IN VIVO. B.E. Derrick *, S.T. Cunningham* and J. L. Martinez, Jr. (SPON: H. Rigter). Dept. of Psychology, Univ. of Calif., Berkeley, CA 94720

Enkephalins excite hippocampal pyramidal cells, in contrast to their depressant effects in most other CNS regions. Studies of evoked responses, primarily in the CA1 region in vitro, show that enkephalins produce an increase in the amplitude and number of population spikes (PSs), without an increase the field EPSP.

In the CA3 region, an area not extensively studied, there are circumscribed opioidergic pathways. We assessed the effects of enkephalins in the CA3 region in vivo. Bipolar nichrome stimulating electrodes were placed stereotaxically in anesthetized (urethane, 1 g/kg) male Sprague-Dawley rats in the hilus of the dentate gyrus (AP -5.0, ML 2.9, DV -3.5). A cannula adjacent to a nichrome recording electrode was placed in the CA3 pyramidal layer (AP -3.5, ML, 2.9, DV -3.7). Following equilibration (1 hr) and collection of baseline data (30 min), [leu]enkephalin (LE, 100 μM or 100 mM) or D-ala^2-[D-leu^5]enkephalin (DADLE, 10 or 100 μM) was applied (0.5 μl) via pressure-ejection into the CA3 pyramidal layer. Test pulses were collected for 2 hrs, followed by a second application of the same drug. Responses to paired pulses were taken before the first, and after the second, drug application.

The primary PS increased from 30 - 350% following application of DADLE (10 μM or 100 μM) or LE (100 mM), and in 2 of 4 rats given 100 μM LE. Double PSs were seen in only 4 of 18 drug-treated animals. CA3 population EPSPs increased as much as 100% following either DADLE (10 μM and 100 μM) or LE (100 mM), and in 2 of 4 rats given 100 μM LE. These effects were of short duration (less than 10-15 min).

DADLE enhanced paired-pulse facilitation at inter-stimulus intervals (ISIs) of 50-300 msec in 6 of 10 rats; this effect was not seen with LE. At ISIs of less than 50 msec, the usual attenuation of the PS amplitude following the second pulse was either unchanged or enhanced by both LE (4 of 6 animals) and DADLE (6 of 8 animals).

These results suggest that the effects of enkephalins in the CA3 region are different from those observed in the CA1 region, in terms of both the increase in population EPSPs and the lack of attenuated PSs in the second response at paired-pulse ISIs of less than 50 msec. These findings suggest that opioids may act through mechanisms other than disinhibition in the CA3 region.

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211.7 SPECIFIC OPIOIDS CAN PROLONG THE CALCIUM COMPONENT OF ACTION POTENTIALS OF MOUSE DORSAL ROOT GANGLION (DRG) NEURONS IN CULTURE* BY DECREASING VOLTAGE-SENSITIVE POTASSIUM CONDUCTANCE. K.-F. Shen & S.M. Crain. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Prolongation of the duration of the calcium component of the action potential (APD) occurs in about 1/3 of the DRG neurons in mouse DRG-spinal cord explants when exposed acutely to the opioid peptide, D-ala-D-leu enkephalin (DADLE) at 1-10uM (in BSS with 5mM Ca, 5mM Ba), whereas the APD is shortened in about 50% of the DRG cells. Tests were made to determine if opioid-induced APD prolongations of DRG neurons are mediated by activation of specific excitatory opioid receptor subtypes that result in an increase in voltage-sensitive Ca-conductance or a decrease in K-conductance. Recordings were therefore carried out under conditions where most types of K-channels were blocked: 5-10mM TEA, 5mM Ba, no Ca in bath; 2M CsCl in recording microelectrode. Under these test conditions, only 1 out of 13 DRG neurons (8%) in DRG-cord explants showed APD prolongation in 1-10uM DADLE, while 11 out of 13 cells (84%) showed APD shortening. Similar attenuation of DADLE-induced APD prolongation was observed in chronic DADLE-treated DRG neurons tested in the presence of K-channel blockers (Crain et al, SN Abstr. 13, ´87).

The results suggest that DADLE-induced APD prolongation of DRG neurons is mediated by excitatory opioid receptor subtypes that decrease a voltage-sensitive K-conductance. Our data also suggest that the DADLE-induced APD shortening which is unmasked during more complete K-channel blockade in DRG neurons is mediated by inhibitory opioid receptor subtypes in the same cells that reduce a voltage-sensitive Ca-conductance. These inhibitory effects were mimicked by low concentrations (1-10nM) of the kappa agonist, dynorphin (1-13), which shortened the APD of most neurons, when tested in Ca, Ba/BSS as well as in the presence of additional K-channel blockers. In contrast to dynorphin, the specific mu and delta opioid agonists, DAGO and DPDPE, appeared to be more selective in eliciting APD prolongations in DRG neurons when applied at low concentrations (1-10nM in Ca, Ba/BSS) that were subthreshold for initiating inhibitory effects (see also Chen et al, SN Abstr. 13, ´87).

The APs of some sensory neurons may therefore be modulated by multiple subtypes of both excitatory and inhibitory opioid receptors functioning within the same cell. These opioid receptor subtypes appear to be coupled to voltage-sensitive K- and/or Ca-conductances via specific GTP-binding proteins in the DRG neurons (Chen et al., ibid.; Shen et al., SN Abstr. 12:1011, ´86). (Supported by grant DA-02031 to S.M.C.)

211.8 INHIBITOR OF CYCLIC AMP-DEPENDENT PROTEIN KINASE BLOCKS OPIOID PROLONGATION OF THE ACTION POTENTIAL OF DORSAL ROOT GANGLION (DRG) NEURONS. G.-G. Chen*, A. Chalazonitis, K.-F. Shen* & S.M. Crain, (SPON: S. Green). Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Application of the opioid peptide, D-ala-D-leu enkephalin (DADLE; 1-10uM), to mouse DRG-spinal cord explants prolongs the duration of the calcium component of the action potential (APD) in about 35% of the DRG neurons and shortens the APD in about 50% of these cells (Shen & Crain, SN Abstr. 13, ´87). In order to determine if the opioid prolongations of the APD are mediated by a cAMP second messenger system, opioid responsivity tests were made on DRG neurons after intracellular application of a protein inhibitor of cAMP-dependent protein kinase (PKI). Monolayer cultures of dissociated fetal mouse DRG neurons were used so that neuron perikarya were accessible to whole-cell patch recording pipettes and intracellular dialysis. The cultures were maintained for 3-5 wks in media modified from that used for the DRG-cord explants.

Control tests were made (current clamp recordings) on 15 DRG neurons (in 6 cultures) via patch pipettes filled with, in mM: 140 K-gluconate, 2$MgCl_2$, 1.1EGTA, 5HEPES. Bath perfusion of 1uM DADLE (in BSS with 5mM Ca, 5mM Ba) prolonged the APD of 5 DRG neurons and shortened the APD of 7 neurons (similar to the effects of DADLE in DRG explants). These changes were reversible by washing. In contrast, in a matched group of 21 DRG neurons (in 5 cultures) tested with similar patch pipettes containing PKI (Sigma, 0.1-1ug/ml), none of the cells showed APD prolongations in 1uM DADLE, whereas the APDs of 50% of the cells were still shortened.

More DRG neurons responded with APD prolongations at lower concentrations of DADLE (as in adult nodose ganglia: Higashi et al, ´82). In a control group of 19 neurons (in 8 cultures), 10nM DADLE prolonged the APD in 16 neurons (reversible by washing) and none showed APD shortening. In a matched group of 12 DRG neurons (5 cultures), when PKI was applied via patch pipettes (1 ug/ml), again none of the cells showed DADLE-induced APD prolongation (nor shortening). The APD prolongations elicited by 10nM DADLE were not reversed by naloxone (3-10uM; n=5). However, the increased proportion of DRG neurons showing these excitatory responses: 84% at 10nM vs. 34% at 1 uM suggests mediation by opioid receptors (see also Shen & Crain, ibid.).

These preliminary data suggest that DADLE-prolongation of the APD of mouse DRG neurons may be mediated by excitatory opioid receptor subtypes that are positively coupled via G_s to the adenylate cyclase/cAMP second messenger system (see also Crain et al, SN Abstr. 13, ´87). The elevated levels of cAMP may in turn result in a decreased voltage-sensitive K-conductance (Shen & Crain, ibid.) or an increased Ca-conductance (Reuter, ´83). (Supported by grant DA-02031 to S.M.C.).

211.9 OPIOIDS EXCITE RATHER THAN INHIBIT SENSORY NEURONS AFTER CHRONIC OPIOID EXPOSURE OF MOUSE DORSAL ROOT GANGLION (DRG)-SPINAL CORD EXPLANTS. S.M. Crain, K.-F. Shen* & A. Chalazonitis. Dept. of Neuroscience, Albert Einstein Coll. Medicine, Bronx, N.Y. 10461

Tests were carried out to determine if the tolerance that develops in dorsal-horn network responses of DRG-spinal cord explants after chronic exposure to opioids (Crain et al. Life Sci. 25,´79) could be due to alterations in the excitability and responsivity of the afferent DRG cells. Intracellular recordings were made from DRG neurons in fetal mouse DRG-cord explants after chronic treatment with 1 uM D-ala-D-leu enkephalin (DADLE) for >3 days starting at 2 wks in vitro. Acute application of 10 uM DADLE (in BSS with 5 mM Ca, 5 mM Ba) shortened the duration of the Ca component of the somatic action potential (APD) in only 5% of the treated neurons, in contrast to 50% of the cells in naive explants (Chalazonitis & Crain, Neurosci. 17, ´86). Furthermore, 77% of the treated cells showed prolongation of the APD in response to increased DADLE concentration vs 36% in naive explants. However, when the DADLE-responsivity tests were carried out in the presence of multiple K-channel blockers (5-10 mM TEA, 5 mM Ba, no Ca in bath; 2 M CsCl, in recording microelectrode), only 13% of the chronic DADLE-treated DRG neurons (2 out of 15 cells) showed APD prolongation and 80% showed APD shortening (as observed in naive DRG neurons: Shen & Crain, SN Abstr. 13, ´87). The results suggest that: 1) DADLE-induced APD-prolongation of the treated DRG neurons is mediated by excitatory opioid receptor subtypes that decrease a voltage-sensitive K-conductance; 2) DADLE-induced APD-shortening effects which are unmasked during more complete K-channel blockade are mediated by inhibitory opioid receptor subtypes in the same neurons that reduce voltage-sensitive Ca-conductance (resembling kappa-opioid effects of 1-10 nM dynorphin on these cells).

The decreases in the proportion of DRG neurons showing opioid-inhibitory responses and increases in opioid-excitatory responses after chronic opioid exposure are remarkably similar to the effects of forskolin or pertussis toxin treatments (Shen et al, SN Abstr. 12:1011, ´86) and suggest that these plastic cellular alterations may be mediated by related mechanisms. Similar increases in excitatory vs. inhibitory opioid receptor functions may also occur at central presynaptic terminals of DRG neurites during chronic opioid exposure, thereby enhancing transmitter release. These in vitro studies may provide clues to compensatory processes (perhaps mediated by enhanced cAMP levels; see Chen et al, SN Abstr. 13, ´87) that could attenuate or block opioid-inhibitory effects on primary afferent synaptic networks in the spinal cord and account for some of the hyperexcitability properties associated with opioid tolerance and dependence.

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211.10 SEROTONIN RECEPTOR SYSTEMS IN SPINAL CORD AND SENSORY GANGLIA: RELATION TO OPIOID ACTION, TOLERANCE AND CROSS TOLERANCE. X.-C. Qiu*, S.M. Crain and M.H. Makman* (SPON: R.W. Ledeen). Depts. of Biochemistry, Neuroscience and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461

We have examined cellular mechanisms of opioid analgesia, tolerance and dependence in fetal mouse spinal cord-dorsal root ganglia (DRG) explant cultures as well as in fetal and adult mouse and rat tissues obtained directly from the animal. We previously found that opioids acutely depressed sensory-evoked dorsal-horn synaptic network responses of explant cultures. Chronic exposure of cultures to opioids attenuate these depressant responses, a phenomenon that may correspond to tolerance that develops in vivo; chronic exposure to opioids also attenuated responses to serotonin (5HT), possibly representing a type of "cross tolerance". The acute depressant effect of opioids was attenuated by cyclic AMP analogs, forskolin (an activator of adenylate cyclase (AC)) and by treatment of cultures with pertussis toxin (PTX). PTX inactivates certain guanine nucleotide regulatory proteins, e.g. the G_i protein that links opioid and $5HT_{1A}$ receptors to AC. We find that forskolin-stimulated AC of explants is inhibited by opioids or $5HT_{1A}$ receptor agonists. Chronic exposure of explants to morphine attenuates opioid inhibition and enhances total activity of AC; PTX treatment attenuates both opioid and 5HT inhibition and enhances total AC.

In addition to $5HT_{1A}$ receptor-mediated inhibition of AC, cord-DRG cultures contain $5HT_{1A}$ binding sites labeled by the selective ligand ^{3}H-8-OH-2-(di-n-propylamino) tetralin (DPAT) (B_{max}: 32 fmoles/mg protein; K_D:1.1nM). High affinity ^{3}H-DPAT binding sites were also found in spinal cord of adult rats (B_{max}: 75 fmoles/mg; K_D:4.5 nM). In rat cord ^{3}H-5-HT itself was found to label both $5HT_{1A}$ sites sensitive to DPAT (B_{max}: 75 fmoles/mg) and additional sites characterized as $5HT_{1C}$ on the basis of sensitivity to mianserin (K_i:5.5 nM in the presence of 10^{-7} M DPAT) as well as insensitivity to DPAT and $5HT_{1B}$ agonists. B_{max} for the mianserin-sensitive component of ^{3}H-5HT binding was 104 fmoles/mg. In order to explore possible mechanisms of cross-tolerance, $5HT_{1A}$ receptors were assessed with 1.2 nM ^{3}H-DPAT as ligand in control cultures and in cultures exposed to 1 μM morphine for 7 days. Specific binding was significantly lower in the treated cultures (9.0 ± 3.0 fmoles/mg) compared with the control (23.1 ± 5.9 fmoles/mg) (n=6). A possible influence of morphine on $5HT_{1A}$ receptor-mediated inhibition of AC remains to be determined. On the basis of the binding studies it is postulated that cross-tolerance to 5HT following morphine treatment involves alteration of a pool of G_i coupled to either opioid or to $5HT_{1A}$ receptors and utilized in common for transduction of morphine and 5HT actions. (AG-00374 and DA-02031)

211.11 OPIOID RECEPTOR SUBTYPES INVOLVED IN THE INHIBITION OF DEPOLARIZATION-INDUCED NA AND ACH RELEASE FROM SLICES OF DIFFERENT RAT BRAIN REGIONS. A.L. Frankhuijzen, F.P.Jansen, A.N.M.Schoffelmeer and A.H.Mulder (SPON: ENA).Department of Pharmacology, Medical Faculty, Free University. v/d Boechorststraat 7, 1081 BT Amsterdam. One of the possible functional consequences of activation of opioid receptors at the cellular level appears to be presynaptic modulation of neurotransmitter release. Recently, we have shown that selective activation of different opioid receptors may in fact differentially modulate neurotransmitter release in a particular brain region. Thus, the depolarization-induced release of (3H)DA and that of (14C)ACh from striatal slices were inhibited selectively by activation of k and δ-opioid receptors respectively, whereas activation of μ-receptors had no effect. However, μ-opioid receptor activation inhibited the release of (3H)NA from cortex, whereas activation of δ or k-receptors was ineffective. In the present study we examined the effects of opioid receptor activation on the release of (3H)NA and (14C)ACh from slices of the amygdala.

Similar to the results obtained in cortex the release of (3H)NA from amygdala slices was inhibited (by 45%) by the selective μ-opioid receptor agonist morphine (pD2 value of 6.50), whereas the highly selective δ-opioid receptor agonist DPDPE (D-Pen2-D-Pen5 enkephaline) and the k-opioid receptor agonist bremazocine were ineffective. However, in contrast to previous results obtained with rat striatal slices, the release of (14C)ACh from the amygdala was not affected by selective δ-opioid receptor activation using DPDPE. Only DADLE and morphine were active (77% and 64% inhibition resp.) in this respect (resp. pD2 values of 7.35 and 6.60). Furthermore, naloxone potently antagonized the effects of morphine and DADLE on the release of both NA and ACh (pA2 value of 8.75 irrespective of the agonist used), while FIT (Fentanyl isothiocyanate), a highly selective irreversible δ-opioid receptor agonist, was ineffective in this respect. However, the release of (14C)ACh from cortex slices was not affected by any of the opioid receptor agonists used.

It is concluded that the opioid receptors involved in the modulation of the release of both NA and ACh in the amygdala are of the μ-type. Moreover, the data indicate that a specific subtype of opioid receptors is not necessarily connected with a specific neuronal system but that regional differences may exist.

211.12 EFFECT OF OPIATES ON THE ACTION POTENTIAL OF NEUROBLASTOMA CELLS. J.W.Day. Department of Neurology, University of California School of Medicine, San Francisco, California 94143.

Opiates hyperpolarize, or shorten the action potentials (APs) of neurons. The hyperpolarization is due to increased potassium conductance (g_K); in dorsal root ganglion (DRG) cells APs are shortened by either increasing g_K (mu- and delta-receptors), or decreasing the calcium conductance (g_{Ca}, kappa-receptors). Opiate receptors have been thoroughly studied biochemically in neuroblastoma cells, specifically the neuroblastoma x glioma hybrid NG108-15. Recent reports show that delta-receptors on these cells decrease g_{Ca}. I have done constant-current experiments on NG108-15 cells to further delineate the effects of opiates on these cells.

NG108-15 cells, plated on coverslips, were studied 5-10 days after inducing differentiation by adding 1mM dibutyrl cAMP or 1mM sodium butyrate to conventional medium. For physiological recordings, cells were continuously perfused with HEPES-buffered saline containing 20mM tetraethylammonium (TEA). Intracellular electrodes filled with 4M K Acetate had resistances of 40-60 MOhms; morphologically well differentiated cells, 40-75 microns in diameter, were studied. Agonist and antagonist solutions were applied from different barrels of a 3-barrel micropipette; micropipette tips were broken to 10 microns (single barrels of 5 microns). Micropipettes were positioned within 10 microns of the cell body, and drugs were ejected by applying 10-15 psi pressure. APs were evoked by anodal break, using the intracellular electrode to both stimulate and record. Voltage signals were digitized and analyzed by computer.

APs had rapid rise and fall times; the duration was greatly increased by the addition of TEA. A 2s application of D-Ala-D-Leu-Enkephalin (DADLE, 200nM in the pipette) caused a 50% diminution in AP duration, a 5% decrease in AP amplitude, and no change in resting potential, rise time or input resistance. APs recovered to near normal in 15 seconds, and fully within 2 min. Ejection of naloxone alone (10 microM in the pipette) did not affect the AP, but blocked the action of DADLE.

These results show that DADLE affects APs in NG108-15 cells much as it does in DRG cells. Whether this is solely due to direct changes in g_{Ca} remains to be determined. Also, this system will allow investigation of opiate receptor function following a prolonged exposure of the cells to ethanol or opiate agonists.

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211.13 EFFECT OF MORPHINE MODULATING PEPTIDE (FLFQPQRF-NH_2) ON ELECTROPHYSIOLOGICAL PROPERTIES OF CULTURED MOUSE SPINAL CORD CELLS. A. GUZMAN*, P.LEGENDRE*, F. DEMOSTES*, S. GEOFFRE*, G. PRECIGNIOUX*, J.D. VINCENT & G. SIMONNET*. I.N.S.E.R.M. U.176, 33077 BORDEAUX cedex France ; Lab. de Cristallographie. Univ. de Bordeaux I, TALENCE, France.

The morphine modulating peptide FMRF-NH_2 was originally isolated from ganglia of venous clam. More recently an octapeptide FLFQPQRF-NH_2 that crossreacts with an antiserum against FMRF-NH_2 was purified from bovine extract (Yang et al., PNAS, 82:7757, 1985). It was found to attenuate the prolongation of the tail-flick latency induced by morphine. The present investigation was done to study the effect of FLFQPQRF-NH_2 on the electrophysiological properties of cultured spinal cord neurones (Legendre et al.Neurosci., 16:753, 1985). Intracellular recordings were made using micropipettes filled with 3M KCl or 4M CH_3COOK. The peptide was applied by pressure from 1μm tip diameter micropipettes (10μM). Application of FLFQPQRF-NH_2 caused a hyperpolarization followed by a long lasting depolarization. This biphasic response was underlied by an increase of the input resistance. No clear reversal potential was observed. However the extrapolated reversal potential for the hyperpolarizing phase was close to -35 mV, while it was close to -90 mV for the deplolarizing phase. In the presence of $BaCl_2$ (6mM) the first phase of the response was increased and the second phase was suppressed. Extrapolated reversal potential of the hyperpolarization was then shifted to + 30 mV. Application of TEA (50mM) or 4AP (10mM) decreased the hyperpolarization and magnified the subsequent depolarization. Furthemore, $CoCl_2$ (3mM) reversibly blocked the hyperpolarization and decreased the amplitude of the subsequent depolarization. Our results suggest that FLFQPQRF-NH_2 has a neurotransmittor-like effect on mammalian spinal cord cells in culture. This biphasic response might be underlied by a decrease in calcium conductance and an increase in K^+ conductance, followed by a decrease in calcium-dependent K^+ conductance.

211.14 **OXYMORPHONE-NALTREXONAZINE, A POTENT MIXED AGONIST-ANTAGONIST.** S. Galetta*, J.A. Clark* and G.W. Pasternak [SPON: R. Price] The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Cornell U. Medical College, New York, NY 10021.

Previous studies from our laboratories have reported the synthesis and pharmacological characteristics of a series of symmetrical opiate azines: naloxonazine, oxymorphonazine and naltrexonazine. These compounds selectively and in a long-lasting manner label mu_1 sites both in vivo and in vitro. To examine the actions of the mixed agonist-antagonist azines of these 14-hydroxydihydromorphinones, we have now synthesized and characterized in binding assays and in vivo two asymmetrical azines: oxymorphone-naltrexonazine and oxymorphone-3-methoxynaltrexonazine. Oxymorphone-naltrexonazine, which theoretically could interact with the receptor as either an agonist or antagonist, displayed antagonist properties in vitro and in vivo. In binding studies, it had a sodium shift indicative of an antagonist. In vivo it antagonized morphine (5 mg/kg, sc) analgesia and had no analgesic activity administered alone. Previous studies have established that blockade of the hydroxyl group at the 3-position virtually destroys the ability of opiates to bind to the receptor. Therefore, oxymorphone-3-methoxynaltrexonazine theoretically can bind only as an agonist since the 3-position of the antagonist portion of the molecule has been substituted with a methoxy moiety. In binding studies, it possessed agonist properties and in vivo 1 mg/kg (sc) elevated tailflick latencies from a baseline of 1.9 * 0.2 to 9.3 * 0.9 sec. These results clearly indicate that the agonist portion of the molecule is capable of binding to the receptor as an agonist. In summary, a compound able to label a receptor as either anagonist possesses antagonist properties both in vivo and in vitro. Chemical inactivation of the antagonist portion of the molecule uncovers a potent agonist activity.

211.15 THE AMINOMORPHINONE DERIVATIVE RX77989 IS A POTENT, SHORT-ACTING OPIATE ANALGESIC. M.H. Ossipov, F. Rudo*, R.L. Wynn*, and T.C. Spaulding*. Anaquest/BOC Health Care, Murray Hill, NJ 07974 and Department of Pharmacology, School of Dentistry, University of Maryland, Baltimore, MD 21201.

The antinociceptive activity of RX77989 (14-β-pentylamino-morphinone) was assessed in standard analgesiometric procedures, including the mouse hot plate (MHP), mouse Haffner assay (MHA), rat hot plate (RHP), rat tail flick (RTF) and rabbit tooth pulp (RTP) tests. After intravenous administration, the potency of RX77989 ranged from 1.5 to 8.2 times that of fentanyl and from 296 to 2590 times that of morphine (see table).

	ED50 (mg/kg)		
Test	RX77989	Fentanyl	Morphine
MHP	0.0039	0.018	10.1
MHA	0.0014	0.01	2.22
RHP	0.0025	0.0086	2.77
RTF	0.0028	0.0043	1.11
RTP	0.0009	0.0074	1.11

The dose-response curves of RX77989, fentanyl, and morphine were parallel within each test.

The durations of the antinociceptive activity of RX77989 in the RHP and RTF tests ranged from 0.7 to 1.5 times that of fentanyl and were considerably shorter than those of morphine at several equi-effective doses.

After intramuscular injection, the ED50's of RX77989 were 0.010 mg/kg (RHP) and 0.003 mg/kg (RTF), indicating a potency 3 to 5 times that of fentanyl and 30 to 1000 times that of morphine. The duration of action was short (1/2 fentanyl) at the lowest maximally effective dose.

When administered intrathecally to mice, RX77989 had an ED50 of 0.012 mcg and was 12 and 21 times more potent than fentanyl and morphine, respectively. Its duration of antinociceptive activity was 20 min while that of fentanyl was 16 min at an equi-efficacious dose.

The antinociceptive effect of RX77989 administered intravenously was completely blocked by naloxone pretreatment in the MHP test. In addition, naloxone caused a 4-fold parallel shift to the right of the dose-response curve for RX77989 in the RTF test, suggesting competitive inhibition at the opiate receptor site.

These data strongly suggest that RX77989 is a very potent, short-acting analgetic agent, pharmacologically similar but chemically dissimilar to fentanyl.

211.16 OPIOID AGONIST PROPERTIES OF TWO NALTREXONE DERIVATIVES, NPC 831 AND NPC 836. K.M. Komer*, J.A. Peterson*, B. Mavunkel*, W.J. Rzeszotarski* and D.L. DeHaven. Nova Pharmaceutical Corporation, 5210 Eastern Avenue, Baltimore, MD 21224.

Two novel oxime derivatives of naltrexone, NPC 831 (6-[2-phenylethyl]-oximino naltrexone) and NPC 836 (6-[3-phenyl-propyl]-oximino naltrexone) are potent agonists at opioid receptors. Both NPC compounds demonstrated nanomolar potency in vitro at all three opioid receptor subtypes, with slight selectivity for the kappa site. These two drugs were equi-potent to morphine and more potent than the kappa-selective agonist U-50,488H to produce analgesia. ED_{50} values of 4.02 mg/kg for NPC 831 and 2.24 mg/kg for NPC 836 were generated for inhibition of the tail flick response in the rat, and ED_{50} values of 0.05 mg/kg for NPC 831 and 0.02 mg/kg for NPC 836 were calculated for inhibition of the writhing response in the mouse. The bombesin-scratch test, proposed as an in vivo measure of agonist activity at kappa sites (Gmerek and Cowan. J. Pharmacol. Exp. Ther. 230: 110, 1984), was used to evaluate the kappa agonist activities of NPC 831 and NPC 836. The A_{50}, defined as the percent antagonism of the bombesin-induced response, was 1.76 mg/kg for NPC 831 and 0.08 mg/kg for NPC 836, compared to an A_{50} of 1.54 mg/kg for U-50,488H. NPC 836 at a dose of 0.6 μg i.c.v. also stimulated total 2 hr food intake by 157%, whereas administration of 10 μg i.c.v. of dynorphin$_{1-13}$ increased food intake by 164%. These data suggest that NPC 831 and NPC 836 possess potent kappa agonist properties, with NPC 836 being approximately twice as potent as NPC 831 to produce analgesia, and twenty times as potent as NPC 831 to inhibit the scratching response produced by bombesin.

211.17 INTERACTION OF A BENZOMORPHAN OPIATE WITH THE CATALYTIC SUBUNIT OF ACETYLCHOLINESTERASE. B.A. Coleman and R.E. Oswald. Dept. of Pharmacology, Cornell University, Ithaca, NY 14853.

The benzomorphan opiate, N-allylnormetazocine [ANMC, SKF10047], has been previously shown to bind two distinct sites in acetylcholine receptor (AChR)-rich membranes from *Torpedo* electroplaque (1). The low affinity site seems to correspond to the site for noncompetitive blockers on the AChR whereas the high affinity site, which can be photoaffinity labeled using UV irradiation, was distinct from this site. Labeling and binding to the latter site were stereospecific with (-)ANMC exhibiting higher affinity than (+)ANMC. Binding of both isomers is inhibited by cholinergic agonists such as carbamylcholine. In the presence of ß-mercaptoethanol, the protein has an apparent MW of 66K daltons on 8% SDS-polyacrylamide gels. The labeled band runs as a dimer in the absence of reducing agents. Results of FPLC gel filtration and sucrose sedimentation velocity gradients of Triton X-100 solubilized protein show that the ^{3}H-ANMC labeled protein and acetylcholinesterase (AChE) activity co-migrate. Further studies indicate that AChE activity is inhibited by ANMC at μM concentrations with the (-) isomer being more potent than the (+) isomer. Classic AChE inhibitors such as physostygmine, neostygmine, and edrophonium inhibited binding of (-)ANMC in a concentration-dependent manner. Propidium, a compound which binds to the regulatory site of AChE, also inhibited binding but at higher concentrations. (-)ANMC was further found to photoaffinity label purified 11S ("lytic") AChE of *Electrophorus*. Antibodies to 11S also precipitated (-)ANMC labeled protein from *Torpedo* electroplaque. Results of these and other experiments indicate that the high affinity binding site of ANMC is the active center of the catalytic subunit of AChE found in these membranes.

(1) Oswald *et al.* (1986). *Mol. Pharm.* **29**:179-187.

Supported by NIH grant 1 R01 NS 18660-05 NEUB and the Muscular Dystrophy Association.

211.18 BEHAVIORAL EFFECTS OF THE l AND d ENANTIOMERS OF U-50,488 (A KAPPA OPIATE RECEPTOR AGONIST) FOLLOWING INTRAVENTRICULAR INJECTIONS. L.B. Estall*, B.de Costa*, K. Rice* and A Pert. (SPON: C.T. Bennett). Biological Psychiatry and NIADDK, Bethesda, MD 20892.

Stereoisomers are often used to discriminate the specific effects of drugs. Recently it has been possible to resolve the racemic U-50,488 into (l) and (d) enantiomers. Preliminary findings indicate that the l enantiomer is 4,000 times more potent than the d enantiomer in kappa receptor binding assays (Rothman et al., in preparation). The purpose of the following studies was to evaluate the behavioral effects of these two compounds following intraventricular injections.

In a 75 min test of motor activity, repeated administration of different doses of d-U-50,488 (10,25,50,100 nmol) had no effect on rats horizontal activity, however 50 nmol did significantly decrease vertical activity ($p< 0.05$). Repeated administration of different doses of l-U-50,488 (10,25,50,100 nmol) significantly increased horizontal activity at the 25 ($p< 0.05$) and 100 nmol ($p< 0.01$) doses. The effects of 100 nmol of l-U-50,488 on horizontal activity were antagonized by 5.0 mg/kg (l.p) naloxone.

In a separate study, the acute administration of 100 n moles of d-U-50,488 produced a significant depression of locomotor activity which was not antagonized by naloxone. The l enantiomer also produced a modest but not statistically significant depression of locomotor output which was surprisingly enhanced by naloxone. The initial depressant effects of the l-U-50,488 were followed by locomotor excitation which was antagonized by naloxone. The d-enantiomer again had no excitatory effect on locomotor output.

Increases in the intake of a highly palatable food were seen in non food-deprived animals after i.c.v. injections of l-U,50-488 (25 & 100 n moles). The increase in food intake induced by l-U,50-488 (100 nmoles) was antagonized by 1.0 mg/kg naloxone (i.p.) the d enantiomer had no effect on food intake.

Depression of water intake was seen in 21 hr water-deprived animals following repeated administration of different doses of l-U,50-488 (25 & 100 n moles). Repeated administration of different doses of the d enantiomer had no effect on deprivation-induced drinking.

These findings clearly demonstrate that some of the centrally mediated behavioral effects of the kappa agonist U-50,488 are stereospecific. The l enantiomer enhanced locomotor activity, increased food intake and decreased water intake. The d enantiomer did not produce similar effects. These compounds will undoubtedly prove useful in analyzing the specific effects of kappa receptor mediated behaviors.

211.19 MORPHINE WITHDRAWAL "IN VITRO": ENHANCEMENT OF AGONIST-DEPENDENT PHOSPHOLIPASE C ACTIVATION. F.Moroni, M.Ruggiero*, S.Giannelli*, V.Chiarugi* and D.E.Pellegrini-Giampietro*. Departments of Pharmacology and General Pathology, University of Florence, viale Morgagni 65, 50134 Firenze, Italy.

Morphine abstinence syndrome has been associated with a number of neurochemical changes including an increase in transmitter release (Collier, Nature, 283:625,1980) and in the function of the adenylate cyclase (Sharma et al., P.N.A.S., 72:3092,1975). No information is available on the morphine withdrawal-induced modification of the phosphoinositide metabolism. In the present study we show that in an "in vitro" model of withdrawal syndrome the norepinephrine (NE)-induced activation of phospholipase C is significantly enhanced.

Rats were made dependent by implanting s.c.morphine base pellets (75 mg X 3). Cortical slices were prepared from dependent animals and incubated in a morphine containing Ringer solution until the withdrawal was challenged by placing the slices in a solution in which naloxone replaced morphine. The slices were pre-incubated in Ringer containing trace amounts of ^{3}H-NE in order to evaluate the transmitter release. Other slices were incubated for 2 h in the presence of ^{3}H-myoinositol (10 uCi/ml) or alternatively in the presence of ^{32}P (20 uCi/ml) in order to subsequently study the breakdown of the formed polyphosphoinositides.

Slices pre-incubated with ^{3}H-NE released an increased amount of label when challenged with K^{+} (30 mM) in the presence of naloxone (10^{-9}-10^{-5} M). The narcotic antagonist did not change ^{3}H-NE release when applied to slices taken from non-dependent animals, thus indicating that the preparation we are using shows a withdrawal-induced increase of transmitter release.

In subsequent experiments, the NE-induced phospholipase C activation was evaluated. Inositolphosphates (IP, IP2 and IP3) and the corresponding phospholipids were separated and measured according to Lapetina et al. (J. Biol. Chem., 260:7078,1985) and to Corradetti et al. (Brain Res., in press). NE (1-100 uM) activated phospholipase C and increased the slice content of both inositolphosphates and phosphatidic acid. This increase was significantly potentiated in slices taken from dependent animals and challenged with naloxone.

Thus, cortical slices taken from dependent rats and incubated in a naloxone containing solution release an increased amount of ^{3}H-NE and have an enhanced activation of phospholipase C.

BLOOD-BRAIN BARRIER I

212.1 DEVELOPMENTAL ASPECTS OF POTASSIUM TRANSPORT BY THE CHOROID PLEXUS IN THE RAT. J.T. Parmelee, M.H. Epstein* and C.E. Johanson. Dept. of Clinical Neurosciences, Brown University, Rhode Island Hospital, Providence, RI. 02902.

The neonatal rat serves as an appropriate model for the study of the maturation of mammalian CSF production because of its immature level of choroid plexus development at birth. We have begun an in vitro study of the ion transport mechanisms in these young animals. In potassium flux experiments ^{86}Rb was used as a tracer for K, and ^{3}H-inulin was included as an extracellular space marker. All flux data were corrected for extracellular label.

Uptake of potassium into lateral choroid plexuses isolated from Sprague-Dawley rats is an active, saturable process the kinetics of which change with development. We have shown that the steady-state accumulation of K per mg wet weight of isolated choroid plexus tissue is 50% higher in young rats aged 11-19 days than in neonatal animals aged 3-7 days. However, the time at which K uptake is half maximum is similar, suggesting that the K uptake transporter (Na,K-ATPase) has a similar affinity but a higher capacity in the more mature animals. The ouabain-inhibitable component of this uptake is approximately 90% in all groups.

Potassium efflux was measured with ^{86}Rb loading and washout experiments in isolated plexuses. After initial, rapid loss of about 40% of the label in the first two minutes of sampling, the percent of label lost from the tissue was linear with time on a semi-log scale. The efflux rate coefficient (ERC) decreased slightly in older animals, suggesting that the cells may become less passively permeable to potassium as the animals mature.

Taken together these data indicate that K transport mechanisms are immature and of limited capability and capacity in the lateral choroid plexus of neonatal rats, but that they develop rapidly in the first 2-3 weeks after birth.

212.2 IDENTIFICATION AND DIFFERENTIATION OF CULTURED CEREBRAL CAPILLARY ENDOTHELIAL CELLS IN THE PRESENCE OF GROWTH FACTORS AND IN CO-CULTURE WITH GLIAL CELLS. U. Tontsch* and H.C. Bauer. Inst. f. Molekularbiologie, ÖAW, 5020 Salzburg, Austria.

Endothelial cells (EC) of cerebral microvessels represent the blood-brain barrier by specific structures (e.g. tight junctions, no fenestrae) of cell membranes. It is proposed that the differentiation of those specific characteristics of brain EC are induced during brain development by the presence of neuronal cells.

An in vitro culture system for porcine and murine cerebral capillary EC was established in order to study differentiation processes of EC and to co-cultivate them with glial cells and neurons from syngeneic animals.

Capillary EC were isolated after several steps of homogenization and centrifugation and were subsequently cultivated under various conditions with respect to media and substrates. Addition of several growth promoting factors (acidic fibroblast growth factor, endothelial cell growth supplement (ECGS) and mouse sarcoma S-180 conditioned medium) resulted in different proliferative responses but did not select for one cell type. Prolonged treatment with ECGS (20 µg/ml) + heparin (100 µg/ml) generated cells of "cobblestone" appearance, which were negative for glial fibrillary acidic protein and desmin, and positive for factor VIII and acetylated low density lipoprotein (Dil-ac-LDL) at given times. These cells retained their phenotype for months and could be passaged up to 11 times. Omission of ECGS and heparin produced spindle-shaped cells similar to smooth muscle cells, but only some of them stained for desmin.

2-D analysis and fluorography of those two different cell types showed significant differences.

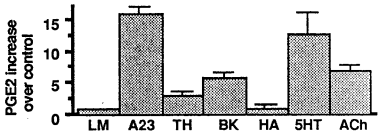
Co-culture of murine EC with syngeneic astroglial cells enhanced the proliferation of EC; the same results were achieved by treating them with conditioned medium of the glial cells.

We raised monoclonal antibodies against cultivated capillary EC and against freshly isolated capillaries. These antibodies are tested on cell cultures and sections in order to locate specific, differentiation dependent cell surface antigens.

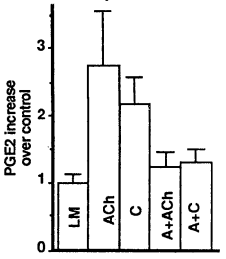
Supported by FWF of Austria, Grant P6268B.

212.3 CEREBRAL ENDOTHELIA RELEASE PROSTAGLANDINS IN RESPONSE TO THROMBIN, BRADYKININ, SEROTONIN AND ACETYLCHOLINE. S.A. Moore, A.A. Spector*, and M.N. Hart*. The University of Iowa, Iowa City, IA 52242

Several hormones, neurotransmitters, and mediators of inflammation and coagulation are known to stimulate prostaglandin (PG) release in endothelium from noncerebral vessels. Many of these same compounds are vasoactive in the cerebral circulation. While acetylcholine (ACh) acts through an endothelial dependant relaxing factor that is not prostacyclin, little else is known about the role endothelial derived PG may play in mediating or modifying these vasoactive responses. Recent studies in our laboratory demonstrating the production of prostacyclin and PGE2 by cultured cerebromicrovascular endothelia in response to exogenous arachidonic acid or calcium ionophore A23187 have suggested that these cultured endothelia may be a useful model for the investigation of PG-mediated events in the cerebral circulation. Endothelial cells are derived from murine brain microvessel isolates and characterized by a monolayer growth pattern, thrombomodulin activity, and Griffonia simplicifolia agglutinin binding. Triplicate cultures of confluent endothelia in passages 6 to 12 were incubated 15 min with serum free Lewis media (LM) or one of the following agents: 2μM ionophore A23187 (A23), 1 unit/ml thrombin (TH), 10μM bradykinin (BK), 50μM histamine (HA), 2μM serotonin (5HT), 20μM ACh, 10μM angiotensin II, 2ng/ml atrial natiuretic factor, and 10μM epinephrine. PGE2 release was quantified by radioimmunoassay.



The graph (to the right) of selected experiments shows that PGE2 release ranged from 3- to 15-times control (mean ± SEM) by TH and A23, respectively. BK, 5HT, and ACh gave intermediate responses. No response was detected with HA (as shown) or the other agents tested (not shown). The ACh response was further characterized by 15 min incubations of triplicate cultures with serum free LM, 20μM ACh, and 100 μM carbamylcholine (C). Both ACh and C stimulated PGE2 release that was inhibited by atropine (A) (fig at right). These cultured cerebromicrovascular endothelia have not only maintained the ability to produce PG, but also appear to have retained receptors for several vasoactive compounds. Specifically, they have muscarinic receptors through which ACh stimulates PG release. These cells should provide a useful model for further investigations into the role of cerebromicrovascular endothelia in the control of the cerebral circulation. [support from NIH grants NS01096 and HL14230]



212.4 SODIUM EFFLUX FROM ISOLATED BRAIN CAPILLARIES IS DEPENDENT ON EXTRACELLULAR POTASSIUM CONCENTRATION. G.P. Schielke, A.L. Betz*, and H.C. Moises. Depts. of Physiology, Pediatrics, Surgery and Neurology, University of Michigan, Ann Arbor, MI 48109

Brain capillary endothelial cells have structural and transport properties which allow them to control the passage of substances into and out of the brain. The presence of Na,K-ATPase primarily in the abluminal membrane, suggests that the brain capillary is capable of pumping Na, K and water across the blood brain barrier. Previous studies in isolated brain capillaries, demonstrated that Na,K-ATPase mediated K transport is half maximal at a K concentration of 3mM (Goldstein, *J. Physiol.* 286:185,1979). We studied the effect of varying extracellular potassium concentration, $[K]_o$, on the kinetics of Na efflux to determine if brain capillary Na transport is also regulated by $[K]_o$ in the physiologic/pathologic range.

Brain capillaries from male rats were prepared by homogenization, dextran gradient centrifugation and glass bead filtration. The rate of ^{22}Na efflux from preloaded capillaries was measured over 6 min. Preliminary studies demonstrated that the rate of efflux was linear for this period. Efflux was terminated by filtration and washing with iced $MgCl_2$. ^{3}H-mannitol was used to correct for trapping or release from the extracellular space.

When capillaries were incubated in K-free buffer, ^{22}Na efflux was stimulated by increasing the extracellular Na concentration, and this effect was inhibited by 1mM amiloride. This finding is consistent with ^{22}Na leaving the capillaries through Na:Na exchange mediated by the Na:H exchanger as reported previously (Betz, *J. Neurochem.* 41:1150, 1983). Efflux of ^{22}Na into buffer containing NaCl, amiloride and varying $[K]_o$ demonstrated a K-dependent efflux which was inhibited by 5mM ouabain. This stimulation in sodium efflux was half maximal at a $[K]_o$ of 2-4mM.

These results demonstrate that the activity of the brain capillary Na,K-ATPase, *in vitro*, is dependent on $[K]_o$ in the physiological range. It has been previously hypothesized (Betz and Goldstein, *Ann. Rev. Physiol.* 48:241, 1986) that blood to brain transport of Na and water is mediated by capillary Na,K-ATPase and, therefore, these processes may be sensitive to changes in $[K]_o$. Since $[K]_o$ may increase markedly during neuronal activity, seizures, spreading depression, and ischemia, our results suggest that brain uptake of Na and water may also be stimulated in these conditions.

Supported by Grant NS 23870 from NIH, and an award to H.M. from The Chicago Community Trust/Searle Scholars Program.

212.5 ADRENERGIC EFFECTS ON 2,4,5-TRICHLOROPHENOXYACETIC ACID (2,4,5-T) TRANSPORT IN THE RABBIT CHOROID PLEXUS. S. McMichael* and C.S. Kim* (SPON: T.J. McCown). Biol. Sci. Res. Ctr., Univ. of N. Carolina Sch. of Med., Chapel Hill, NC 27514

Edvinsson et al. have provided evidence that there is a sympathetic neural influence on CSF production (Exp. Neurol. 48:241, 1975). The sympathetic nerve terminals within the choroid plexus innervate both the resistance vessels and the secretory epithelium. This finding led us to undertake the effects of adrenergic agents on the organic acid transport system in the choroid plexus using the organic acid herbicide, 2,4,5-T. Adult rabbits (New Zealand) were sacrificed by exsanguination and lateral ventricular choroid plexuses were removed. Individual choroid plexus was placed in 2.5 ml of artificial CSF for the control and for the experimental media containing ^{14}C-2,4,5-T 0.02 uCi/ml (sp. act. 23 mCi/mmol) and incubated for 10 min. Tissue uptake was expressed as tissue-to-medium ratio (T/M). T/M ratio of 37.2 ± 4.1 was achieved in the control media. The uptake of ^{14}C-2,4,5-T in the choroid plexus was enhanced in a dose-related manner by the presence of isoproterenol. This enhancement started at 10^{-6} M with maximum enhancement (25%) seen at 10^{-4} M ($p < 0.05$). Unexplainably, the enhancing effect of isoproterenol disappeared and returned to control level at 10^{-3}M. The specific beta-adrenergic antagonist, propranolol, inhibited significantly (50%) 2,4,5-T uptake at 10^{-3}M ($p < 0.001$) and enhanced (22%) the uptake at lower concentration, 10^{-7}M ($p < 0.05$). These results suggest the possibility of selective neurogenic modulation of organic acid herbicide, 2,4,5-T, transport in the choroid plexus. Further study of such interactions may give new insights into the pathogenesis of neurotoxicity and more selective treatment of neurotoxic encephalopathies. Supported in part by NIH grants: HD-03110 and ES-03458.

212.6 LOW CEREBROVASCULAR PERMEABILITY OF VINCRISTINE AND VINBLASTINE LIMIT THEIR BRAIN UPTAKE IN THE RAT. Nigel H. Greig*, Seiji Momma*, Quentin R. Smith, Stanley I. Rapoport. (SPON: Robert Steinman). Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

The vinca alkaloids, vincristine and vinblastine, are widely used in the treatment of neoplastic disease, particularly in acute lymphoblastic leukemia and in lymphomas, usually in combination with others drugs. Both agents are lipophilic, with octanol/water partition coefficients of log 2.14, for vincristine sulfate, and of log 1.68, for vinblastine sulfate. Neither, however, demonstrates significant activity within the brain, although neurotoxicity is the dose-limiting toxicity for vincristine. The cerebrovascular permeability surface area product, PA, of both agents was quantified using an isolated brain perfusion technique (Takasato et al., Am. J. Physiol. 247: H484, 1984) to measure brain uptake in relation to drug binding to plasma constituents and/or drug degradation. In barbiturate anesthetised rats, the right cerebral hemisphere was perfused with physiological saline or plasma containing either 3H-vincristine or 3H-vinblastine, 0.5 nmol/ml. After 20 s, the rat was decapitated and 6 brain areas and perfusion fluid were analysed for radioactivity. Samples of perfusion fluid and blood were chromatographed and showed no metabolites. The mean PA's of vincristine and vinblastine were 1.2 ± 0.2 (S.E) x 10^{-4} s^{-1} and 5.3 ± 1.1 x 10^{-4} s^{-1}, respectively, using saline perfusion. These values are far lower than would be predicted from their octanol/water partition coefficients. The binding of vincristine and vinblastine to plasma constituents was measured by centrifugal ultrafiltration and equal 60.4% and 83.7%, respectively. The PA's of vincristine and vinblastine fell to 0.5 ± 0.1 x 10^{-4} s^{-1} and an unmeasurable value, respectively, during their brain perfusion with plasma. These data demonstrate that the brain uptakes of both vincristine and vinblastine are extremely low, despite their lipophilicity. We suggest that for these compounds, the additive rules that determine lipid solubility (Hansch, Acc. Chem. Res. 2: 232, 1969) are inapplicable to diffusion across the blood-brain barrier, in so far as a small hydrophilic region will prevent transfer of the entire molecule across the vascular endothelial membranes. The minimal brain entry of vincristine and vinblastine is primarily a result of their low cerebrovascular permeabilites and not their binding to plasma constituents or degradation. However, it is probable that the neurotoxicity of vincristine is due to a metabolite that enters the brain but does not possess significant anticancer activity.

212.7 MOVEMENT OF VINCRISTINE ACROSS THE BLOOD-BRAIN BARRIER OF THE RAT AS AFFECTED BY THE PRESENCE OF ALCOHOL IN THE PRESENCE OR ABSENCE OF ACETYLCHOLINE OR HISTAMINE. F.R. Domer and M.E. Smith. Department of Pharmacology, Tulane Univ. Sch. of Med., New Orleans, LA 70112

Vincristine sulfate is an alkaloid from the periwinkle plant that is used in the treatment of leukemia and gliomas in the central nervous system. Access to the central nervous system is limited by the function of the blood-brain barrier (BBB). Because alcohol has effects both in the central nervous system and on permeability of a number of tissues, its effect on the movement of vincristine across the BBB was thought to be of interest. Additionally, possible interactions with acetylcholine or histamine might be of importance in the movement. Male Sprague-Dawley rats weighing 260 to 390 g were anesthetized with pentobarbital sodium (50 mg/kg) intraperitoneally. The femoral artery was cannulated to permit the recording of the mean systemic blood pressure and the juqular vein was cannulated to permit the administration of saline, drugs and radioactivity. The permeability of the BBB to vincristine was evaluated by the use of ^{3}H-labeled vincristine sulfate. Ten µCi were given intravenously 5 minutes after the intraperitoneal administration of 13.3 ml/kg of saline or 3% ethanol. One minute after the administration of the vincristine, either saline, acetylcholine (1 or 2 µg/kg) or histamine (1.25, 2.5 or 5 µg/kg) was given intravenously. At 15 minutes the thoracic cavity was opened, a sample of blood was obtained from the right ventricle, the venae cavae were cut, and 125 ml of saline was infused into the left ventricle to remove blood from the cerebral vasculature. The brain was removed and samples of cerebral cortex, midbrain and cerebellum were placed in vials, dissolved with Soluene and placed in a liquid scintillation counter to permit determination of the content of radioactivity. The permeability of the BBB was assessed as the ratio of ^{3}H-vincristine in brain relative to that in plasma. That ratio was 0.48 in cerebellum, 0.53 in cerebral cortex and 0.66 in midbrain in control animals. When ethanol was given and resulted in a concentration of 20.3 mg/dl at the end of the experiment, the ratios decreased significantly, i.e. 0.088 for cerebellum, 0.044 for cerebral cortex and 0.063 for midbrain. When acetylcholine was given to rats that had also received ethanol, the permeability was increased somewhat and significantly only selectively. Likewise, histamine caused selectively variable changes in the permeability of the BBB, but the changes weren't as large or as consistent as those obtained with acetylcholine. Ethanol significantly decreased the permeability of the BBB to ^{3}H-vincristine sulfate. In the presence of ethanol acetylcholine and histamine caused rather small, variable changes in the permeability. Supported in part by a grant from the Cancer Crusaders and USPHS 5T 35H07299 and 5D 18 MB00009.

212.8 ALTERED CERERROVASCULAR PERMEABILITY AND ULTRASTRUCTURE FOLLOWING THE INFUSION OF rIL-2 AND/OR ITS VEHICLE. M.D. ELLISON*, J.T. POVLISHOCK, and R.E. MERCHANT* (SPON: J. ASTRUC). Dept. of Anat., VA. Commonwealth Univ., Richmond, VA 23298

Clinical trials have shown that rIL-2 administration can induce marked regression of metastatic lesions in some patients. At efficacious doses, however, rIL-2 exhibits significant toxic effects many of which appear to be secondary to increased systemic microvascular permeability. Moreover, patients receiving rIL-2 therapy have exhibited neurological abnormalities including disorientation, somnolence and coma. Despite such clinical reports, to date, the effects of IL-2 on the cerebral vasculature and brain parenchyma have not been investigated. This study, performed in cats and rats, examined the effects of rIL-2, infused at clinical dosage levels, upon blood-brain barrier (BBB) status and cerebral vascular/parenchymal ultrastructure. Animals were infused i.v. with either rIL-2 or rIL-2 vehicle alone. Following transcardial perfusion with aldehydes, brains were processed for ultrastructural visualization of either horseradish peroxidase (HRP), injected prior to IL-2 infusion, or endogenous IgG. Blood-borne HRP and IgG are normally restricted to the intravascular compartment by the BBB. In both species, following light microscopic examination, brain tissue from all rIL-2 infused animals and from the majority of vehicle-infused animals showed extravasated HRP or IgG reaction product throughout the brain, predominately within subcortical white matter. Ultrastructural examination of brain tissue from animals with increased permeability revealed reaction product within perivascular basal laminae and within the brain parenchymal extracellular space. The observation that numerous endothelial cells were completely or partially inundated by tracer suggested direct transendothelial passage. Neither junctional cleaving nor increased pinocytotic activity was observed. All animals studied, regardless of permeability status, exhibited perivascular glial and neuronal swelling together with an expansion of the associated extracellular compartment. Additionally, highly vascuolated periarteriolar phagocytic cells were identified in all brains examined. These dramatic effects of a single rIL-2/vehicle infusion suggest that multiple infusions, used in man, may have a profound impact on the cerebral vasculature and the surrounding brain parenchyma. Furthermore, it appears that many of the cerebrovascular consequences may be mediated by the vehicle itself. In the interest of accelerating the development of an effective cancer therapy, free of adverse effects, these findings indicate that further study of cerebrovascular rIL-2/vehicle toxicity is mandated.

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212.9 BRATTLEBORO RATS DEMONSTRATE NORMAL CEREBROMICROCIRCULATORY RESPONSIVITY TO ALTERATION IN ARTERIAL C02. Sheldon H. Preskorn, M.D., Steve J. Bupp, M.D.*, Melissa Croskell*, Heather Croskell*, Department of Psychiatry, University of Kansas School of Medicine-Wichita, Wichita, KS 67214.

A central adrenergic system (CAS) has been implicated in the regulation of: 1) cerebral capillary permeability to diffusion-limited substance (PS), 2) the surface area of the capillary bed, and 3) cerebral blood flow (CBF). There are several reasons to postulate that the CAS may mediate these effects indirectly through the release of vasopression (VP). First, the VP rich areas of the hypothalamus receive dense CAS innervation. Second, intracerebroventricular administration of arginine VP produces qualitatively similar effects on PS and CBF as CAS stimualtion. Third, effects of CAS stimulation on local glucose consumption has been reportedly blocked by CAS denervation of the hypothalmus.

We therefore elected to test whether PaC02-induced changes in PS, which we have previously shown is dependent on an intact CAS, are also dependent upon an intact VP system. Given that the previously reported surgical means to denervate the hypothalamus causes appreciable damage to other systems, we elected to first test this hypothesis in the Brattleboro rat which is genetically deficient of VP.

A dual label isotope procedure (Irwin and Preskorn, Brain Res., 1982) was used to simultaneously measure CBF and PS to water. Rats were passively ventilated with 02, N02, and varying amounts of C02. PS and CBF were measured in five brain regions: rostral telencephalon, caudal telencephalon, diencephalon, medulla-pons, and cerebellum. Three strains of rats were studied in an identical fashion in a latin square manner: Sprague-Dawley (SD), Long-Evans (LE), and brattleboro (BB). The SD strain has been used in our other studies. The BB is the pivotal strain to test the hypothesis. The LE strain is the one from which BB was derived. The LE and BB strains were obtained from the same supplier. All three strains were housed and handled in a similar manner.

Our results demonstrated no differences between these three strains in terms of PaC02 induced changes in PS or CBF in any brain region studied. In fact, the curves were superimposable. While PaC02-induced changes in these parameters are dependent on an intact CAS, they do not require an intact VP system. These findings are consistent with our theory that the CAS affects these parameters through adrenergic receptors located on cerebral capillaries.

212.10 **PERMEABILITY INCREASES CORRELATE WITH CHANGES IN LUMINAL SURFACE ANIONIC BINDING SITES IN THE RETINAL MICROVASCULATURE OF SPONTANEOUSLY DIABETIC RATS.** M.E.C. Fitzgerald and R.B. Caldwell, Department of Anatomy and Neurobiology, The University of Tennessee, Memphis, TN. 38163.

In diabetes, changes in microvascular barrier functions may contribute to the development of serious visual disability. Previous horseradish peroxidase (HRP) studies in diabetic rats suggest that permeability of the retinal microvasculature increases due to an increase in pinocytotic transport (Ishibashi et al., 1980). In brain arterioles of hypertensive rats, increases in pinocytotic transport are associated with decreases in anionic binding sites along the endothelial cell luminal surfaces (Nag, 1984). To see whether permeability increases in the diabetic retinal vasculature are also associated with alterations in endothelial cell luminal surface charge distribution, we studied cationized ferritin binding and HRP permeability in the diabetic rat retina.

We injected HRP (Type VI) into tongue veins of spontaneously diabetic (BB-WorUtm) rats. Animals were intracardially perfused with fixative at various time intervals after the injections, and the posterior pole of the retina was cut into 20-40 micron sections. HRP was then visualized using a diaminobenzidine reaction, and the tissue was processed for electron microscopy. Tissue sections from HRP injected animals were also incubated in cationic ferritin (0.5 mg/ml, 37°C, 1hr). All levels of the retinal microvasculature (outer plexiform, inner plexiform and nerve fiber layer) showed a greater HRP uptake in the diabetic endothelial cells as compared with the control vessels from the same regions. The tight junctions in all animals were intact. In control rats, HRP was confined to vesicles, cisternae, and multivesicular bodies. Cationized ferritin binding appeared generally uniform on the plasmalemma. Particles were also observed in coated pits, on the stomata of pinocytotic vesicles, in membrane pits continous with the plasmalemma, and on the luminal end of the interendothelial space. In the diabetic animals, HRP label was present in the same structures as the controls; however, some HRP-filled cisternae and vesicles were open to the basal lamina. Cationized ferritin binding in the diabetic animal was similar in distribution to that of the control, but the luminal surface binding was reduced in all levels of the retinal microvasculature. Particle-free areas were commonly observed. Furthermore, in the blood vessels where HRP-labeled cisternae or vesicles opened to the basal lamina, ferritin binding was not only reduced but also appeared patchy.

The presence of HRP-labeled vesicles and cisternae fusing with the basal lamina in the endothelial cell may signify early changes in the breakdown of the blood-brain barrier. The concomitant reduction of anionic binding sites on the luminal surface of the retinal microvasculature suggests that alterations in plasma membrane proteins or associated cell surface proteins may contribute to the permeability increases in the diabetic retina.

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212.11 GLUCOSE INFLUX IS SUPPRESSED DURING CHRONIC HYPERGLYCEMIA IN RATS. R.B. Duckrow. Department of Medicine, Division of Neurology, The Pennsylvania State University, Hershey, PA 17033.

Chronically hyperglycemic diabetics may have cerebral symptoms if their plasma glucose is corrected suddenly. These symptoms could be caused by a relative substrate limitation to brain energy metabolism. This would imply that the kinetics of the facilitated diffusion of glucose from blood to brain is subject to adaptation. There are data indicating that the maximum rate of glucose transport into the brain decreases during chronic hyperglycemia. These data rely on the consistancy of cerebral blood flow and cerebral blood volume during acute and chronic hyperglycemia. Because cerebral blood flow and blood volume may change during hyperglycemia, the effect of acute and chronic hyperglycemia on glucose influx and cerebral blood volume was re-examined. Male Sprague-Dawley rats (350 g) were prepared for study using halothane-nitrous oxide anesthesia and restrained with plaster hip-casts to allow measurements to be made with the rats awake. Wounds were infiltrated with 1% procaine and all experiments ended with rapid decapitation. Acutely hyperglycemic rats were studied 10 minutes after intraperitoneal injection of d-glucose. Chronically hyperglycemic rats were studied at 1 and 3 weeks after intravenous injection of streptozotocin (60 mg/kg). The plasma glucose was 30 μm/ml in hyperglycemic rats and 10 μm/ml in sham-injected control rats. Cerebral blood volume was measured using [^{3}H]inulin and [^{14}C]sucrose. Glucose influx was measured using the intravenous tracer injection method described by Bachelard and by Ohno as modified by Hawkins (J. Neurochem. 40:1013, 1983). Clearance is measured at multiple time points up to 18 seconds after tracer injection in separate rats and the instantaneous influx expected at time zero is calculated. This method is relatively insensitive to changes in cerebral blood flow. Cerebral blood volume did not change from control measurements during acute or chronic hyperglycemia. Glucose influx increased and was similar during acute hyperglycemia and chronic hyperglycemia of 1 week duration. However, after 3 weeks of chronic hyperglycemia, glucose influx returned to levels equal to that measured in control animals with lower plasma glucose concentrations. This relative reduction in glucose influx implies that the kinetics of glucose transport is altered during chronic hyperglycemia. This "down-regulation" requires more than 1 week to occur and does not involve a change of cerebral blood volume. Because cerebral blood flow is decreased during both acute and chronic hyperglycemia it is unlikely that blood flow changes are involved in this adaptive process. (Supported in part by PHS NS24109. RBD is an Established Investigator of the AHA.)

212.12 ACUTE LOW DOSE MICROWAVE IRRADIATION ALTERS METHYL-NALTREXONE POTENCY IN MICE. D.G. Lange, A.M. Phelan, C. Fredricks, H. Kues and R.M. Quock. Department of Anesthesiology, and Applied Physic Laboratory, The Johns Hopkins Medical Institutions, Baltimore, MD 21205, and Department of Basic Sciences, Marquette University, Milwaukee, WI 53233.

Previous studies by a number of groups have suggested that acute as well as chronic microwave exposure may alter the CNS availability of systemically administered drugs. This alteration is thought to be due to changes in blood-brain barrier (BBB) permeability. These earlier studies have required high, thermogenic, doses of microwave exposure to achieve measurable changes in BBB permeability. Male ICR mice (20-25g) received morphine sulfate (MS), 10 mg/kg, ip (10 mg/ml). Five minutes after MS, saline, the narcotic antagonist naltrexone (0.2 mg/kg, ip), or its quaternary derivative methyl-naltrexone (2.5 mg/kg, ip) were administered and the animal placed into the microwave anechoic exposure chamber. Five minutes following exposure to sham or far-field microwave irradiation (2.45 gHz, 5 mW/cm^2, SAR = 3 W/kg, duration of 10 min) the animals were tested for nociceptive responsiveness on a hot plate. Both continuous and pulsed microwave exposures were examined. Microwave irradiated animals given morphine/methyl-naltrexone showed a significant decrease in the time to respond to applied nociceptive stimulus (16.46 $\pm$ 1.37 sec, n=8) versus sham exposed morphine/methyl-naltrexone treated animals (24.59 $\pm$ 1.96 sec, n=12). Neither microwave irradiation nor methyl-naltrexone administration alone altered morphine's antinociceptive activity. Thus, microwave irradiation, at power densities and exposure durations designed to avoid a hyperthermic response in the animal model produces a change in the CNS pharmacologic potency of methyl-naltrexone, an agent normally excluded from the brain. It is suggested that low dose microwave irradiation may significantly alter BBB function, in the absence of hyperthermia. (Supported by ES 03386).

212.13 MIDDLE CEREBRAL ARTERY THROMBOSIS: ACUTE BLOOD-BRAIN BARRIER CONSEQUENCES. W.D. Dietrich, R. Prado, B.D. Watson and H. Nakayama. Cerebral Vascular Disease Research Center, Departments of Neurology, Anatomy and Neurosurgery, University of Miami School of Medicine, Miami, FL 33101.

The effect of middle cerebral artery (MCA) thrombosis on the behavior of the blood-brain barrier (BBB) was studied in rats. MCA thrombosis was produced by a dye-light insult and the BBB assessed by the protein tracer horseradish peroxidase (HRP). The beam of a tunable argon ion laser operated at 514.5 nm at a power level of 25 mW was first focused onto the MCA of anesthetized rats just proximal to the olfactory tract. Rose bengal (1 mg in 0.133 ml saline/100 g animal weight) was infused simultaneously with the start of irradiation. On an average, 2-3 min were required for partial occlusion while 5 min of irradiation was carried out for complete occlusion of the MCA. Rats were then injected intravenously with 20 mg/ml HRP. Fifteen min following the end of the irradiation period, rats were perfused for scanning (SEM) and transmission electron microscopy (TEM). The irradiated MCA segment was removed from the surface of the brain, cut longitudinally, and processed for SEM. Vibratome sections were incubated with 3,3' diaminobenzidine and processed for light or TEM analysis.

In sham-operated control rats (irradiation without rose bengal infusion), the MCA appeared unremarkable and no extravasation of HRP was observed. In rats which underwent complete occlusion of the MCA, a large platelet thrombus occupying the entire vessel lumen was seen with SEM. Proximal and distal to the thrombus, the artery appeared constricted and the endothelial cell layer was damaged. Vibratome sections demonstrated widespread protein leakage in both ipsilateral and contralateral hemispheres. HRP staining was most intense within the ipsilateral striatum and deeper layers of the neocortex. A similar but less intense pattern of HRP leakage was apparent in rats following non-occlusive thrombosis. In addition to diffuse barrier leakage, focal sites of HRP leakage within both gray and white matter were seen in 3 out of 4 rats. With TEM, enhanced endothelial transport of HRP was demonstrated. In summary, experimentally induced MCA thrombosis results in an immediate breakdown of the BBB to HRP. The extent of barrier leakage was widespread and not confined to the occluded MCA territory. It is hypothesized that unidentified neurohumoral substances or factors released at the thrombotic site and not cerebral ischemia are responsible for the acute barrier alterations. Supported by NIH grants NS5820 and NS23244. WDD is an Established Investigator of the American Heart Association.

212.14 BLOOD-BRAIN BARRIER BREAKDOWN AND RESTITUTION FOLLOWING BRAIN INJURY J.B. Farrell*, G.S. Sarna*, and M.E. Carey* (Spon. J.S. Soblosky) Department of Neurosurgery, LSU Medical Center, New Orleans, LA. 70112.

Brain injury may be associated with damage to the blood-brain barrier (BBB) and vasogenic brain edema (VBE). In prior experiments studying the occurrence of VBE following a 0.9 Joule missile wound we determined that VBE peaks at 24-48 hours, then recedes (Abstract: Annual Meeting of the American Association of Neurological Surgeons, 1986). Repair of the damaged BBB, whereby plasma electrolytes, water and proteins are prevented from entering the extracellular fluid, is necessary for the resolution of VBE. We recently studied BBB breakdown and restitution following an experimental missile wound to the brain by means of Evans blue dye (EBD)(MW 68,000) to ascertain when BBB integrity was reestablished to the large EBD-albumin molecule and to learn whether BBB damage occurred only locally about the missile track or in distal areas as well.

Cats were anesthetized with pentobarbital (44 mg/kg) and placed in a stereotaxic frame. We created a fronto-occipital right cerebral hemisphere wound using a 31 mg steel sphere fired by a custom made gun at $\simeq$ 240 m/sec (0.9 Joules). The cats were injected intravenously with 2% EBD (2.5 cc/kg) at 10 minutes and 1, 6, 24, 48 and 72 hours after injury. The cats were then painlessly euthanized and their brains fixed-perfused. The brains were then removed, sliced and photographed in order to make a permanent record of BBB breakdown.

We observed EBD extravasation about the missile track up to 24 hours after wounding, but not thereafter. For large molecules such as EBD-albumin, the BBB regains its integrity at about 24 hours after a 0.9 Joule missile wound. This time interval correlates well with peak occurrence of VBE which we have documented in our model, as well as with the beginning of VBE resolution after this time. Our EBD studies have shown areas of BBB breakdown distally in the brain stem in some animals. Thus, structural damage appears to occur in the brain stem, which may be associated with "brain stem effects" commonly seen after wounding (i.e. hypertension, respiratory depression and bradycardia).

212.15 ALTERATIONS IN SPINAL CORD VASCULATURE AFTER SPINAL CORD INJURY. L.J. Noble, J.A. Ellison* and M. Kwok*. Dept. of Neurology, Sch. of Med., University of California, San Francisco, Ca 94122.

We have previously examined the permeability of spinal cord vasculature to the protein horseradish peroxidase (HRP) after transection and reported that there was an axial spread of tracer both proximal and distal (caudal) to the injury. Vascular permeability to the tracer was maximal at 1 day after transection. However, by 2 weeks the barrier to HRP was re-established (Brain Res., In Press). Because the neural axis undergoes degeneration for an extensive period of time, we postulated that although spinal cord vessels are no longer "leaky" to HRP, they may still be influenced by these degenerative events.

In an initial study we wished to determine whether the vascular response to injury is altered in segments of spinal cord undergoing degeneration. One month after transection at the T2 segmental level, a second transection was made 2-3 segments distal to the first. The barrier response to HRP was evaluated at 1, 3, and 6 h and 1 day after the second transection. The distribution of reaction product (RP) and hemorrhage was examined at the light microscopic level in samples at 0.5, 1.0, and 2.0 cm distal to the second transection and 0.5, 1.0, and 2.0 cm proximal to the first transection. The areas of RP and hemorrhage were determined at each sampling site. These results were compared with the same parameters at sites distal to a single transection.

The pattern and extent of hemorrhage was similar in the distal cord after either a single or double transection. Hemorrhage was typically restricted to a central zone in the dorsal columns and only rarely extended into the adjacent gray matter. In contrast, there appeared to be a marked increase in RP after a double transection as compared to a single transection. RP was associated with dorsal column hemorrhage, appearing as a halo around the extravasation. In addition, it was also noted in the gray matter and in the peripheral white matter in the absence of hemorrhage. Although RP was primarily extracellular, it also appeared to be present in neurons, ependymal cells, and glia.

After a double transection, RP was not restricted to sites distal to the second transection, but was also present in segments of cord proximal to the first transection. RP appeared as early as 3 h after the second transection and became more extensive with time. It was typically located in the peripheral white matter, dorsal columns, and occasionally within gray matter.

This data indicate that there is a prolonged and extensive sensitivity of the barrier after spinal cord injury. It is not readily apparent until challenged, in this case by a second transection, and then responds with marked permeability to protein along the axis of the cord.

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212.16 IgG, IgM, AND ALBUMIN BLOOD NERVE BARRIER INDICES IN HUMAN DIABETIC NEUROPATHY.
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A highly sensitive and specific solid-phase antibody-capture assay was developed to measure IgM and IgG in fascicular biopsies of human sural nerve from healthy controls and patients with diabetic neuopathy. Assay amplification was obtained by utilizing biotin-labeled anti-IgM or anti-IgG antibody and ^{125}I-streptavidin. When this assay was applied to normal fascicular biopsies from human sural nerve, the percent of IgM and IgG, respectively, of the total endoneurial protein was 0.021±0.005% and 0.50±0.21% (Mean ± SD; N=3). When these endoneurial concentrations of IgM and IgG were correlated with the plasma concentrations (mg IgM or IgG/mg total plasma protein), an IgM-BNB-Index of 3.17±0.48% and an IgG-BNB-Index of 3.82±1.67% were obtained (Mean ± SD; N=3). These values were also correlated with the albumin (Alb) concentration in the biopsies (1.58±0.18% mg) and with the Alb-BNB-Index (2.53±0.61%) (Mean ± SD; N=3).

The Alb and IgG concentrations in the endoneurium of fascicular sural nerve biopsies were significantly elevated in 5/5 diabetic neuropathy patients, and the IgM concentration was significantly elevated in 3/5 patients. Plasma concentrations of these macromolecules in patients was normal, except for a significant increase in the IgG of 4/5 patients. After normalization to total endoneurial protein and plasma protein, BNB indices were significantly increased for Alb (19.20±7.70%; P=0.006; 5/5), IgG (8.61±3.42%; P=0.034; 5/5), and IgM (6.65±1.57%; P=0.011; 3/5). The endoneurial compartments containing these plasma proteins consist of the endoneurial space occupied by the endoneurial fluid, the vascular compartment, and the cellular compartment. Since it is likely that these compartments are not altered in diabetic neuropathy patients, it is hypothesized that the observed increase of these plasma proteins in patients results from altered transport through the capillary endothelial barrier. Increased routes of entry might involve leaky tight junctions and/or plasma membranes, or an increase in intracellular vesicle transport. The endoneurial ratio of Alb/IgG was decreased 2.5 fold, even though plasma IgG was elevated, and the endoneurial ratio of Alb/IgM was decreased 3.3 fold compared to controls. Although the endoneurial IgG and IgM levels are increased in diabetic neuropathy patients, the Alb increase is greater suggesting a mechanism other than extravasation of plasma proteins. (Supported by NINCDS Grant NS-14304 and by the Borchard Fund).

212.17 MORPHOMETRIC ANALYSIS OF FROG BLOOD-NERVE BARRIER DURING CHRONIC WALLERIAN DEGENERATION. C.H. Latker*, K. Wadhwani, A. Weerasuriya, A. Balbo and S.I. Rapoport (Spon:Don Newman) Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

The entry of water-soluble substances into the endoneurial compartment of the peripheral nerve is limited by endoneurial blood vessels and by the perineurium, which together comprise the blood-nerve barrier (BNB). We previously showed that during chronic Wallerian degeneration (WD), there was an increase in permeability of the BNB to [14C]sucrose and horseradish peroxidase (HRP) and an increase in the vascular space (Latker et al., Soc. Neurosc. Abst, '86). Proliferation of non-neuronal cells of the peripheral nerve also occurred. In this study we investigated during chronic WD proliferation of BNB cells in relation to changes in the endoneurial vascular space and BNB permeability. Sciatic nerves of adult frogs were severed in the abdominal cavity, ligated to prevent regeneration, and examined at distal points in the thigh at intervals of 3 days to 7 months. One day after intraperitoneal injection of [3H]thymidine, the animals were killed and the nerves were processed for autoradiography, and light and electron microscopy. The cells in the endoneurium and perineurium that incorporated [3H]thymidine and the number and area of endoneurial blood vessels were quantified. In endothelial cells (EC) of endoneurial blood vessels proliferation occurred at 7 days, corresponding to the increased permeability to [14]sucrose and HRP. Both vascular permeability and EC proliferation increased to day 14, returned toward normal at 6 weeks and continued at near normal levels to 7 months. The blood vessels increased in number and frequently in size resulting in an increased vascular space. Proliferation of perineurial cells was seen at 7 days, but incresed perineurial permeability was not detected until 10 days. After 6 weeks proliferation ceased, but perineurial permeability remained elevated for 7 months. This study suggests that following WD: 1) the permeability of the endoneurial vasculature increased temporarily in tandem with proliferation of the EC cells, 2) the increase in vascular space was due to EC proliferation, and 3) after proliferation ceases, the perineurial cells did not form a tight barrier. The presence of nervous elements may be necessary for the maintenance of the perineurial, but not the vascular, component of the BNB.

212.18 IRREVERSIBLE INCREASE OF PERINEURIAL PERMEABILITY AND WATER CONTENT OF THE FROG PERIPHERAL NERVE DURING WALLERIAN DEGENERATION. K.C. Wadhwani, C.H. Latker, and S.I. Rapoport. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

In the vertebrate peripheral nerve, the perineurium and endoneurial blood vessels form a blood-nerve barrier (BNB), which limits entry into the endoneurial compartment of proteins and water-soluble substances from blood. This study investigates whether the integrity of the perineurial barrier requires the presence of neuronal factors. Lumbo-sacral plexuses in the abdominal cavity of adult female frogs, R. pipiens, were cut and tied to prevent regeneration. At 7, 10, 14, 21, and 147 days, during Wallerian degeneration, perineurial permeabilities to ^{3}H-sucrose and ^{14}C-dextran (MW=70,000) of the sciatic nerve were determined and compared with the contralateral uncut nerve. Water content, nerve conduction velocity and maximal voltage of compound action potential (CAP) of the degenerating nerve were also measured. Seven days after transection, the water content of the degenerating nerve [(73.5 ± 0.6) % wt wt] (mean ± S.E., n=8) was significantly higher ($p<0.05$) than in the contralateral uncut nerve (71.9 ± 0.4 % wt wt), but the maximal voltage and conduction velocity of CAP, as well as the perineurial permeabilities to sucrose and dextran were unchanged. After 10 days, the degenerating nerve showed significant increases in permeabilities of the perineurium to ^{3}H-sucrose [$(3.71 \pm 1.00)\times10^{-7}$ cm/sec] and ^{14}C-dextran [$(2.37 \pm 0.29)\times10^{-7}$ cm/sec] when compared to values in the uncut nerve [$P_{sucrose} = 0.78 \pm 0.09$ and $P_{dextran} = 1.12 \pm 0.19$] ($p<0.05$). Similarly, the maximal voltage and the conduction velocity of CAP was significantly decreased. Between 14 days and 147 days, no CAP was detected and perineurial permeability as well as water content of the degenerated nerve increased with time. After 147 days, perineurial permeabilities to ^{3}H-sucrose and ^{14}C-dextran in the degenerated nerve were 7 fold higher than in the uncut nerve. The water content [(83.4 ± 0.8) % wt wt] also was significantly higher [(73.5 ± 0.7) % wt wt]. We conclude that: 1) the perineurium of the frog peripheral nerve may need neuronal factors to maintain its barrier function, and 2) the edema observed in the degenerated nerve may be a consequence of increased perineurial permeability.

213.1 ANATOMICAL DISTRIBUTION OF IMMUNOREACTIVITY FOR PRE-PRO-TRH160-169, A POSSIBLE MARKER FOR TRH NEURONS. M.W. Wessendorf, R. Wohlhueter*, and R. Elde, Dept. Cell Biology and Neuroanatomy, and Inst. Human Genetics, Univ. Minnesota, Minneapolis, MN 55455

Attempts at immunohistochemical staining for thyrotropin-releasing hormone (TRH) have frequently been unsuccessful. In addition, even when staining has been obtained different antisera have sometimes produced different patterns of staining. For instance, not all groups have observed staining for TRH in the superficial dorsal horn of the spinal cord. Because of these considerations, an alternate method of localizing TRH would be desirable. Recently, the sequence for the precursor to rat TRH was described. Within one molecule of pre-pro-TRH were 5 molecules of pro-TRH, each separated by one of several novel peptide sequences. It was decided to test whether an antiserum directed against one of these intervening peptide sequences might be useful for localizing TRH-synthesizing cells and axons.

The decapeptide pre-pro-TRH160-169 (SFPWMESDVT; abbreviated ppT) was synthesized by solid-phase methods and purified using HPLC. Amino acid analysis was used to confirm the identity of the purified fraction. ppT was conjugated to bovine thyroglobulin (BTG) using glutaraldehyde, and rabbits were hyperimmunized with the conjugate. Rat brain and spinal cord were examined for ppT-like immunoreactivity using fluorescence immunohistochemistry.

The neuronal distribution of ppT-like immunoreactivity closely resembled that reported for TRH. Fibers and terminals strongly immunoreactive for ppT were observed in the median eminence of the hypothalamus. Strongly-labeled fibers and terminals were also observed in the spinal cord in the ventral horn, the intermediolateral cell column, and around the central canal. Fibers and terminals more lightly stained for ppT were also observed in the superficial dorsal horn. In rats pretreated with colchicine, ppT-immunoreactive cell bodies were visible in the paraventricular nucleus and in the medullary raphe nuclei. Using simultaneous 2-color immunofluorescence it was found that ppT-immunoreaactive cell bodies in the vicinity of the medullary raphe frequently were also immunoreactive for serotonin. Using the same technique in the spinal cord it was found that fibers and terminals immunoreactive for serotonin were generally also immunoreactive for ppT. However, such double-labeling was seldom observed in the superficial dorsal horn. ppT-like immunoreactivity was abolished when tissue was stained with antiserum preincubated with 10 µg/ml ppT-BTG conjugate.

It is concluded that the distribution of ppT closely resembles that of TRH, and that ppT may be useful as a marker peptide for neurons containing TRH. In addition, the presence of ppT-like immunoreactivity in the superficial dorsal horn suggests that TRH is also present in that region.

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213.2 7-AMINO-4-METHYLCOUMARIN-3-ACETIC ACID (AMCA): A BLUE FLUOROCHROME USEFUL FOR THREE-COLOR IMMUNOHISTOCHEMISTRY. N. Appel, M. Wessendorf and R. Elde. Dept. Cell Biol. Neuroanatomy, U. Minn. Mpls. MN 55455

We have been investigating the hypothesis that the neurochemical identity of a population of neurons may reflect its anatomical and physiological character. To this end we characterized a method of two-color fluorescence immunohistochemistry in order to observe pairs of neurotransmitter-related antigens in single sections of nervous tissue (Wessendorf and Elde, *J. Histochem. Cytochem.* 33:894, 1986). Recently, Khalfan *et al.* (*Histochem. J.* 18:497, 1986) and Staines *et al.* (in press) suggested the use of 2° antibodies labeled with coumarin derivitaves for fluorescence immunohistochemistry. These substances appear blue under ultraviolet (UV) excitation. We found that AMCA-labeled 2° antibodies could be used in combination with 2° antibodies labeled with fluorescein and with lissamine rhodamine to observe simultaneously three neurotransmitters in single tissue sections.

Transverse, slide-mounted cryostat sections of spinal cord from rats which had been perfused with Zamboni fixative were rehydrated with phosphate-buffered saline (PBS) and incubated for 10 min with 50 µl of10% normal goat serum in PBS/0.3% Triton X-100. Subsequently they were incubated overnight at 4°C with 50 µl of a solution containing rabbit antiserum to serotonin (5HT), rat antibody to substance P (SP) and mouse antibody to leucine enkephalin (Enk) (1:100, 1:100 and1:50) in PBS/0.3% Triton X-100. The following day they were incubated for 1 hr at room temperature with AMCA-labeled goat immunoglobulins directed against rabbit IgG (GAR), 1:24 in PBS/0.3% Triton X-100, in combination with fluorescein labeled goat antibody to rat IgG (1:10; preabsorbed with mouse liver powder) and lissamine rhodamine labeled antibody to mouse IgG (1:30; preabsorbed with rat liver powder). AMCA (BioCarb, Lund Sweden) was conjugated to GAR as described by Khalfan *et al.*

Under UV illumination, blue AMCA-stained 5HT-immunoreactive fibers were visible in ventral horn, around the central canal and in the intermediolateral cell column. Under green or blue illumination Enk- or SP-immunoreactive fibers, respectively, were observed in superficial dorsal horn, lateral spinal nucleus, intermediolateral cell column, ventral horn and around the central canal. In some of these areas we observed fibers and terminals immunoreactive for combinations of these neurotransmitters. Specifically, in ventral horn we observed single motor neurons apposed by one fiber labeled for both coexisting SP and Enk, but not 5HT and another fiber labeled for both coexisting SP and 5HT, but not Enk. In addition, when sections containing neurons previously retrogradely labeled with Fluoro-Gold (FG) were immunostained in this manner, we could observe the retrogradely labeled neurons in addition to their immunostained neuropil. Thus, a four-color fluorescence histochemical detection system had been accomplished. Moreover, since FG and AMCA are both excited by UV light, FG labeled neurons and the AMCA-stained fibers apposing them were visible simultaneously.

This technique offers a convenient method for simultaneously detecting three neurotransmitter- related antigens in single tissue sections. In addition, it provides an increased level of resolution for establishing the neurochemical identity of a population of neurons. Supported by grants DA 05275, NS 22665 and DA 02148.

213.3 DIRECT SIMULTANEOUS VISUALIZATION OF GABA INNERVATION AND SEROTONIN UPTAKE IN ADRENAL MEDULLARY CELLS. M.F. Franzoni,+ M. Beltramo,+ M.L.Sapei,+ C.Decavel+ and A.Calas (SPON:ENA).Dip.Biol.Animale Università,10123 Torino,Italy; Lab.Physiol.Interactions Cell. UA339CNRS,Université Bordeaux I,33405-Talence,France.

We have previously demonstrated the presence of a content and of a selective neuronal uptake of ^{3}H serotonin (5-HT) by adrenaline cells of mouse and rat adrenal medulla (AM). Biochemical approaches have shown that rat AM cells contain,take up,release and bind GABA which can control the catecholamine secretion. Moreover GAD has been detected in AM cells and fibers by immunohistochemistry.

In order to further determine in a comparative approach possible relations between GABA and 5-HT in the AM we have combined a radioautographic (RAG) study of ^{3}H 5-HT uptake with a simultaneous detection of endogenous GABA content. Either mice and frogs were intracardially injected with 3.7 10^{6}Bq of 10^{-4}M ^{3}H 5-HT together with 10^{-3}M cold Noradrenaline (NA)or adrenals were in vitro incubated in 10^{-6}M ^{3}H 5-HT with 10^{-5} M NA. The fixative was 4% glutaraldehyde.Vibratome sections were treated with 1:5000 anti-GABA antibodies(Immunotech,Luminy,France) revealed by Fab coupled with peroxidase,then radioautographied with K5 Ilford Nuclear emulsion exposed for 20 days. Intense,although variable RAG reactions occurred on AM cells of all species,especially at the periphery of the gland in the mouse. Noticeable bundles of nerve fibers and terminals among the AM labeled cells were immunopositive in the mouse and in the frog,although with a lesser density and a thinner aspect.The GABA immunoreactivity of the AM cells was difficult to assess although silver grains seemed to occur either on immunopositive as well as negative cells.

These techniques suggest in both groups intercellular relations between GABA innervation and 5-HT related AM cells which can be further explored at E.M. level. They can help to define other types of interrelations between these transmitters. (Work supported by NATO 86/0796)

213.4 GOLGI STAINING AND CHOLINE ACETYLTRANSFERASE IMMUNOHISTOCHEMISTRY ON THE SAME BRAIN SECTION. L.K. Gorman, T.W. Farris, N.J. Woolf, and L.L. Butcher. Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024-1563, U.S.A.

A procedure has been developed for routine use that permits the exquisite morphologic detail evinced by use of the Golgi technique to be demonstrated on the same brain section processed immunocytochemically for various neurochemical indices. Using a modification of the single-section Golgi method of Gabbott and Somogyi (1984, J. Neurosci. Meth. 11: 221-230) and choline acetyltransferase (ChAT) immunohistochemistry (e.g., Gould and Butcher, 1986, Neurosci. Lett. 63: 315- 319), we have examined the morphologies of putative cholinergic and non-cholinergic neurons in the basal forebrain and caudate-putamen complex. Rats aged 25-30 days were perfused transcardially with a 4% paraformaldehyde-2% picric acid solution and postfixed for 24 hrs in a solution with the same composition as the perfusate. Sections 100 µm thick were then prepared on a Vibratome. Free-floating sections were processed for ChAT-like immunoreactivity (Eckenstein-Thoenen monoclonal antibody; gift of Dr. Felix Eckenstein) according to the avidin-biotin method. Following immunostaining, brain sections were Golgi stained as follows: sections were rinsed once in phosphate- buffered saline (pH, 7.4) and then placed into 3.5% potassium dichromate for 24 hrs. Sections were then mounted onto a coverslip, and another coverslip was placed over the mounted sections. This assembly was then placed between two standard-size glass slides. The resulting "Golgi sandwiches" were then put into a Coplin jar containing a 1.5% silver nitrate solution. At least 4 days were allowed for neuronal impregnation. Microscopic analyses were performed on fully impregnated cells, and photographs were taken of these neurons. The tissue sections were then removed carefully from the coverslips, mounted onto pig-gelled slides, and affixed to a petri dish. The entire preparation was then covered with a 5% sodium thiosulfate solution and microscopically examined during the dissolving of the silver chromate deposits. Spiny neurons in the caudate-putamen complex and basal forebrain were not found to be ChAT positive. Some Golgi impregnated cells that were large (20-40 µm in maximum soma extent) and aspiny or sparsely spined were demonstrated to be ChAT positive, thereby confirming previous suggestions that cholinergic neurons in the striatum and basal forebrain cholinergic system evince few or no spines (Woolf and Butcher, 1981, Brain Res. Bull. 7: 487-507; Bigl, Woolf, and Butcher, 1982, Brain Res. Bull. 8: 727-749). [Support: NS 10928 to L.L.B.].

213.5 SERIAL 2 MICRON CRYOSTAT SECTIONS: A MODIFIED COLOCALIZATION TECHNIQUE FOR IMMUNOCYTOCHEMISTRY. B. Quinn & E. Weber. Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

Three general approaches are available in light microscopy for the colocalization of different antigens within the same cell: (1) differential visualization of primary antibodies generated in different species; (2) repeated staining of the same section after elution of previous antibodies; and (3) use of serial sections in which the same cell is visible on several sections. The serial method requires no special reagents, but demands thin sections with good morphology. Because polyethylene glycol (PEG) is used in immunohistochemistry as a water-soluble wax (Smithson et al., J. Neurosci. Meth. 7:27, 1983), we investigated its use in thin cryostat sectioning.

Rats were perfused with 4% buffered paraformaldehyde/4% sucrose and blocked brains were immersed overnight in one of four cryoprotectants: 20% sucrose, 5% sucrose, 3% PEG (MW 400), or 20% sucrose + 3% PEG. Tissues were frozen in freon at either dry ice or liquid nitrogen temperature, stored in sealed vials at -70°, and sectioned at -20° to -30° C. Unlike 20% sucrose, 5% sucrose allowed sectioning in the 3-5 micron range, but morphology was somewhat poor. 3% PEG alone had a plasticizing effect and thus allowed 1 micron sections, but with very poor morphology. The combination of 20% sucrose and 3% PEG allowed routine cutting of 1-2 micron sections with good morphology, when tissue was frozen in freon/N_2 and sectioned at -25° to -30°.

In summary, we found that combined PEG/sucrose infiltration can be readily incorporated into existing frozen section protocols, and provides a simple and rapid method of evaluating possible antigen colocalizations. This approach should also be compatible with the multiple-species antisera or serial elution methods, possibly allowing the analysis of four or more antigens when those methods are available.

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213.6 **MICROWAVE POST-FIXATION ENABLES RAPID PROCESSING OF CNS TISSUE FOR ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDIES.** A.J. Hodgson, I. Llewellyn-Smith*, J. Minson*, J.P. Chalmers*, P. Pilowsky*. Centre for Neuroscience, Flinders University, Bedford Park 5042, South Australia.

The treatment of brain and spinal cord with microwaves enables rapid fixation and preserves antigenic and ultrastructural integrity. Adequate fixation of fine structure with preservation of immunoreactivity is often difficult to achieve. Commonly used aldehyde mixtures can give poor preservation of heavily myelinated regions (Peters, In:Contemporary Research Methods in Neuroanatomy, pp 56, 1970). Furthermore, high concentrations of glutaraldehyde, whilst giving good ultrastructure, are acknowledged to affect many antigens adversely. We explored the utility of post-fixation by microwave treatment. We reasoned that, by speeding the fixation and reducing the time that tissue is exposed to aldehydes, both good ultrastructural and antigenic preservation would be achieved. Rats were anaesthetized and perfused briefly through the descending aorta with tissue culture medium gassed with 95% O_2 and 5% CO_2 followed by a fixative containing 10% formalin, 0.5% glutaraldehyde, 0.2% picric acid in phosphate buffer pH 7.4, at a rate of 200-300ml/min for 2-4 mins. The brain and spinal cord were rapidly removed, divided longitudinally, immersed in the same fixative, and rapidly heated to 60-65°C in a domestic microwave oven operating at full power (1 kW, 2.45 GHz). This took 20-60 sec. depending on the volume of fixative. The fixative was maintained at 60-70°C in the microwave oven ("defrost" setting) for 2,4,8 or 16 minutes. Post-fixation continued for a further 15,30 or 60 minutes. Different fixation protocols were compared using semithin (1um) and ultrathin sections of osmicated, plastic-embedded tissue. The preservation of the cell bodies, neuropil and myelin sheaths, particularly in large fibre tracts, was evaluated. The optimal fixation was produced by heating rapidly to 60-65°C, maintaining this temperature for at least 8 minutes followed by 60 minutes in the cooling fixative. With this rapid fixation protocol the fine structure of neuronal perikarya, axons and dendrites, myelin sheaths, mitochondria, synapses, synaptic vesicles, ribosomes and microtubules was well preserved. In light microscopic studies using 50um Vibratome sections labelled in the presence of 0.3% Triton X-100, immunoreactivity for substance P, neuropepide Y and serotonin was present. Microwave post-fixation has the following advantages. (1) The method is rapid, with tissue being fixed for ultrastructural studies within 90 minutes. For LM studies the material can be cut on a Vibratome after only 4-8 minutes of fixation. (2) Ultrastructure is well preserved. (3) Immunoreactivity for neuropeptides and serotonin is retained. (Supported by grants from the NH & MRC of Australia).

SOMATIC AFFERENTS II

214.1 A FINK-HEIMER STUDY OF THE MEDULLARY PROJECTIONS OF CERVICOTHORACIC DORSAL ROOTS IN THE CAT, WITH SPECIAL REFERENCE TO C1 FIBRES. C.K. Tan* and W.C. Wong. Department of Anatomy, National University of Singapore, Kent Ridge, Singapore 0511.

20 adult cats (1.5 - 3.0 kg) were used for the present study. All animals were anaesthetized with sodium pentobarbitone at a dosage of 30 mg per kg body weight both for operation and sacrifice. Intradural rhizotomies of C1 to T2 dorsal roots were performed through a hemilaminectomy under aseptic conditions (3 cats for C1, 2 for C2, 2 for C3, 2 for C4, 1 for C5, 1 for C6, 3 for C7, 1 for C8, 1 for T1 and 1 for T2). Sham operations were carried out in 3 cats in which C3, C5 and C8 dorsal roots, respectively, were exposed but not cut. All cats were sacrificed five days after operation by vascular perfusion with 10% formalin and processed by the Fink-Heimer method to demonstrate terminal degeneration.

The present study confirms the findings of other previous studies that the dorsal root fibres of cervicothoracic origin terminate both in the dorsal cell nest region as well as in the ventrocaudal and rostral, reticular regions of the ipsilateral cuneate nucleus. In the cell nest region, the upper cervical fibres terminated more laterally than the lower ones but the ventrocaudal and rostral regions of the nucleus, no somatotopia was observed.

After C2 and C3 dorsal rhizotomy, terminal degeneration was observed not only in the ipsilateral cuneate nucleus but also in the ipsilateral nucleus of the tractus solitarius. But after C1 dorsal rhizotomy, terminal degeneration was observed to be more widespread. Degeneration was observed bilaterally in both the cuneate and gracile nuclei and was more dense ipsilaterally. In the ipsilateral cuneate nucleus, terminal degeneration was observed in the lateralmost part of the dorsal cell nest region; in the ventrocaudal and rostral, reticular zones of the nucleus, it was less dense. In the contralateral cuneate nucleus, terminal degeneration was sparse and was observed only in the ventralmost part of the nucleus. In the gracile nucleus, no degeneration was observed rostral to the obex. In the ipsilateral gracile nucleus, terminal degeneration was most dense in the region of the obex but in the contralateral nucleus, it was sparse. Terminal degeneration was also observed throughout the entire rostrocaudal extent of the ipsilateral nucleus of the tractus solitarius. Degeneration was also observed in the dorsal part of the ipsilateral spinal nucleus of the trigeminal nerve above the rostral end of the pyramidal decussation. Sparse degeneration, was also observed in the dorsolateral part of the ipsilateral medullary reticular formation.

The present findings indicate that the first three cervical dorsal roots project not only to the dorsal column nuclei but also to a cranial nerve visceral afferent nucleus (nucleus of the tractus solitarius), and a cranial nerve general somatic afferent nucleus (spinal nucleus of the trigeminal nerve). Of particular interest is the observation that C1 dorsal root fibres, unlike others, also terminate in the medullary reticular formation. These observations suggest that the first three cervical dorsal roots, in particular C1, are concerned not only with afferents arising from the neck but possibly also with others arising from areas supplied by the cranial nerves.

This study was supported by a grant from the Singapore Turf Club.

214.2 TERMINATIONS WITHIN THE CENTRAL NERVOUS SYSTEM OF PRIMARY JOINT AFFERENTS FROM THE RAT KNEE AND ANKLE. C.N.R. Henderson* and S. Saporta. Dept. of Anat., Coll. of Med. Univ. of S. Fla., Tampa, FL 33612.

The rat has become a promising animal model for the study of rheumatoid arthritis and chronic joint pain. This has served to focus interest on joint receptors and their central terminations. We have used the transganglionic transport of wheatgerm agglutinin conjugated to horseradish peroxidase (WGA-HRP) to demonstrate the central nervous system terminations of primary afferents from the joint capsules of the rat knee and ankle. Rats of either sex were anesthetized with methohexitol sodium (50 mg/kg). Each joint capsule was injected twice, under fluoroscopic guidance (Levine, J.D. et al., Science, 226:547, 1984), with 5% WGA-HRP dissolved in radiocontrast medium (43% iothalamate meglumine). The knee was initially injected with 20 µl of WGA-HRP, followed 24 hours later by a second 20 µl injection. The contralateral ankle was given two 10 µl injections following the same time sequence. Seventy-two hours after the initial injection, each animal was anesthetized with sodium pentobarbital (40 mg/kg) and perfused transcardially with saline, followed immediately by cold 1% paraformaldehyde-2% glutaraldehyde in phosphate buffer (pH 7.4). Frozen 40 µm sections of the caudal medulla, spinal cord segments T8 through L6 (inclusive), and dorsal root ganglia L3, L4 and L5 were reacted with tetramethylbenzidine (TMB) to demonstrate the presence of WGA-HRP.

Small, medium and large perikarya were labeled in the dorsal root ganglia. Ankle injections produced a large number of labeled cells in both L3 and L4 ganglia, while only L4 was heavily labeled by the knee injections. Three distinct areas of terminal label were observed within the central nervous system from injection of either the knee or ankle joints. Knee injections produced a small cluster of terminal label in the medial portion of the medial half of spinal dorsal horn laminae I and II. Ankle injections produced a larger cluster of terminal label in the lateral portion of the medial half of laminae I and II. In some sections, terminal label from the ankle extended into lamina III. Label was present in the central portion of the nucleus gracilis caudal to the obex following knee or ankle injections. As in the spinal cord, more reaction product was present in the nucleus gracilis from the ankle injection, as compared to label from injection of the knee. Terminal label was also present in the nucleus dorsalis following knee or ankle injections. In all instances, label was only present ipsilateral to the side of joint injection.

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214.3 CALCITONIN GENE-RELATED PEPTIDE CONTAINING NERVES IN PERIOSTEUM MAY BE PRIMARY AFFERENT IN ORIGIN. Esther L. Hill and Robert Elde. Dept. of Cell Biology and Neuroanatomy, Univ. of Minnesota, Minneapolis, MN 55455

Recently, it has been suggested that peptidergic neurons play a role in the local regulation of bone mineralization. The neuropeptide vasoactive intestinal peptide (VIP) increases bone resorption in vitro, while calcitonin gene-related peptide (CGRP) has been shown to inhibit bone resorption in vitro and, at high doses, to reduce plasma calcium levels in vivo. CGRP and VIP circulate at levels too low to evoke hormonal responses from bone. However, nerve fibers may release high concentrations of neuropeptides into small areas of tissue . Sympathetic nerves with VIP- immunoreactivity innervate bone and periosteum. We sought to determine if CGRP fibers, like VIP fibers, exist in periosteum and what their origin might be.

Male rats (175-200g) were anesthetized and the lateral mandibular periosteum subjacent to the molars and beneath the deep portion of the masseter was exposed. Fast Blue or Fluorogold (2-3µl) was injected into the subperiosteal space. Fast Blue and Fluorogold were used interchangeably. In some animals injected subperiosteally with one tracer, the other was injected into the surrounding masseter muscle as a control. Animals were allowed to recover and after 4--7 days were perfused with Zamboni's fixative and tissues prepared for immunohistochemistry. Routinely, the following tissues were taken: brainstem, trigeminal, nodose and superior cervical ganglia. Cryostat sections (10µ) were incubated with antiserum to CGRP, substance P (SP), and VIP. After rinsing, sections were then incubated with fluorophore-labeled secondary antisera, rinsed and coverslipped. Sections were examined with epifluorescence and ultraviolet illumination for coexistence of peptide immunofluorescence and retrograde tracer. In other rats, fresh periosteum was removed from the mandible in the region subjacent to the molars, immersed in Zamboni's fixative, and processed for whole mount immunofluorescence for CGRP, SP, and VIP.

In all animals with subperiosteal injections retrogradely labelled cells were seen in ipsilateral trigeminal ganglia, superior cervical ganglia, and nodose ganglia. In animals injected in both the masseter and subperiosteal space with different labels, both labels were seen in cell bodies in these same areas. Additionally the label injected into the masseter was also seen in the mesencephalic trigeminal nucleus (MeV).

In animals with subperiosteal injections only, cells double-labelled with CGRP-immunoreactivity and retrograde tracer were seen only in the mandibular portion of the trigeminal ganglion. In animals injected in masseter muscle a few moderately CGRP-immunoreactive cells were also observed in the retrogradely labelled cells in MeV.

In periosteal whole mounts, nerve fibers immunoreactive for CGRP and VIP were present. In preparations using two-color immunofluorescence most CGRP- immunoreactive fibers were also immunoreactive for substance P. Although the CGRP- and VIP- immunoreactive fibers were primarily perivascular, small collaterals could be seen to depart the vascular trunk and approach the bone surface.

These data suggest that CGRP-immunoreactive nerve fibers in periosteum may be of primary afferent origin. Given the reported effects of CGRP on bone mineralization , the present results suggest that primary afferent nerves containing CGRP and SP, as well as sympathetic nerves containing VIP, may play a role in focal bone remodeling. Supported by a grant from 3M.

214.4 QUANTITATIVE STUDIES OF PEPTIDE-CONTAINING VARICOSITIES ASSOCIATED WITH PRIMARY AFFERENT NEURONS IN THE DORSAL HORN OF THE RAT SPINAL CORD. M.M.Tuchscherer and V.S. Seybold. Univ. of Minnesota, Dept. of Cell Biology and Neuroanatomy , Minneapolis, MN 55455 USA.

Many peptides have been localized in primary afferent neurons of the rat. Some of these peptides include calcitonin gene related peptide (CGRP), galanin (GAL), dynorphin A(1-8) (DYN), somatostatin (SOM) and substance P (SP). We determined the proportion of immunoreactive (IR) varicosities for each of these peptides in the spinal cord which are of primary afferent origin. In addition we determined the percentage of coexistence for each of the peptides with SP. Two groups of animals were used: one (n=6) underwent unilateral dorsal rhizotomy (L1-L6) to destroy primary afferent input to spinal segment L4; the other (n=4) received no surgery and served as a control. The contralateral side of the spinal cord from animals which underwent dorsal rhizotomy also served as a control. Tissue sections (5um) through spinal segment L4 were immunostained for SP and one of the other peptides using a method for the simultaneous visualization of two antigens by immunofluorescence. Serotonin (5-HT) immunoreactivity was also studied as a control. Computerized image processing was used to quantify the densities of the IR varicosities within the superficial laminae of the dorsal horn. For the analysis, these laminae were divided into two equal portions: Laminae I+II outer (LI/IIo) and Lamina II inner (LIIi). With respect to the total number of varicosities immunoreactive for each substance, the data from normal animals indicated that LI/IIo contained higher densities of varicosities immunoreactive for SP, GAL, SOM, CGRP, and 5HT in comparison to LIIi. The density of DYN-IR varicosities was the same in both LI/IIo and LIIi. To determine the proportion of IR-varicosities for each peptide which are associated with primary afferent neuron input, densities of IR varicosities from normal animals were compared with those determined following dorsal rhizotomy. In LI/IIo, densities of varicosities IR for SP, SOM, and GAL were reduced approximately 50% following rhizotomy. The density of CGRP-IR varicosities was reduced by 85% in LI/IIo, while the density of DYN-IR varicosities was unaffected by rhizotomy. Within LIIi following dorsal rhizotomy, no change was seen in the density of varicosities IR for GAL, SOM, DYN, or 5HT. In contrast, the density of SP-IR varicosities was reduced by one third, and the density of CGRP-IR varicosities was reduced by 90-95%.

The extent of coexistence of SP with the other substances within varicosities was also quantified. Approximately 30% of the total varicosities immunoreactive for GAL, DYN, and CGRP also contained SP in both LI/IIo and LIIi of normal animals. Following dorsal rhizotomy, densities of varicosities IR for SP+CGRP and SP+GAL were depleted from both LI/IIo and LIIi. In contrast, the density of varicosities IR for SP+DYN was unaffected in either LI/IIo or LIIi ipsilateral to the rhizotomy. Interestingly, the density of varicosities IR for SP+DYN in both LI/IIo and LIIi of the side contralateral to the lesion was reduced following dorsal rhizotomy. Five conclusions can be made from these studies: 1) GAL- and SOM-containing axons of primary afferent origin project primarily to LI/IIo; 2) CGRP- and SP-containing axonsof primary afferent origin project to both LI/IIo and LIIi; 3) Very few, of the DYN-containing axons in LI/IIo or LIIi arise from primary afferent origin; 4) Varicosities exhibiting SP+GAL and SP+CGRP coexistence are primarily of primary afferent origin; and 5) Significant amounts of SP-, SOM-, GAL-, and DYN-containing varicosities are of intrinsic or descending origin in the laminae studied. These studies were funded by NS17702.

214.5 BOTANICAL LECTINS AS HIGHLY SPECIFIC PROBES FOR VISUALIZATION OF PERIPHERAL NON-PEPTIDERGIC NOCICEPTIVE AXONS. J.D. Silverman* and L. Kruger. Depts. of Anatomy & Anesthesiology & the Brain Res. Inst., UCLA Center for Health Sciences, Los Angeles, CA 90024.

Small sensory ganglion (SG) cells giving rise to thin, predominantly nociceptive axons, comprise a biochemically and functionally diverse class of neurons whose peripheral terminals subserve a variety of afferent and efferent roles. These neurons have been roughly grouped into those containing one or more known neuropeptides vs. those displaying fluoride-resistant acid phosphatase (FRAP) activity. While the cell bodies and central terminals of these cells have been further characterized through labeling with carbohydrate-specific monoclonal antibodies and plant lectins, progress in understanding their structural and functional relations in the periphery has been limited by the difficulty in specifically visualizing the large FRAP(+), non-peptidergic axon population.

In searching for a specific marker of non-peptidergic thin fibers, we initially compared FRAP staining patterns in formaldehyde-fixed rat SG and spinal cord (SC) sections adjacent to those stained with: a) calcitonin gene-related peptide (CGRP) immunoreactivity (IR), the most prevalent of the neuropeptides; b) monoclonal antibody 2C5 IR, known to co-localize with FRAP; and c) several plant lectins specific for α-D-galactose (GAL) or N-acetyl-D-galactosamine (D-galNAc). FRAP and CGRP labeled distinct, minimally overlapping SG cell subsets, but taken together stained virtually all small cells. The less numerous 2C5(+) cells comprised a subset of the FRAP(+) population. The GAL-specific lectin griffonia simplicifolia I B_4 (GSA) labeled most FRAP(+) and 2C5(+) cells, but also co-localized with a small number of CGRP(+) cells. The D-galNAc lectin sophora japonica stained a subpopulation of GSA(+) neurons. In SC dorsal horn, CGRP labeled the marginal zone and outer substantia gelatinosa (sgel), while the lectins were primarily restricted to sgel, with FRAP and 2C5 confined to inner sgel. Therefore, using 2C5 and the lectins as probes for FRAP(+) non-peptidergic axons, we then stained whole mounts of peripheral tissues innervated primarily by thin fibers, including cornea, tympanic membrane, meninges, cerebral vessels and testicular vascular sheath. 2C5 IR axons were demonstrable in cornea, whereas both lectins readily labeled numerous presumptive thin fibers in all regions examined.

We conclude that, although the significance of distinctive carbohydrate expression by small SG cells is unclear, lectin histochemistry appears to serve as a valuable and highly specific tool for examining non-peptidergic thin fiber innervation in the periphery, and for developing a more complete taxonomy of small sensory ganglion cells. (Supported by the National Institutes of Health NS-5686 and DE-5447.)

214.6 CGRP INNERVATION OF TEETH AND JUNCTIONAL EPITHELIUM: DEVELOPMENT CORRELATES WITH UNUSUAL TISSUE SPECIALIZATION. M. R. Byers Anesthesiology & Biological Structure, Univ. Washington, Seattle, WA 98195

Trigeminal nerve fibers labeled by axonal transport innervate dentin of erupting rat molars (Byers, J.Comp.Neurol 191:431, 1980) and develop extensive innervation of molar dentin and adjacent junctional epithelium (JE) (Byers and Kish, J. Dent. Res. 55:419, 1976; Byers and Holland, Anat. Rec. 188:519, 1977). A subpopulation of those fibers have calcitonin gene-related peptide-like (CGRP) immunoreactivity (Silverman et al, Anat. Rec. 214:122A, 1986). Developing rat molars and JE have been studied here to determine the initial sites of CGRP innervation and subsequent development in relation to tissue maturation. Molars of rats aged 21,28, 35, or 49 days were compared with those of 3-6 mo. old adults. Anesthetized rats were perfused with p-formaldehyde/picric acid and the excised jaws were post-fixed, decalcified, frozen sectioned and reacted by standard immunocytochemistry for CGRP, using primary antisera raised against synthetic CGRP, 0.5% Triton-X 100, avidin-biotin and diaminobenzedine, with appropriate controls.

Just before rat molars erupt, dentin begins to be innervated by CGRP fibers. The developing innervation patterns are not symmetrical in each cusp but focus at particular regions characterized by columnar odontoblasts, wide predentin, and numerous patent dentinal tubules. Maxillary and mandibular first molar cusps are oriented at opposing angles; innervated patches are most prominent on the side closest to the occlusal plane-.i.e. the anterior side for max. cusps and posterior side for mand. cusps. As the maturing teeth are used, their surfaces wear down exposing larger areas of crown to occlusal contact. The CGRP innervation territory increases to match the progressively wider tooth surfaces. The number of dentinal tubules, their patency, and the continual outflow of fluid from pulp through dentin are greatest in the innervated zones. Junctional epithelium (JE) is specialized to allow leukocyte transmigration into the oral cavity and fluid outflow, in addition to tooth attachment to gum. CGRP innervation is present as soon as JE begins to develop (during tooth eruption), and reaches mature density when JE is fully formed in young adults. The extent of JE and its CGRP innervation do not change in older adults.

CGRP sensory fibers have been shown to be involved in vasodilation and neurogenic inflammation in other tissues, in addition to nociceptive functions. The patterns of CGRP innervation in developing rat molars and junctional epithelium appear to correlate with sites of greatest fluid outflow from those tissues. Since dental crowns and JE are continually exposed to oral bacteria, their CGRP innervation may be involved in regulating the response to infection. In addition, the vitality of dentin and JE may depend on special fluid dynamics, which could be influenced or controlled by the CGRP innervation. Finally, the fluid outflow in dentin and JE may simply form a permissive environment allowing CGRP fibers to innervate for somatosensory functions. (Supported by NIH grant DE05159.)

214.7 ALTERATIONS IN MYSTACIAL DEVELOPMENT: CHANGES IN THE SPATIAL SEQUENCE FOLLOWING MANIPULATION OF THE TRIGEMINAL GANGLION.
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It has been shown that for some peripheral structures (e.g., rete ridges and muscle spindles), proper development is dependent on sensory innervation. Critical periods exist in which manipulation of the nervous innervation of these structures results in a significant alteration in their normal development, and for certain structures, sensory innervation is essential for initiation of development. The present study was undertaken to determine the relationship between sensory innervation from the trigeminal ganglion and normal development of mystacial vibrissae.

The model takes advantage of the precocious birth and relative immature development of the neonate opossum, Monodelphis domesticus. The neonate opossum is an "extrauterine embryo" roughly equivalent to a rat embryo of 15.0 days or a mouse embryo of 13.5 days. Manipulation of the neonate nervous system effectively alters the normal trigeminal influence at a time before development has been completed. In this manner, we can assess the role of sensory innervation on the development of peripheral structures.

Monodelphis domesticus pups were operated on 18-24 hours after birth; the delay helped prevent cannibalization. The left trigeminal ganglion, located immediately below the skin surface in the neonate, was removed by surgical cauterization with no adverse affect on feeding observed.

Vibrissal development on the lesioned side was altered. Follicles of the rostral part of the horizontal rows were neither found in the characteristic pattern nor the normal number of structures seen in the adult, although every animal demonstrated follicular development. Rete ridge development, characteristic of glabrous snout skin, failed to develop on the operated side.

We conclude that sensory innervation is not critical for the initiation of mystacial development, but does play an important role in determining the spatial sequencing of follicular development.

214.8 RESPONSES OF LAMB SPINAL TRIGEMINAL NUCLEUS NEURONS TO MECHANICAL, THERMAL AND CHEMICAL STIMULATION OF THE ORAL CAVITY AND EPIGLOTTIS. R.D. Sweazey and R.M. Bradley. Dept. of Oral Biology, Sch. of Dentistry, Univ. Michigan, Ann Arbor, MI 48109.

We have shown previously that neurons in the lamb nucleus of the solitary tract (NST) receive converging inputs from the oral cavity and epiglottis, and respond to more than one stimulus modality. We have now recorded neural responses from an area of the spinal trigeminal nucleus (SPVn) where primary afferent fibers from the oral cavity and epiglottis terminate. The locations of receptive fields were determined for each neuron and its response to mechanical, thermal and chemical stimuli recorded.

Forty-eight neurons with receptive fields on the tongue, palate or epiglottis were isolated in the dorsomedial interpolaris and caudalis subnuclei of SPVn. Thirty-seven percent of the cells had receptive fields restricted to the tongue, 39% to the palate, and 13% of the neurons responded to stimulation of the epiglottis. Only 10% of SPVn neurons received converging inputs from anatomically separate receptive fields. This is a smaller percentage than that observed for lamb NST neurons (17%). Furthermore, neurons with opposing receptive fields observed frequently in the NST were rarely found in SPVn.

All neurons isolated in the SPVn responded to either mechanical or thermal stimuli. No multimodal responses were observed. In addition, no responses to stimulation with 0.5 or 1.0 M KCl, NH_4Cl, NaCl, 0.01 N HCl or water (epiglottis) were observed in SPVn neurons. The most effective stimulus for SPVn neurons was mechanical which produced responses in 80% of the cells. The majority of these neurons had a rapidly adapting response. The remaining neurons responded to thermal stimulation showing increases in activity to cooling and decreases to warming. These results are quite different from those observed in the NST where over 69% neurons were multimodal.

While the presence of convergence and multimodal neurons in the NST suggested an important role in the integration of sensory information important to upper airway reflexes, the area of the SPVn sampled in the present study appears to function as a modality specific relay for mechanical and thermal information from the oral cavity and epiglottis.

Supported by NIH Grant DE05728.

214.9 THE JAW-OPENING REFLEX EVOKED BY TOOTH-PULP STIMULATION IN THE RAT AND THE EFFECT OF NEONATAL CAPSAICIN TREATMENT.
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Rats were prepared under general anaesthesia (alphaxalone and alphadolone acetate; Saffan, Glaxo Laboratories) by placing wire electrodes into one anterior digastric muscle and a stimulating electrode into each lower incisor (Matthews,B. and Myslinski,N., J.Physiol. 365:14P,1985). We have confirmed previous findings that a long latency (20-45ms, depending upon stimulus intensity) digastric reflex is produced by electrical stimulation of nerves in the incisor pulp and that a shorter latency (6-14ms) reflex replaces this when the stimulus spreads to excite nerves in the periodontal tissues. The reflex due to stimulation of pulpal nerves is seen only under optimal conditions and the short latency response alone may be obtained if the electrodes are placed too close to the gingival margin, the animal is too deeply anaesthetized or if it has been subjected to more than minimal surgery, as noxious stimulation even in remote areas of the body depresses the pulpal reflex for several hours. In several recent papers it is implied that the short latency reflex may, at least in part, be due to pulpal nerves (e.g. Tal,M., Behav. Brain Res. 13:197,1984; Vassel,A. et al., Archs oral Biol. 31:159, 1986).

Almost all the nerve fibres throughout the length of the pulp of the rat incisor are unmyelinated (Bishop,M.A., Am. J. Anat. 160:213,1981), which would account for the long latency of the reflex they evoke. Neonatal capsaicin treatment is known to destroy a large proportion of the non-myelinated axons in peripheral nerves. However, contrary to the results of Tal, we were able to evoke long latency reflexes by stimulating incisor pulp in 5 rats that had been treated neonatally with capsaicin (50mg/kg I.P.). There were no significant differences between the thresholds of the reflex in these animals and in untreated, age-matched controls. Counts of the numbers of unmyelinated fibres in the saphenous nerves and incisor tooth-pulps are in progress and preliminary results indicate that the numbers in the saphenous nerves were reduced markedly in the treated animals. It has been shown that the ratio of the numbers of unmyelinated to myelinated fibres in the pulps of rat molars is not affected by capsaicin treatment (Holje,L. et al., Brain Res., 266:133,1983).

The results could be explained if either rat tooth-pulp was innervated by C-fibres which were resistant to capsaicin or, at least in the case of the afferents responsible for the jaw-opening reflex from incisor pulp, they were myelinated outside the tooth but had unmyelinated terminals throughout the length (appox. 20mm) of the pulp.

214.10 RESPONSES OF UNMYELINATED NOCICEPTIVE AFFERENTS TO HISTAMINE.
A. A. Khan*, R. A. Meyer, and J. N. Campbell. Dept. Neurosurgery and Applied Physics Lab., Johns Hopkins U., Baltimore, MD 21205

The peripheral neural basis for itch sensation is not well understood. Although several lines of evidence indicate that itch is signalled by activity in C-fibers, a specific cutaneous receptor which responds preferentially to pruritic agents has not been identified. In this study, we sought to determine if a subclass of C-fiber nociceptors preferentially responded to a pruritic substance. Standard teased-fiber techniques were used to record single fiber activity from 24 C-fiber nociceptors in monkey with cutaneous receptive fields sensitive to both mechanical and heat stimuli (CMHs). Intradermal injections of 5 nmoles (1 μg) of histamine dihydrochloride in 10 μl of normal saline (pH = 7.4) were used as the pruritic agent. Similar injections in human subjects have been reported to produce itch sensation without pain (Simone, et al., Somatosensory Res., 1987). Two of the 24 CMHs in this study developed a pronounced response following the injection. Immediately after the injections, both fibers responded with rates as high as 10 impulses/s. Within 30 s, the response decreased to a plateau with an average response of 2 impulses/s for the next 5 min. The total discharge during the five minutes after the injection was 656 and 674 impulses for these two fibers. The response waxed and waned during this time with responses ranging from 0 to 26 impulses per 5 s sampling interval. During the next 5 minutes, the response gradually decreased to a rate of approximately 1 impulse/s. In contrast, the other 22 CMHs responded markedly less to the histamine. The total discharge of these fibers during the 5 min. after the injection ranged from 0 (n=5) to 103 impulses (mean ± SEM = 24 ± 7 impulses). The two histamine-sensitive CMHs did not differ from the other 22 CMHs with respect to mechanical or heat threshold, receptive field size, or conduction velocity. We previously reported that C-fiber nociceptors could be classified into two distinct groups based on their quickly adapting (QC) or slowly adapting (SC) response to stepped heat stimuli (Meyer and Campbell, Brain Res., 1981). Fibers in both groups responded to histamine, though there were more low-responders (i.e., 5 impulses during 5 min. after injection) in the QC group (7 of 10) than in the SC group (2 of 14). We conclude that a small subset of the CMH population exhibit a high sensitivity to histamine and likely signal itch sensation. Further studies are needed to determine the prevalence of these histamine-sensitive CMHs and whether they have other distinguishing properties. (Funded by N.I.H. grant # NS-14447)

214.11 EFFECT OF BRADYKININ AND [DES-ARG9]-BRADYKININ ON CULTURED HUMAN AND MOUSE DORSAL ROOT GANGLION NEURONS. D. M. Rock, C. P. Taylor, and M. J. McLean Dept. Pharmacology, Warner Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor MI 48105 and Dept. Neurology, Vanderbilt Univ., Nashville, TN 37232.

Bradykinin (BK) is a naturally occurring nine-amino-acid peptide that is enzymatically formed following tissue injury. Experiments were undertaken to study the response of cultured dorsal root ganglion neurons to BK.

Dorsal root ganglion neurons were obtained from humans by selective ganglionectomy for intractable pain (experiments approved by Vanderbilt Hospital Human Use Committee) or from mature mice and were maintained in primary dissociated culture for 4 to 16 weeks. Conventional intracellular recording techniques were used and BK or [des-Arg^9]-BK were applied by pressure ejection from blunt micropipettes within 100 μm of the impaled cell. Neurons with electrophysiological properties similar to those of A- and C-fiber dorsal root ganglion neurons of intact ganglia were observed in cell culture. A-like neurons fired single tetrodotoxin-sensitive action potentials of less than 2 msec duration in response to 450 msec depolarizing current steps. C-like neurons fired action potentials (3-8 msec duration, tetrodotoxin-resistant) throughout 450 msec depolarizations. Both A- and C-like cells were quiescent at rest.

Application of BK (5-10 sec, 10^{-7} M) did not change resting potential or elicit action potentials in 5 of 5 mouse A-like cells. BK ejection (10^{-9} to 10^{-6} M) caused concentration-dependent depolarizations (up to 20 mV) in 21 of 24 human C-like cells that began after 2-4 sec and lasted up to 120 sec after BK application. BK responses were usually reduced in amplitude with repetition and could not be repeated at intervals less than about 3 min. Rapid trains of action potentials (up to 80 Hz) were usually elicited. Similar responses were seen with mouse dorsal root ganglion neurons. Application of 10^{-6} M [des-Arg^9]-BK (a selective agonist of B1 bradykinin receptors) had no effect on 4 human C-like cells that responded to BK.

These findings suggest that the response of C-like dorsal root ganglion neurons to bradykinin is mediated by B2 bradykinin receptors. Cultured dorsal root ganglion neurons may be a useful model to study cellular mechanisms of sensory transduction and inflammation mediated by BK.

214.12 DIFFERENTIAL EFFECTS OF POTASSIUM AND CALCIUM CHANNEL BLOCKERS ON ACTION POTENTIALS OF FROG DORSAL ROOT GANGLION (DRG) NEURONS. S. David Stoney, Jr., Dept. of Physiology & Endocrinology, Med. Coll. of Georgia, Augusta, GA 30912

Somatic action potential (AP) shape and axon conduction velocity (CV) of frog sensory neurons correlate with the least conduction interval for pairs of impulses through their axon branchpoint in DRG. Branchpoints of neurons with fast, smooth somatic APs had short least conduction intervals (LCIs) regardless of axonal CV. Neurons whose APs had a hump on the falling phase had significantly slower CVs and longer LCIs (Stoney, Neuroscience Letters 59, 1985). These in vitro experiments on Rana pipiens DRG neurons aimed to establish ionic determinants of AP shape. Most were carried out on F neurons (fast, smooth AP; average CV=22.6 m/s; average LCI=1.8 msec), H1 neurons (fast AP with -dV/dt inflection; CV=27.6 m/s; LCI=1.7 msec) and H2 neurons (significantly broader AP with hump on falling phase; CV=17.7 m/s; LCI=2.2 msec). APs were recorded in 5-10mM Mn^{++}, 10mM TEA, combined TEA & Mn^{++}, and in TEA & 10mM Ca^{++}. Mn^{++} eliminated the shoulder or -dV/dt inflection on the falling phase and significantly increased the maximum rate of repolarization (Rmax = -dV/dtmax) for H1 & H2, but not F, neuron APs. TEA significantly increased AP duration and decreased Rmax for all neurons, but enhanced or caused the appearance of or a hump only for H neuron APs. H1-Ca^{++} increased the duration of H neuron TEA APs by enhancing the shoulder. Mn^{++} significantly decreased duration and increased RFmax of TEA APs only for H1 & H2 neurons. The results suggest that Ca^{++} involvement in somatic spikes is ordered H2>H1>F. Mn^{++} did not eliminate the hump on H4 neuron APs (very broad; CV=0.36 m/s; LCI=9.1 msec). Experiments are underway to see if H4 neurons have TTX-insensitive Na^+ channels like C-fiber DRG neurons of mice (Yoshida & Matsuda, J. Neurophysiol. 42, 1979). Yoshida has suggested that TTX-insensitivity and Ca^{++} components in the somatic spike are indicative of relatively undifferentiated types of DRG neurons. If that is the case, then relatively undifferentiated neurons, at least in frog DRG, can also be regarded as relatively poor neural signalers. The maximum frequency of APs they can conduct to the CNS ($<LCI^{-1} \times 1000$) is limited by a high degree of filtering action imposed by their axon branchpoints. (Supported by BRS 2 S07 RR 05365 23)

214.13 DEPENDENCE OF C-FIBER PRIMARY AFFERENT AND MAST CELL-EVOKED PLASMA EXTRAVASATION ON SYMPATHETIC POSTGANGLIONIC NERVE TERMINALS. T.J. Coderre, A.I. Basbaum and J.D. Levine*. Depts. of Medicine & Anatomy, Univ. of California, San Francisco, CA 94143.

Although plasma extravasation can be produced by substances released from the peripheral terminals of C-fiber primary afferents (PAs), and mast cells (MCs), the underlying mechanisms are unknown. In this study we assessed the extent of plasma extravasation produced by pharmacological activation of C-fiber PAs and MCs, in rats in which either PAs, MCs or sympathetic postganglionic neurons (SPGNs) were eliminated.

We used a push pull perfusion (rate: 0.15 ml/min) of the rat knee joint to study extravasation of Evans Blue (50 mg/kg, i.v.) in response to PA stimulation by capsaicin (5 mg/ml) or MC degranulation elicited by compound 48/80 (0.1 mg/ml). These doses produced equivalent extravasation, as measured by absorption at 650 nm. To destroy PAs, rats were treated with capsaicin (50 mg/kg, s.c.) on neonatal day 2. To deplete MCs, a total dose of 0.25 mg i.v. of compound 48/80 was injected over 3 days, ending two days before testing. To deplete SPGNs, 6-OHDA was injected over 7 days (50 mg/kg day 1 & 2; 100 mg/kg day 6 & 7, i.p.), ending one day before testing.

Compared with untreated rats, the peak of capsaicin evoked extravasation was reduced by 48% in rats pretreated with neonatal capsaicin, by 67% with compound 48/80 and by 47% with 6-OHDA. Compared to controls, the extravasation induced by compound 48/80 was reduced by 78% in rats pretreated with compound 48/80 and by 65% with 6-OHDA, but was increased by 2.5% in rats pretreated with neonatal capsaicin.

The data not only confirm a dependence of PA effects on MCs but, importantly, suggest that PA and MC effects are dependent on the presence of intact SPGNs. We, therefore, repeated the studies using pharmacological activation of SPGNs with 6-OHDA (50 mg/ml) and tyramine (25 mg/ml). Extravasation in response to sympathetic stimulation, by either agent, was not significantly reduced by a combined pretreatment with neonatal capsaicin and compound 48/80 (18% and 6% reductions for 6-OHDA and tyramine, respectively) but was significantly reduced--in comparison to control rats--by 58% and 82%, respectively, in 6-OHDA sympathectomized rats.

These data support previous studies which indicate that substances released from primary afferents can degranulate MCs. In addition, they suggest a focal role for the SPGN in the production of plasma extravasation. Together, the data suggest that there is a sequential and unidirectional activation of C-fiber PAs, MCs and SPGNs in the production of plasma extravasation.

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214.14 **SPATIAL FREQUENCY TUNING IN CUTANEOUS TYPE I MECHANORECEPTORS**, Fred J. Looft, Department of Electrical Engineering, Worcester Polytechnic Institute, Worcester MA 01609

Swept period grating stimuli were used to study cat, hairy skin, slowly adpating Type I (T1) mechanoreceptors. These receptors are of interest because of their similarity to the primate SA I units, their multiple dome puncate organization and the possibility of action potentials (APs) from one dome resetting and/or blocking the AP responses of other domes. It was hypothesized that this would result in the receptors being tuned to specific periods of a grating stimulus.

The swept period grate had 39 periods (40 grates), a minimum period of 0.25 mm and a maximum period of 2.0 mm over a stimulus length of 40 mm. The period between successive sets of grates was a constant distance increment larger than the period between a previous set of grates. Different scan directions and velocities were used to study directional and spatio-temporal frequency response sensitivities. Data analysis consisted of the generation of cycle histograms showing the probability of a unit firing as a function of stimulus position.

Single domes exhibited no spatial frequency tuning and could easily encode the minimum grate period (250u) for all scan velocities (10-80mm/sec) and scan directions. Multi-dome receptors exhibited a spatial frequency tuning characteristic that was dependent on the scan diretion, the scan velocity and the number and organization of a receptor's domes. Under ideal conditions, the matched spatial frequency encoding characteristics of two dome units could be predicted from the spatial organization of the domes.

A computer program was developed to model the tuning characteristics of two dome T1 receptors. The model was based on the single assumption that the first dome to generate APs completely blocked the AP responses of the other dome. Although not exact, the model results compared favorably with the results from several experiments. For other data sets, the experimental results could not be fitted or predicted form the model.

While the T1 and primate SA I units are not identical, it is hypothesized that the more graded SA I response profiles will result in less definition to the tuning regions, but that they will still exist.

214.15 REPRESENTATION OF GRATINGS IN THE FINE STRUCTURE OF MECHANORECEPTIVE AFFERENT DISCHARGE. K. Sathian*, A.W. Goodwin, K.T. John* and I. Darian-Smith. Department of Anatomy, University of Melbourne, Parkville, Victoria 3052, Australia.

When gratings are run sinusoidally across the monkey's fingerpad, the mean response rate, in all three major classes of mechanoreceptive afferents innervating the fingerpad - slowly adapting (SA), rapidly adapting (RA) and Pacinian (PC) afferents, increases as a monotonic function of groove width (Sathian et al, Soc. Neurosci. Abstr. 12: 90.2). Apart from the mean afferent response rate, information can also be conveyed by the fine structure of afferent discharge. Gratings were run sinusoidally across the fingerpad of anesthetized macaque monkeys, with a constant indentation amplitude of 1.0 mm. The groove width (G) of the gratings was varied systematically while keeping ridge width (R) constant, and vice versa. The motion, being sinusoidal, varies in speed throughout the sinusoidal cycle. However, over the central 42-degree segment of each half-cycle, the speed is constant to within 6.6% of its peak value. These segments were chosen for systematic analysis of the fine structure of afferent discharge. Since grating temporal frequency f is given by s/P where s is speed and P is spatial period, and since P = G+R, f decreases as G (or R) increases when s is constant. In order to separate the effects of temporal and spatial parameters, the gratings were run both at constant s and at constant f.

Afferent responses to moving gratings are phase-locked to the occurrence of successive grating spatial cycles. A measure of the fine structure of afferent discharge that lends itself to quantitative analysis is the mean number of impulses per grating spatial cycle (ipg). The mean number of ipg increased as a monotonic function of groove width when ridge width was constant. This held whether s or f was constant, for all three afferent classes. The mean number of ipg increased as a monotonic function of ridge width when groove width and s were constant, in the case of RAs as well as SAs. When groove width and f were constant, however, the mean number of ipg was relatively invariant as a function of ridge width for these two afferent types, indicating that f rather than ridge width was represented in this measure of response. PCs did not fit into this pattern of response. Thus the stimulus parameters represented in the mean number of ipg, for RAs and SAs, are groove width and grating temporal frequency. It was possible to describe the mean number of ipg concisely in terms of these two parameters by simple empirical models.

214.16 SELECTIVE ACTIVATION OF THERMAL NOCICEPTORS BY RADIANT ENERGY --- A THERMODYNAMIC MODEL. D. L. Tanelian, and J. D. Murphy*. Dept. of Anesthesia, Stanford Univ. Sch. of Med., Stanford, CA 94305 and NASA Ames Research Center, Moffitt Field, CA 94035

A method to noninvasively and selectively activate nociceptors has been difficult to achieve, but would be of great value in studying the transduction process of sensory afferents and also integral to the development of nociceptive sensory evoked potentials. Radiant energy can produce a thermal noncontact stimulus with a rapid rise time and be easily controllable for energy delivered, duration and stimulus area. Because the receptor endings of sensory afferents are located below the surface of cutaneous tissues and at a superficial depth, determination or prediction of the temperature rise at the receptor portion of the nerve ending is difficult to obtain and influenced by the wavelengh of energy used and the thermal transport and optical properties of the tissue.

To determine the ideal set of stimulus parameters necessary to activate thermal nociceptors in a given tissue, we have developed a mathematical model which can calculate the temperature distribution in tissue following a pulse of radiant energy. The thermodynamic equation used is the basic one-dimensional Fourier heat conduction law , $\partial T/\partial t = a^2 \partial^2 T/\partial x^2 + I/\rho c$ where $I = pHe^{-px}$ is the energy deposition per unit volume in a semi-transparent medium by laser light. The boundary conditions and initial conditions are : at t=0, $T=T_0$ for all x; at x=0 , $-\partial T/\partial x = h/k\,(T_{surface} - T_{ambient}) + \epsilon \sigma T^4$ surface ; at $x = x_{max}$, $T = T_0$ for all t . T_0 = tissue temperature, t = time, x = distance measured from the tissue surface, p = absorption coefficient, a = thermal diffusivity, ρ = the tissue density, σ = the Stefan-Boltzmann constant, ϵ = emissivity, c = specific heat, H =power density per unit area , h / k = surface heat transfer coefficient .

The accuracy of the one-dimensional approximation depends on the fact that the depth of concern (0-200um) is very small compared with the radius (2-5mm) of the area to be stimulated. A numerical solution based on the Crank- Nicolson time marching procedure is used and spatial discretization is carried out by means of splined cubic Hermite polynomials. The resulting matrix is solved by a special solution technique which takes advantage of the special sparcity structure resulting from the Hermite formulation (Murphy and Prenter, 1985).

Solving the conduction equation with and without the convective and radiative heat loss terms demonstrates that > 99% of the heat loss following a pulse of radiant energy is due to conductive heat loss alone. By using the appropriate thermodynamic values for skin we are able to identify the combinations of stimulus parameters necessary to reach the thermal nociceptive threshold of 45° C .

Variation of the absorption coefficient (p), which is a function of wavelengh for a given tissue, enables maximization of heat deposition at the receptor terminals while minimizing energy delivery to adjacent tissue elements. This wavelengh optimization allows for minimal tissue damage,temporally discrete receptor activation and minimizes bulk tissue heating after multiple stimulus applications. A value of 50-100 cm^{-1} for p maximizes heat deposition to nociceptive receptors located at 150-200um below the surface of human skin.

With this model one can design a radiant energy source with the appropriate stimulus parameters (wavelengh, pulse duration, rise-time, interstimulus interval, power input) to activate thermal nociceptors in any tissue type for the study of pain transduction and perception.

214.17 THE EFFECT OF VOLUNTARY ISOMETRIC CONTRACTION ON THE DETECTION THRESHOLD OF ELECTRICAL AND PAINFUL THERMAL STIMULI IN MAN. J.S.Feine*, C.E.Chapman, M.C.Bushnell, G.Duncan and J.P.Lund. Département de stomatologie et Centre de recherche en sciences neurologiques, Univ. Montréal, Québec H3C 3J7.

Previous experiments have shown that the ability to detect the presence of weak electrical shocks is diminished during isotonic movement of the stimulated arm (Chapman et al., Exper. Brain Res., In press). Although the suppression is much greater during active than passive movement, it is not known if the sensory threshold changes during motor acts in which there is little movement about the joints. Indeed, cortical evoked potentials that are reduced by movement are reported to be unchanged during isometric contraction (Lee, R.G., and White, D.G., EEG Clin. Neurophysiol., 36: 53, 1974). Thus, in an initial series of experiments, we compared the effects of isotonic and isometric contractions about the elbow at a frequency of 0.5 Hz on the ability to detect the occurrence of weak electrical shocks applied to the ipsilateral forearm and hand (n=8 subjects). The results indicate that the detection of stimuli applied to the forearm is signicantly reduced during isometric contractions of muscles acting about the elbow (Wilcoxon, $p < 0.01$), although not to the extent occurring during isotonic movement. The effects on stimulation of the dorsum of the hand were weaker, but again more pronounced during isotonic than isometric movement ($p < 0.02$). In conclusion, it appears that isometric, as well as isotonic, contractions modify the perception of electrical shocks, and that this effect increases with proximity to the fulcrum.

Since sensory fibers of several modalities are simultaneously activated by an electrical stimulus, the functional significance of these results is unknown. Therefore, we have begun to study the effects of movement upon the ability to detect natural events by stimulating small-diameter fibers using near-painful and painful heat (43° to 48°C). Isometric contractions were performed with the right arm as described above, and subjects were asked to detect pain and to rate the intensity of the thermal stimulus. Preliminary results indicate that the pain threshold increases during muscle contraction. These results suggest that the reduction in sensory transmission is not restricted to pathways conveying information from large diameter cutaneaous afferents.

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214.18 TACTILE DISCRIMINATION OF SOFTNESS. M.A. Srinivasan*, J.M. Whitehouse*, and R.H. LaMotte (SPON:J.G. Collins) Dept. of Anesthesiology, Yale University Sch. of Med., New Haven, CT 06510.

A measure of the softness of an object is the ratio of the depth of indentation of the object to the force causing the indentation. During active touch, information about both the force and indentation depth of the object is available to the subject through sensory receptors in skin, joint and muscle. In contrast, when an object is applied to the passive skin surface, the subject can assess the force from the cutaneous receptor response, but the depth of indentation of the object is unknown. In order to determine the importance of non-cutaneous cues in judging softness, we investigated the capacity of humans to discriminate softness under both active and passive touch.

Twelve transparent cylindrical disks (35mm dia. x 10mm thick) made of silicone rubber of varying softness (controlled by varying the amount of a diluent) were cast. Objective measures of softness, or compliance, were determined by measuring the average slopes of force versus displacement traces obtained by constant velocity indentations with a rigid, flat probe (6mm diameter).

When human subjects actively indented each specimen with the fingerpad, their subjective ranking of softness was the same as the ordering based on the objective measure of compliance. Five specimens (2.8, 4.1, 5.7, 9.2, and 10.2 μm/gm), 100% discriminable with active touch, were chosen for passive touch discrimination. In pairwise discrimination tasks, each specimen of the pair was brought onto the subject's stationary fingerpad at a constant velocity, maintained steady for 1 s and then withdrawn. The subject stated which one of the pair was softer. Subjects could discriminate only the specimens 2.8 and 10.2 from the 5.7um/gm specimen at levels greater than 75% correct. In separate tests, when one member of each pair was applied at a slower or faster velocity than the other, threshold discriminations of softness were not possible even between specimens of 2.8 and 5.7 um/gm compliance. Thus human discrimination of softness was significantly better under active than passive conditions. Passive discrimination deteriorated when indentation velocity was varied.

In order to determine the relevant cutaneous cues for discrimination, contact areas between the passive fingerpad and the specimens were videotaped. Off-line analyses indicated that the variation in contact area versus time for the specimens was quite small, but the corresponding traces of total force (and thus pressure) versus time were distinct from each other. Thus active softness discrimination may be based on the combined responses of mechanoreceptors in skin (encoding the rate of change of pressure) and joint or muscle (providing the velocity of indentation of the object). The absence of the proprioceptive information leads to a considerable deterioration of discriminability.(PHS grant 15888).

214.19 EFFECT OF TIMING OF INSPIRATORY OCCLUSION ON SPINAL AND CEREBRAL EVOKED POTENTIALS. W. Robert Revelette and Paul W. Davenport*. Dept. of Physiology and Biophysics, Univ. of Ky., Lex, Ky. 40536 and Dept. of Physiological Sciences, College of Vet. Med., Univ. of Fla., Gainesville, Fla. 32610.

Davenport et al. (J. Appl. Physiol. 60:1843-1848, 1986) reported that interruption of airflow (occlusion) from the onset of inspiration (OCC-ONSET) was associated with cerebral evoked potentials in the awake human subject. Revelette et al. (Fed. Proc. 46:826, 1987) found that mid-inspiratory occlusion (OCC-MID) was associated with spinal and cerebral evoked potentials. The peak latencies of the cerebral potentials were similar to those reported by Davenport et al. It is unclear from these studies whether there is any significant difference in the results obtained using these two methods.

This study was designed to determine if the abrupt decrease in mouth pressure (and rise in inspiratory muscle pressure) associated with OCC-MID alters the amplitudes and peak latencies of spinal and cerebral evoked potentials, and the latency for detection. The present study involved random presentations of 64 OCC-ONSET and 64 OCC-MID to five subjects. Recordings (bandpass 3-300 Hz) were made from electrode pairs Cz-C3, Cz-C4 (scalp) and Fz-spC5 (spinal). Data were stored and averaged by computer. Occlusions were performed by the computer using a Rudolph balloon occlusion assembly connected to the inspiratory port of a two-way non-rebreathing valve. The results indicated there was no difference in the mean peak latencies of the evoked potentials recorded at electrodes Cz-C3 and Cz-C4. The amplitudes of the potentials recorded from Cz-C4 were 30% greater than those recorded from Cz-C3. There was no difference in the mean peak latencies of the evoked potentials for OCC-ONSET and OCC-MID. However, the amplitudes of the potentials were 205% greater for the data recorded during the OCC-MID. The mean detection latency was 310 msec for OCC-ONSET and 245 msec for OCC-MID. From the results of this study it appears that: the afferents responsible for the initial potentials project bilaterally to the cerebral cortex; despite the more abrupt stimulus, the peak latencies for cerebral and spinal potentials are not shorter with OCC-MID; OCC-MID produces evoked potential waveforms of significantly greater amplitude. We suspect that the greater amplitude with OCC-MID is the result of recruitment of afferents and/or elevation of the activity of responsive afferents. Supported by UK PSP RR05374 and NIH HL33163.

214.20 EFFECTS OF SEXUAL AROUSAL ON RESPONSES TO DORSAL PENILE NERVE STIMULATION. S. Hendricks, B. Graber, D. Fitzpatrick*, J. Gartner*, M. Fleisher*. Dept. of Psychiatry, Univ. of Nebr. Med. Ctr., Omaha, NE 68105.

Electrophysiological measures were obtained in response to percuteneous stimulation of the dorsal penile nerve (DPN) and the posterior tibial nerve (PTN) stimulation in human males before and during sexual arousal, and after orgasm. Stimulation of the PTN was by 5 mm silver disk electrodes 2 cm apart, applied at the ankle. DPN stimulation was via 5 mm tin disk electrodes taped to the glans of the penis. The subject initiated stimulus delivery by button press and was instructed not to stimulate faster than once every two seconds. Recordings were obtained from segments of the nerves proximal to stimulation sites and from the scalp. Measures were obtained during a baseline condition, an arousal condition with full erection, a condition just after ejaculation, and a condition 15 minutes after ejaculation.

A marked decrease in the amplitudes of the somatosensory evoked potentials measured at the scalp to DPN but not PTN stimulation was seen during penile erection. Subjects also reported reduced sensation to DPN stimulation during arousal, possibly related to the degree of erection. Further results to be presented will be from evoked potential to DPN stimulation on the shaft of the penis during the same conditions and effects of these conditions on conduction velocities in the DPN.

SPECIAL LECTURE

215 SPECIAL LECTURE. EXCITATORY NEUROTRANSMISSION. Charles F. Stevens, Yale Medical School, New Haven, CT.

Excitatory neurotransmission has been studied in cultures of hippocampal neurons using whole cell and single channel recording. These neurons express several classes of excitatory amino acid receptors and develop synaptic connections that can be studied in identified pairs of cells.

217 SYMPOSIUM. MOLECULAR GENETIC ANALYSIS OF NEURONAL DEVELOPMENT. C.S. Goodman, Stanford Univ. (Chairperson); S. Artavanis-Tsakonas*, Yale Univ.; Y.N. Jan, U.C.S.F.; G. Rubin, U.C. Berkeley.

The last few years have witnessed great progress in the application of molecular genetic approaches to the study of neuronal development, particularly in simple organisms with advanced genetics such as Drosophila and the nematode. Here we focus on four examples of such developmental studies using a simple genetic system: the fruitfly Drosophila.

We will first examine the development of the embryonic nervous system. We begin with two talks on the molecular mechanisms controlling neurogenesis. The first talk by Artavanis-Tsakonas will focus on neurogenic genes such as Notch which are involved in the decision of whether a cell becomes a neuronal precursor cell (neuroblast) or a non-neuronal cell in the CNS; he will also describe the expression of other genes which share the EGF sequence homology with Notch and which are transiently expressed by subsets of cells during neurogenesis. The second talk by Jan will describe genes such as Cut which are involved in the decision of which types of neurons a neuronal precursor cell generates in the PNS; he will focus on the network of genes that are involved in specifying cell fates and the overall pattern of neurons in the developing PNS. The third talk by Goodman will describe genes such as fasciclin I and III which encode surface glycoproteins which are expressed on subsets of axon fascicles during development; genetic analysis indicates that these proteins are involved in the recognition of specific axonal surfaces by neuronal growth cones. The final talk by Rubin will examine many of these same events during the development of the visual system, focusing in particular on genes such as sevenless which are involved in the cell surface interactions which specify particular neuronal cell types. He will also discuss genes such as disco which are involved in guiding retinal growth cones towards their targets.

218 WORKSHOP: INFORMATION PROCESSING IN THE MACAQUE RETINA

Robert Shapley, New York University (Chairman); David Williams*, University of Rochester; William Merigan, University of Rochester, V. Hugh Perry, Oxford University; Ehud Kaplan, Rockefeller University; Stan Schein, Harvard Medical School; Barry Lee, Max-Planck-Institut Goettingen

The initial stages of vision seem remarkably similar in Old World monkeys and man. This similarity has stimulated a surge of research on the visual pathways of the macaque monkey and in particular has motivated speakers in this Workshop to study the monkey's retina as a model for the human retina. Several characteristics of human visual perception may be explicable in terms of retinal processes and structures. One of the striking features of human vision is high visual acuity. Dr. Williams will describe the arrays of cone photoreceptors in the eyes of macaques and will relate cone densities, which vary with distance from the fovea, to acuity. Another facet of human vision is a human observer's ability to see both black-white and color contrast with high sensitivity. These independent abilities may result from activity in different neural channels that arise in the retina. Such channels have been studied by the remaining speakers in the Workshop. Dr. Merigan compares chromatic and achromatic contrast sensitivity in macaques with severe loss of small P-type retinal ganglion cells. Dr. Perry stains different functional classes of macaque ganglion cells with HRP, and defines the dendritic morphology of the P and M (the large ganglion cells) types as well as their central visual connections. Dr. Kaplan (in collaboration with Dr. Shapley) finds that M cells have a higher sensitivity for black-white contrast than P cells, while P cells are more sensitive for coarse patterns of color contrast. The mechanisms of these differences in achromatic and chromatic contrast sensitivity will be discussed. Dr. Schein studies the spatial scaling of receptive field size and ganglion cell density in the P and M populations and compares this scaling with spatial scaling of human performance. Dr. Lee measures the responses of P and M ganglion cells to heterochromatic exchange in an attempt to understand which cell type underlies perception of brightness. In these talks, the relation of macaque retina to human vision is a repeated theme. New understanding of the monkey's retina is leading to a clearer view of how humans see.

TRANSPLANTATION FOR MOVEMENT DISORDERS

219.1 SURGICAL TRIALS ON TREATMENT OF PARKINSON'S DISEASE IN MICE. K. Shimizu*, N. Tsuda*, Y. Matsui*, Y. Okamoto*, Y. Miyao*, K. Tamura*, S. Nakatani*, H. Mogami* Department of Neurosurgery, Osaka University Medical School, Osaka 553, Japan (SPON: T. Tsumoto)

Attempts to reconstruct the damaged nigrostriatal pathway in experimental models of Parkinson disease have thus far been carried out in animals with neurotoxically induced dopamine deficiency. Our study established that unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriataldopamine (DA) neurons produced a well-characterized functional asymmetry in the behavior of the C57BL/6 ($H\text{-}2^b$) mice.The intraperitoneal administration of methamphetamine induced ipsilateral rotation at 7-20 turns/min. $1x10^6$ syngenic DA-rich cells of embryonic ventral mesencephalon were stereotaxically transplanted in the caudate-putamen. A complete recovery of methamphetamine-induced rotational response was produced around the 60th day after the syngenic cell suspension graft. And a complete compensation of the rotational response was also brought about with the DA-rich cells from embryonic ventral mesencephalon (crown-rump length; 10-13 mm)of allogenic C3H/HeN ($H\text{-}2^K$) mice. The FACS IV analysis revealed no H-2 (K^K and Ia^K) antigens before transplantation of these embryonic cells. Immunohistochemistry showed that the dopaminergic fibers had grown predominantly into the ipsilateral caudate-putamen. These results provide evidence of integration of syngenic and allogenic grafts and host tissue. And the immunological response in the transplanted brain are under investigation.

219.2 EFFECT OF DOPAMINE NEURONAL GRAFTS ON THE LOCOMOTOR AND COGNITIVE DEFICITS INDUCED BY 6 HYDROXYDOPAMINE LESIONS OF THE MESOLIMBO-CORTICAL DOPAMINE SYSTEM. R. E. Strecker, P. Brundin*, F. H. Gage and A. Björklund. Dept. of Histology, Univ. of Lund, Lund, Sweden. (RES Current address: Hana Biologics, Alameda, CA).

In Parkinsons disease, although the reduction of dopamine (DA) levels is greatest in the mesostriatal DA system, there are also significant DA reductions in cortical and limbic regions of the mesolimbocortical DA system, which receive their inputs from DA neurons in the ventral tegmental area (VTA, A10 of Dahlström and Fuxe). This system has been experimentally implicated in a variety of behaviors including regulation of locomotor activity and cognitive performance, functions that may also be involved in the clinical symptomology of Parkinson's disease.

Lesion experiments: Consistent with previous reports, we found that extensive bilateral 6-hydroxydopamine (6OHDA) lesions of the VTA DA cell bodies produced a blockade of amphetamine-induced locomotor activation and an increased locomotor activity in response to apomorphine. We also found that lesioned rats could not learn a conditioned rotation task, and had deficits in cognitive performance as measured in the Morris watermaze. In the watermaze rats with lesions showed a prolonged latency to find the hidden escape platform and generally exhibited reduced swim speeds. In addition, these rats showed little indication of using spatial cues to find the platform. Neurochemical analysis revealed a strong correlation between DA depletion in limbic forebrain/anteromedial caudate and these cognitive deficits in the watermaze. Note that these behavioral effects were not related to a depletion of norepinephrine, since rats were treated with desmethylimipramine prior to 6OHDA injection. Compared to normal rats, these lesioned rats were also unable to learn a conditioned learning paradigm, in which water-deprived rats were placed in a hemispherical bowl and trained to turn in circles for a sucrose-water reward.

Graft Experiments: Lesioned rats showing deficits in the watermaze received bilateral suspension grafts of DA-rich rat fetal ventral mesencephalon (donor age 13-15 days) into both the nucleus accumbens and caudate-putamen. These grafts completely reversed the lesion induced hyporesponsiveness to amphetamine. However, the grafts did not improve the performance of lesioned rats on either of the more complex behaviors tested, i.e., cognitive performance in the watermaze or conditioned learning in the rotation paradigm. Possible explanations for the intractability of these latter deficits to amelioration with DA grafts include either the need to replace DA in cortical areas, or alternately, the innervation of the graft by host afferents may be insufficient to regulate these more complex behaviours, even though amphetamine-induced locomotor behavior was completely normalized.

219.3 COULD GRAFTED HUMAN FETAL DOPAMINE NEURONS PROVIDE A THERAPY FOR PARKINSON'S DISEASE ? - AN EXPERIMENTAL STUDY IN THE RAT. P. Brundin*, R.E. Strecker, D.J. Clarke*, H. Widner*, O.G. Nilsson*, B. Åstedt*, O. Lindvall* and A. Björklund. Dept. of Histology, Univ. of Lund, Biskopsgatan. 5,S- 223 62 Lund, Sweden. (SPON.: B. Ehinger)

Recent studies with autografts of adrenal medullary tissue to the basal ganglia of patients with Parkinson's disease (PD) have reported of some beneficial graft effects. As experimental studies indicate that neural tissue grafts have a greater functional potential in animal models of PD it seems warranted to try to develop sources of *neural* donor tissue. Two of the critical issues concern the choice of donor tissue, and the possible need for immunosuppression of the patient. We have investigated human fetal dopamine (DA) neurons as a potential donor tissue. Ventral mesencephalic tissue was obtained from aborted human fetuses of 6.5-19 weeks gestation. The tissue was grafted to the striatum of cyclosporin A immunosuppressed rats with 6-hydroxydopamine lesions of the mesostriatal DA pathway. There evidently exist donor age restrictions as only rats receiving cell suspension grafts from the 9-week old or younger fetal donors reliably showed reversal of amphetamine-induced motor asymmetry and contained large numbers of surviving grafted mesencephalic DA neurons (Brundin et al. Exp. Brain Res. 65 (1986) 235-240). The need for immunosuppression is supported by data showing that grafted rats were immunized against human antigens and that if the recipient rats were not immunosuppressed the human DA neuron grafts did not survive. Estimates of the number of DA neurons innervating the human striatum and estimates of the survival rate of grafted human DA neurons in the rat taken together suggest that the grafting of one fetal human ventral mesencephalon may be enough to reinstate a functional unilateral DA reinnervation in a PD patient. The functional effects in the rats appeared much later (12-15 weeks after grafting) than with similar transplants utilizing rodent donors (2-4 weeks after grafting). One group of recipients also showed compensation of spontaneous motor asymmetry and apomorphine-induced rotation. Moreover, using the intracerebral dialysis technique we have monitored spontaneous DA release from grafted human DA neurons. The intrastriatally grafted DA neurons grew an an extensive terminal network stretching several millimetres throughout the host caudate-putamen, nucleus accumbens and globus pallidus, whereas when the grafted DA neurons were located in the neocortex overlying the striatum they exhibited little fiber outgrowth. The DA neurons seemed to retain some human morphological characteristics in that they were larger and possessed more prominent dendrites than their rodent counterparts. Electron microscopy revealed that the grafted human DA neurons form abundant tyrosine-hydroxylase (TH) immunoreactive synaptic contacts with host striatal neurons. In addition, TH-immunoreactive dendrites, that extended from the graft into the host, received non-labelled afferents, possibly arising from the host. These results show that DA neurons from 6.5-9 week old human fetuses survive cell suspension grafting to immunosuppressed hosts, reinnervate the host striatum, form synapses with host striatal neurons, spontaneously release DA and can reverse drug-induced and spontaneous motor asymmetry. These results thus clearly indicate the potential usefulness of human fetal mesencephalic DA neurons for clinical grafting in PD patients.

219.4 INTRACEREBRAL GRAFTING AND CULTURE OF CRYOPRESERVED PRIMATE AND RAT DOPAMINE NEURONS. T.J. Collier, M.J. Gallagher*, B.F. Daley*, W.F. Silverman, D.E. Redmond Jr., R.H. Roth, C.D. Sladek and J.R. Sladek Jr. Departments of Neurobiology and Anatomy and Neurology, University of Rochester School of Medicine, Rochester, N.Y. 14642 and Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT. 06510.

The availability, storage and transportation of donor tissue are significant practical problems associated with the potential use of grafted neural cell replacements in human neurodegenerative diseases. In connection with ongoing studies utilizing dopamine neuron grafts to ameliorate the experimental parkinsonism produced by MPTP in African green monkeys, we have begun to assess the applicability of cryopreservation techniques to the storage of neural tissue. Mesencephalic dopamine neurons were frozen (Houle and Das, Brain Res. 192:570, 1980), stored in liquid nitrogen (1-70 days), thawed, and tested for survival and growth in intrastriatal grafts and in culture. Ventral midbrain tissue was obtained from 3 fetal African green monkeys (CRL: 8.0, 17.5 and 19.0 cm). Tissue placed in culture was derived from the 19.0 cm CRL fetus and tissue from all three donors was used for intrastriatal grafting into 3 adult monkey hosts. Within the storage intervals examined (4 and 28 days), recently thawed fetal neural tissue yielded suspensions of intact cells (99% viable by trypan blue exclusion) that developed normal neuronal morphology and stained for tyrosine-hydroxylase (TH) after 2 weeks in culture. Grafted TH-positive neurons derived from all 3 donors also were identified in the striata of host monkeys. Grafts of frozen-thawed tissue appeared to be smaller and contain fewer TH-positive neurons than grafts of fresh tissue, but the neurons that were present exhibited extensive neurite outgrowth. We pursued evaluation of the cryopreservation technique utilizing midbrain tissue from 32 15-day gestation F344 rats. Estimates of cell density from suspensions of rat mesencephalon made prior to culturing or implantation suggest that frozen-thawed tissue yields fewer total cells, but appears to have no significant effect on the proportion of TH stained neurons counted in culture two weeks later. Samples of rat midbrain tissue freeze-stored for 1, 14 or 70 days exhibited a 50% decline in cell density as compared to fresh tissue. Comparisons of staining for TH and neuron specific enolase indicated no marked change in the percentage of neurons expressing TH; ranging from 72-89% fresh and frozen samples. Thus, a marked loss of dopamine neurons does not appear to be a consequence of freeze-storage. Intrastriatal implantation of fresh versus freeze-stored (70 days) midbrain tissue into nigrostriatal-lesioned rats (6 each) confirmed our impression from the primate studies that cryopreserved tissue yielded smaller grafts containing fewer TH-positive neurons. As in the primates, the neurons that were present exhibited normal morphology at both the light microscopic and ulturastructural levels. While further evaluation of the physiological and behavioral efficacy of transplanted cryopreserved tissue is needed, our initial findings indicate that both primate and rat midbrain dopamine neurons remain viable in culture and survive intracerebral grafting following cryopreservation periods of up to 70 days. (ADRDA FSA-85-015, NS24032, AG00847, 1-F32-AG05384)

219.5 BEHAVIORAL EFFECTS OF FETAL DOPAMINE CELL TRANSPLANTATION IN BONNET MONKEYS WITH MPTP-INDUCED PARKINSONISM C.R. Freed, J.B. Richards, C. Hutt*, J. Whalen*, R. Peterson*, and M. Reite, Depts. of Med., Pharm., and Psych., Univ. Colo. Health Sci. Ctr., Denver, CO 80262.

The neurotoxin N-methylphenyltetrahydropyridine (MPTP) which produces a Parkinsonian syndrome in man and other primates has provided a useful model for studying new therapies for Parkinson's disease. Preliminary reports indicate that fetal substantia nigra dopamine cells may survive when transplanted into Parkinsonian monkeys (Bakay et al., Soc. Neurosci. Abstracts 11: 1160, 1985; Redmond et al., Lancet i, 1125, 1986) although behavioral improvement is uncertain. We have studied the behavioral effect of fetal substantia nigra tissue transplanted unilaterally into caudate and putamen of five MPTP lesioned Bonnet monkeys (maccaca radiata). Animals were food deprived and trained to pull a lever for a food reward. Because preliminary experiments showed that MPTP lesions eliminated trained behavioral responses in some animals, simpler tasks were also tested. Animals were timed as they reached for food pellets in their field of view. After training, animals were lesioned with MPTP 0.5 mg/kg i.m. every other day for 5 injections. All animals which survived the acute effects of MPTP showed spontaneous improvement over weeks to months. Most required repeat courses of MPTP (up to 4 courses of 2.5 mg/kg) to establish persistent deficits. Fetal tissue was obtained from 18 to 45 mm crown-rump embryos with substantia nigra dissected from the ventral third of the mesencephalic arch and disrupted by passage through a needle. Recipient animals were injected with tissue unilaterally into caudate and putamen at a total of 20 sites along 5 tracks. The side of transplant was randomly chosen. Motor performance was assessed with lever pulling, with the reach tasks and with amphetamine 0.5 mg/kg i.m. Because of spontaneous improvement, transplant effects were measured as a change in the slope of behavioral improvement. Four to 6 months after transplant, animals were killed and tissues examined for transplant survival with tyrosine hydroxylase immunocytochemistry. Results showed that behavioral improvement was gradual, appearing 4 to 8 weeks after transplant. While there was greater motor improvement on the side contralateral to the transplant, most animals also showed improvement on the ipsilateral side. One severely affected animal regained the ability to maintain a posture, to feed and drink, to display paw preference with the hand contralateral to the transplant, and to circle after amphetamine to the side contralateral to the transplant. The animal showed no restoration of trained behavior. Animals with milder deficits had recovery to normal in trained pull tasks in the extremity contralateral to the side of transplant.

219.6 GRAFTS OF ADRENAL MEDULLA PROMOTE RECOVERY OF TYROSINE HYDROXYLASE IMMUNOREACTIVE (TH-IR) FIBERS IN STRIATUM OF THE MPTP TREATED MOUSE. M.C.Bohn, F.Marciano*, L.Cupit*, and D.M.Gash. Dept.Neurobiology and Behavior, State Univ. of New York, Stony Brook, N.Y. 11794 and Dept. Neurobiology and Anatomy, Rochester Medical Center, Rochester, N.Y. 14642.

The drug, MPTP, depletes striatal dopamine (DA) in primates and certain rodents, and produces Parkinsonian-like symptoms in humans and non human primates. In mice, the dopaminergic neurons in the substantia nigra are severely damaged resulting in a rapid loss of striatal dopamine (DA) and catecholamine fluorescence. However, some neurons survive and slowly recover.

To investigate the consequences of grafting adrenal medulla tissue into the brain of a rodent model of Parkinson's disease, adult mouse adrenal medulla was grafted unilaterally into mouse striatum 1 week after MPTP treatment (2x50mg/Kg,16hrs.apart). This MPTP treatment depleted DA levels by 86% at 5 weeks and caused the virtual disappearance of TH-IR fibers in the striatum at 2 weeks. In contrast, at 2,4 and 6 weeks after adrenal grafting, dense TH-IR fibers were observed in the grafted striatum and only sparse fibers in the contralateral striatum. Sham grafts of gelfoam were without effect. The TH-IR fibers were dopaminergic since they did not stain for dopamine B-hydroxylase(DBH) and appeared to arise from the host rather than from the grafts in which only a few cells survived. Grafted cells which survived expressed TH, DBH, PNMT (phenylethanolamine N-methyltransferase) and/or leu-enkephalin. Some surviving cells retained their chromaffin cell morphology while others had neuronal-like processes. These processes remained within the graft and did not penetrate into the brain parenchyma.

These observations suggest that, in mice, adrenal medullary grafts exert a neurotrophic action on the host to promote recovery of dopaminergic neurons, an observation which may be pertinent to similar approaches presently being taken to treat humans with Parkinson's disease.

Supported by NIH grant NS20832, a Jacob Javits Award to D.M.G.,NS15109 and a Research Career Development Award to M.C.B., NS00910.

219.7 STRIATAL TRANSPLANTS PROMOTE CENTRALLY-MEDIATED BEHAVIORAL EFFECTS OF N-METHYL-SCOPOLAMINE WHICH DOES NOT NORMALLY CROSS THE BLOOD-BRAIN BARRIER. P.R. Sanberg, S.F. Calderon,* E.M. Weissman,* M. Giordano and A.B. Norman, Laboratory of Behavioral Neuroscience, Depts. of Psychiatry, Neurosurgery, Psychology and Physiology, University of Cincinnati Medical Center, Cincinnati, OH 45267.

Neural tissue transplants have proven to be very exciting as a potential method of treatment for many CNS disorders. Recently, however, it has been reported that the blood-brain barrier (BBB) does not develop normally within the transplanted tissue. Thus, compounds such as horseradish peroxidase, which do not normally enter the brain following systemic injections can be found within the transplanted tissue, surrounding host tissue and CSF (Rosenstein, Science, 230, 772-774, 1987). We examined whether N-methylscopolamine (NMS), which does not normally pass the BBB, but has psychoactive properties when administered intracerebrally, can influence behavior in animals following systemic injections, presumably by entering the brain via an altered BBB within the transplant.

Day 17 fetal striatal tissue or vehicle alone was injected either bilaterally (2 ul per side), or unilaterally (4 ul) into the striatum of adult male Sprague Dawley rats. Two to four weeks later, the animals were put into automated Digiscan Activity Monitors (Omnitech Electronics), habituated for one hour, and then injected i.p. with NMS (vehicle 2, 4 and 8 mg/kg) and their locomotion was tested for one more hour.

In the control animals NMS did not produce any locomotor changes. However, in the transplant groups, especially the bilateral groups, there was a significant change in the pattern of locomotion and a small hyperactivity. Present studies are examining the extent to which systemically injected [^{3}H](-)NMS will label transplant and host brain tissue.

It is possible that these differences in behavior may be due to the differential responsiveness of the transplanted and host tissue to the relatively small concentrations of NMS which may cross an intact BBB. Alternatively, these preliminary data suggest that transplants which are located at a pharmacologically active site may serve as a potential access point for allowing some drugs which do not normally enter the brain to produce a centrally-mediated behavioral effect. If this latter hypothesis is correct, this may have important clinical significance by allowing possible therapeutic agents which would not normally cross the BBB to have a direct effect within the brain via neural transplants. Supported by Pratt Family and Friends, Hereditary Disease Foundation, Huntington's Disease Society of America and Omnitech Electronics.

219.8 EXPERIENCE WITH ADRENAL MEDULLARY AND FETAL MESENCEPHALIC TRANSPLANTATION FOR THE TREATMENT OF BILATERAL AND UNILATERAL MPTP PARKINSONISM IN PRIMATES. R.A.E. Bakay, M.S. Fiandaca, K. Sweeney*, P.M. Invone and D.C. Collins, Dept. of Surgery Div. Neurological Surgery, Yerkes Regional Primate Research Center and Veterans Administration Medical Center, Emory Univ. Sch. of Med., Atlanta, GA 30322.

We have gained experience with both the bilateral and unilateral MPTP nonhuman primate Parkinson model. In both model systems spontaneous recovery does occur Most of this recovery is within the first two weeks but may occur up to two months. Both models appear to more reliably maintain an appropriate level of Parkinson like features by titrated multiple dose applications administered two to four weeks apart. Properly prepared animals can be maintained for many months with very little behavioral variability.

The bilateral model displays the complete array of Parkinson-like features whereas the hemiparkinson model features very few. The hemiparkinson animals have a contralateral neglect of the upper extremity. Differentiation from a hemiplegic extremity can be made by noting the increased tone and ability to use the extremity when aroused similar to the paradoxical kinesia. The clear advantage of the hemiparkinson model is the ease of preparation and maintenance.

We have experience with both the adrenal and fetal tissue transplantation for correction of the MPTP induced Parkinson-like behavior. Most of our experiences with fetal mesencephalic tissue which we have been able to demonstrate significantly improves clinically observable behavior ($P<.001$). The younger animals appear to recover more function than do older animals and those less severely appear to recover more function than those with the most severe deficits. There is a correlation with the degree of recovery and the elevation of CSF catecholamine metabolites as determined by HPLC.

This project was funded by the American Parkinson Disease Association and supported by the Veteran's Administration, Yerkes Regional Primate Research Center, Emory University, (NIH Core Grant RR-00-165) and NIH Grant ROI-NS17524 to PMI.

219.9 **ATTENUATION OF APOMORPHINE-INDUCED ROTATIONAL BEHAVIOR BY FETAL STRIATAL TISSUE TRANSPLANTS IN RATS WITH UNILATERAL STRIATAL KAINIC ACID LESIONS.** A.B. Norman, T. McGowan,* S.F. Calderon,* M. Giordano and P.R. Sanberg. Laboratory of Behavioral Neuroscience, Departments of Psychiatry, Neurosurgery, Psychology and Physiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Transplants of rat fetal striatal tissue into rats with bilateral striatal kainic acid (KA) lesions reverses the lesion-induced spontaneous locomotor abnormalities suggesting a functional integration of host and transplanted tissue (Sanberg et al., Pharm. Biochem. Behav. 25, 297, 1987). However, it is unclear at present whether the transplanted tissue develops similar pharmacological properties as the original host tissue.

Rotational behavior is produced in response to apomorphine and other dopamine receptor agonists in unilateral KA-lesioned rats (Schwarcz et al., Brain Res. 170, 485, 1979) presumably by an asymmetry in the dopamine effector systems. Any reduction in this asymmetry might be expected to reduce the rotational behavior. We therefore assessed the effects on apomorphine-induced turning behavior of rat fetal striatal tissue transplants into the lesioned striatum.

Male Sprague Dawley rats (180-220 g) were stereotaxically administered KA (5nmol) unilaterally into striatum. Between 4 and 6 weeks post-lesion, rats were injected s.c. with 0.5-0.75 mg/kg apomorphine and the number and topography of rotations assessed in an open-field environment. Rats were tested at 4-5 day intervals on three occasions prior to transplantation in order to obtain an accurate baseline for rotational behavior. Interestingly, rats turned contralateral to the lesion in contrast to the results obtained by Schwarcz et al. Day 17-19 fetal striatal tissue (4 ul) was stereotaxically implanted into the lesioned striatum. Three and five weeks post-transplant, apomorphine-induced rotational behavior was reassessed using the previous dose. Post-transplant the topography of the rotations changed from tight pivotal rotations to walking in a circle, with a marked reduction in both the total number and maximal rate of rotations. Stereotypic behavior in one location tended to replace ambulation.

These results suggest that the transplanted tissue reduced the asymmetry in the dopamine effector systems in the rat striata and, therefore, that the developing transplanted tissue possesses similar pharmacological properties as the original host tissue with respect to dopaminergic neurotransmission. Fetal striatal tissue transplants, therefore, appear to be capable of functionally and pharmacologically restructuring damage to a complex neurochemical system.

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219.10 AFFERENTS TO AND EFFERENTS FROM SEROTONERGIC CELL SUSPENSIONS TRANSPLANTED IN THE CAUDATE-PUTAMEN COMPLEX OF THE YOUNG AND MIDDLE AGED RAT. A COMBINED IMMUNOHISTOCHEMICAL AND ANTEROGRADE TRACING STUDY. R.J. Vermeulen* and H.W.M. Steinbusch (SPON: ENA). Dept. Pharmacology, Free University, 1081 BT Amsterdam, The Netherlands.

Transplantation of monoaminergic/cholinergic fetal cell suspensions into either the rat caudate-putamen (cp) or the hippocampus has resulted in an increased understanding of the outgrowth arising from these cell suspensions. However most of these studies were focussed either upon the neuromorphological delineation of the outgrowth or behavioral observations. In this study we were interested in two matters. To start with we want to show that serotonergic neurons implanted into the cp of young and middle aged rats receive afferents from the host tissue. In addition we were interested if these afferents only arise from adjacent brain regions, thus i.e. the cp, or also from areas which are more remote like caudally the substantia nigra, pars compacta (snc) or rostrally the prefrontal cortex. The latter two areas are known to project upon the cp. For this reason we injected into the cp of young (3 months old) and middle aged (21 months old) rats a fetal mesencephalic raphe cell suspension in the previously denervated cp. After a survival time of at least four weeks the animals received an injection with PHA-L into either the rostral part of the cp, immediately adjacent to the transplantation sites or a small injection into the prefrontal cortex or in the snc. After 8 days the animals were processed for PHA-L and serotonin-immunocytochemistry using a combined DAB/ DAB-Ni double staining technique. We observed that in particular fibers arising from the snc make contacts with the transplanted serotonergic cells in rats of both ages. Injection into the neighboring cp did not result in an innervation of the transplanted cells. Finally to determine the total outgrowth of a mixed cell suspension in relation to its monoaminergic cell component PHA-l was injected into the area which previously received a cell suspension. Double staining experiments revealed that it was difficult to reach the exact injection site since, due to prolonged survival time, the coordinates have partially changed. Accordingly we used a new approach by mixing PHA-l with the cell suspension. We demonstrated that after a survival time of 4 weeks some of the implanted cells have taken up PHA-l and started to give efferents. Using double staining experiments we demonstrated that some of these cells actually contain serotonin. These studies show that fiber networks in the central nervous are not static elements, but have the capacity to change their localization during their life and adapt to new circumstances. Finally these lightmicroscopical observations give only a first impression about these contacts and they should be followed by detailed electronmicroscopical investigations.

219.11 ULTRASTRUCTURAL ANALYSIS OF NEURONAL AND SYNAPTIC DEVELOPMENT WITHIN NEOSTRIATAL TRANSPLANTS IN AN ANIMAL MODEL OF HUNTINGTON'S DISEASE. Paul D. Walker* and James P. McAllister II (SPON: J. A. Kenning). Depts. of Anatomy and Neurosurgery, Temple University School of Medicine., Philadelphia, PA 19140.

We previously showed that neostriatal (NS) transplants do not establish axonal connections with host NS afferent and efferent regions up to 2 months post-grafting. Instead, grafted neurons project axons to different locations within the transplant and possibly synapse on other grafted neurons (Walker and McAllister, Brain Res., in press). Recently, we analyzed the neuronal and synaptic maturation of NS grafts to determine if transplanted neurons can establish a NS-like connective organization in the absence of normal developmental cues, such as extrinsic connectivity. Five days after intrastriatal kainic acid injections, adult rats received grafts of dissociated E14 NS. Animals containing 1, 3, and 5 week old grafts and age-matched (E21, 2, and 4 week) normal control animals were processed for transmission electron microscopy. Seven days after grafting, microglial cells comprised a large proportion of cells within the transplant. Cells resembling normal NS neuroblast cells were also abundant in the graft. As in the normal prenatal NS, graft neuropil contained much extracellular space. This space was filled with cellular debris which most likely resulted from kainate neurotoxicity and spontaneous death of some grafted neurons. Apposed membranes of cellular processes were seen, but synaptic densities were rare and no synaptic vesicles were present at these contacts. Neurons within the 3 and 5 week grafts were seen individually and in clumps of 3-4 neurons. Cells with ultrastructural characteristics resembling normal spiny neurons were most numerous. In contrast to the normal NS, medium and large neurons with deeply indented nuclei (aspiny neurons) were more frequent in the transplant. However, these cells were less abundant than spiny neurons. Axosomatic synapses were present on all types of transplanted neurons. Neuropil in 3 and 5 week grafts was filled with neural and glial processes and many axospinous and axodendritic synapses. Axo-axonal synapses were also present. When compared with control NS, the transplant neuropil contained enlarged dendrites and fewer synapses. These data indicate that although NS transplants do not establish connections with normal NS afferents and efferents, neurons within the graft develop a complex synaptic organization amongst themselves. Although similar synaptic types as the normal NS were found in the transplant, fewer synapses were present and the morphology of dendrites were altered. These results may reflect differences in developmental cues between grafted and normally maturing NS neurons.

219.12 QUANTITATIVE ANALYSIS OF DENDRITES FROM TRANSPLANTED NEOSTRIATAL NEURONS. James P. McAllister II, Mark C. Zemanick and Paul D. Walker. Depts. of Anatomy and Neurosurgery, Temple University School of Medicine, Philadelphia, PA 19140

Morphologic and biochemical results suggest that grafted embryonic neostriatal neurons have similar characteristics to normal adult neostriatal neurons. Our previous Golgi analysis showed that 4-6 week old neostriatal transplants are populated by neurons qualitatively similar to the neuron-types comprising the normal neostriatum. As in the normal neostriatum, the Spiny I neuron was the type most commonly found in transplants. However, our recent horseradish peroxidase study has indicated that no axonal connections exist between the host and the transplant at 35 days post-transplantation. To determine if transplantation and lack of extrinsic connectivity affect dendritic morphology, the present morphometric study compared the spine density of 35-day old transplanted Spiny I neurons to age-matched controls. Host rats received bilateral neostriatal transplants 5 days after intra-striatal kainic acid lesions. Transplants consisted of cell suspensions of neostriatal tissue taken from embryos on the 14th day of gestation. Hosts were sacrificed at 35 days post-transplantation; control tissue was obtained from 28-day old animals to correlate with the actual age of the transplanted tissue. All tissue was processed using rapid Golgi methods, and well-impregnated neurons were analyzed quantitatively by light microscopy. Control spine densities (0.92 ± 0.25/10 um) were similar to those observed in adult animals by others. The overall spine density of transplant dendrites, however, showed a significant 39.1% reduction when compared to controls ($p < .001$). This reduction was due to a significantly decreased spine density of intermediate and distal branches. Specifically, there was a 38.0% reduction in the spine density of intermediate branches and a 39.1% reduction of distal branches (both $p < .001$). Proximal branches of transplanted neurons, however, demonstrated a significant increase in spine density over controls ($p < .05$). Because of the short length of proximal branches, this increase had little effect on overall spine density. In addition, transplanted neurons were found to have a significant 23.7% reduction ($p < .001$) in the total number of proximal dendrites. This study thus provides evidence that 35 day old transplanted spiny I neurons show quantitative differences in dendritic morphology compared to age-matched controls. These differences may be due to aberrant maturation of grafted neurons as well as a lack of extrinsic input to the transplant.

NEUROTOXICITY I

220.1 BARREL ROTATION AND TOXIC EFFECTS OF NEUROPEPTIDES: ADDITIVE EFFECTS OF INTRACEREBROVENTRICULAR (I.C.V.) ARGININE-VASOPRESSIN (AVP) AND SOMATOSTATIN (SRIF) IN RATS. Carey D. Balaban* and Walter B. Severs. Depts. of Anatomy, Surgery and Pharmacology, Col. Med., Penn State Univ., Hershey, PA 17033

Injections (i.c.v.) of SRIF and AVP can elicit barrel rotation (BR), convulsions and lethal pulmonary edema [Brain Res., 365 (1986) 21-29; Fed. Proc., 46 (1987) 1449 (2 abstracts)]. In addition, i.c.v. SRIF (40 μg) can destroy cerebellar Purkinje cells. This study examined whether these multiple effects are additive. Alert adult SD rats received either (a) 0.5 μg AVP, (b) 20 μg SRIF or (c) 20 μg SRIF + 0.5 μg AVP through a lateral ventricular cannula implanted four days previously (volume: 5 μl; vehicle: artificial CSF). BR incidences of SRIF, AVP and SRIF+AVP groups were 13/16, 6/25 and 17/18, respectively. The AVP incidence was lower ($p<0.01$, Fisher's exact test) than the other groups. The incidence of mortality, preceded by convulsion, was 0/16 for SRIF, 1/25 for AVP and 12/18 for SRIF+AVP; mortality in the latter group was elevated ($p<0.001$, Fisher's exact test vs either peptide alone). Hazard plots of BR latencies were consistent with a two parameter exponential model. Minimum latencies (μ) were 78 s for SRIF, 46 s for AVP and 31 s for SRIF+AVP; fast phase hazard (θ^{-1}) was lower for SRIF (0.62 ± 0.26%/s, slope $\pm$ 95% C.I., r=0.98) than for SRIF+AVP (3.96 ± 0.82%/s, r=0.995; $p<0.05$, t test). For the smaller AVP sample (n=6), $\theta^{-1} = 0.47 \pm 0.32$%/s (r=0.95). The fast phase θ^{-1} for SRIF+AVP was also greater than observed for either SRIF (40 μg) or AVP (1 μg) [*loc. cit.*]; however, the SRIF+AVP value represents the sum of these higher dose θ^{-1} values for single peptides. Similarly, SRIF+AVP mortality represents a sum of mortality observed for SRIF (40 μg) and AVP (1 μg) [*loc. cit.*]. Sample sizes did not permit estimation of slow phase θ^{-1}. BR latencies were longer for SRIF than either AVP or SRIF+AVP rats ($p<0.01$, Mann-Whitney U test); the AVP and SRIF+AVP groups were similar ($p>0.05$). Cupric-silver staining of brains after a 4 day survival period revealed degenerating Purkinje cells in midline cerebellum of SRIF and SRIF+AVP rats. These data imply that i.c.v. SRIF and AVP have additive effects on BR incidence, BR latency (θ^{-1}) and mortality. Since CSF [SRIF] is elevated after metrazole-seizures in rats [Neuropeptides, 9 (1987) 19-24] and both [SRIF] and [AVP] are elevated in CSF from patients with elevated intracranial pressure [Horm. metab. Res., 18 (1986) 555-557; J. Neurol. Neurosurg. Psychiat., 48 (1985) 50-57], we propose that interactions between neuropeptides may be a risk factor for development of multiple motor sequelae, which may be lethal. This motivates simultaneous examination of CSF levels of multiple neuropeptides in both clinical and experimental contexts. (C.D.B. supported by RCDA NS00891).

220.2 CATECHOLAMINES ARE TOXIC TO RAT CEREBRAL CORTEX IN DISSOCIATED CELL CULTURE. P. A. Rosenberg and M. A. Dichter. Departments of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115, and Graduate Hospital and University of Pennsylvania Medical School, Philadelphia, PA, 19146.

We have been interested in understanding the actions of norepinephrine (NE) in cerebral cortex, one of the major projection areas for noradrenergic neurons in the central nervous system. A role for NE in facilitating cortical synaptic plasticity was suggested by reports by Kasamatsu that NE depletion using intracortical 6-hydroxydopamine prevented the ocular dominance shift produced by suturing one eye closed in cats during a critical window of development. These studies interested us in the possibility that a developmental effect of NE on neurons might be demonstrable in tissue culture. To test for an effect on neuronal survival, NE was added to growth medium of cultures plated 24 hours before at a final concentration of 0.25, 2.5, 25 or 250 uM. Control cultures received similar volume of vehicle (100 uM EDTA, 1 mM HCl) only. Cultures were observed 24, 48, and 72 hours later. NE at a concentration of 25 uM was, surprisingly, associated with a large loss of both neurons and nonneuronal cells from the culture. No cells survived exposure to 250 uM NE. Time course experiments comparing cultures in 25 uM NE with control cultures showed a steady decline in cell numbers over at least 48 hours following addition of NE. In other experiments designed to determine the minimal interval of exposure required to demonstrate toxicity it was found that there was no effect of exposure to NE for 1 and 4 hours, but nearly the full toxic effect was manifest following a 24 hour exposure to NE. In order to further define the parameters contributing to toxicity, the effect of cell density on toxicity was examined, and it was found that halving the culture plating density, from 450,000 to 225,000 cells per 35 mm dish, significantly decreased the number of surviving cells. The pharmacology of catecholamine toxicity was characterized, and it was found the effect could be mimicked by any catecholamine, including isoproterenol, but not blocked by beta adrenergic antagonists, suggesting that catecholamine toxicity is not mediated by an adrenergic receptor. On the other hand, toxicity could be blocked by catalase, superoxide dismutase, and tocopherol, suggesting mediation by free radicals generated during oxidative degradation of catecholamines.

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220.3 MPP+ UPTAKE STUDIES IN DISSOCIATED DOPAMINERGIC CELL CULTURES. S. Schinelli*, U di Porzio, A. Zuddas and I.J. Kopin, Sect. of Immunopharmacology and Lab. of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892.

The discovery that the administration of the neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to primates and mice can partially reproduce behavioral and biochemical changes observed in idiopathic Parkinson's disease has provided a new tool to study the ethiology of this neurological disorder. Two steps are required for onset of the neurotoxicity: first the bioactivation of MPTP by monoamino oxidase B (MAO-B) into N-methyl-4-phenylpyridine ion (MPP^+) and then the uptake of this active toxin into dopaminergic neurons.

We have studied MPP^+ uptake and its accumulation in DA neurons using dissociated cell cultures from E13 mouse embryonic mesencephalon and compared to that of dopamine (DA). The neurons were grown in serum-free conditions (di Porzio et al., Nature 1980, 288, 370-373). Uptake kinetic parameters were determined in one week old mesencephalic cultures and control cultures from the striatum.

The K_m for DA (0.204 ± 0.079 uM) showed that the affinity was greater than for MPP^+ (1.080 ± 0.227 uM) whereas the V_{max} for MPP^+ (55.25 ± 3.65 pmoles/mg prot/min) was greater than that for DA (24.8 ± 3.44 pmoles/mg prot/min). Under the same conditions, mazindol (a DA uptake inhibitor) showed similar potency in inhibiting both DA and MPP+ uptake. In striatal cell cultures uptake of DA and MPTP was negligible and MPP+ uptake was only about 10% of that in mesencephalic cultures. DA neurons were identified using tyrosine hydroxylase (TH) immunostaining and formaldehyde-induced catecholamine fluorescence. They accounted for about 1% of the total neuronal population in mesencephalic cultures.

Depolarization induced by veratridine (0.1 mM) released comparable proportions of [^{3}H]DA and [^{3}H]MPP^+ from mesencephalic cultures (presumably from DA neurons). These experiments are consistent with uptake of MPP^+ into dopaminergic neurons in vitro by the same system as DA. A small percentage of non-DA neurons may take up small amounts of MPP^+, probably through a low affinity uptake. Furthermore, DA and MPP+ seem to be stored in the same intracellular compartments, at least shortly after entering the cells, and appear to be released by similar mechanism.

220.4 CULTURED SEROTONIN NEURONS ARE SENSITIVE TO MPP+ TOXICITY BUT RESISTANT TO MPTP. L. K. Friedman and C. Mytilineou. Department of Neurology, Mount Sinai School of Medicine, New York, N.Y. 10029.

We have previously shown that the toxic effect of MPTP and its metabolic by-product MPP+ is similar for both dopaminergic (DA) and noradrenergic (NE) neurons in dissociated cell cultures (Friedman and Mytilineou, Soc. Neurosci. Abst. 12:268, 1986). Since the serotonin (5-HT) neurons are resistant to MPTP toxicity in vivo, we examined the effect of the toxins in an in vitro preparation. Varied concentrations of MPTP and MPP+ were added to the feeding medium for 7 days beginning on the 8th day in vitro. Following a 24 hour washing of the cultures were analyzed for toxicity by measuring [3H]NE or [3H]5-HT uptake. MPP+ toxicty increased with increasing concentrations (1 to 10 uM) for both neuronal populations. However, the 5-HT neurons were less affected than the NE neurons at all concentrations of the toxin (65-82% vs. 95-99% inhibition of uptake). In contrast, MPTP toxicity differed markedly in the two neuronal populations. In the NE neurons MPTP at concentrations between 1 and 5 uM resulted in 75% inhibition of NE uptake, while higher concentrations (10 to 100 uM) were less toxic. In the 5-HT neurons MPTP produced no toxic effect at concentrations up to 10 uM. A toxic effect to 5-HT neurons was seen only after 100 uM MPTP (65% inhibition of uptake). The differences in the response of NE and 5-HT neurons to the toxins could be the result of the high inhibitory effect of MPTP on the 5-HT uptake pump. The presence of MAO B within 5-HT neurons could explain the toxicity of MPTP at high concentrations. Supported by NIH grant NS-18979 and American Federation for Aging Research.

220.5 MPTP METABOLISM AND TOXICITY IN DISSOCIATED CELL CULTURES OF EMBRYONIC RAT BRAIN. C. Mytilineou Dept. of Neurology, Mount Sinai School of Medicine, New York, N.Y. 10029.

The metabolic oxidation of MPTP was studied in dissociated cell cultures established from embryonic rat rhombencephalon. The water-soluble metabolite, MPP^+, was separated from MPTP by ether extraction, according to the method of Glover et al. (Neurosci. Lett., 64:216, 1986). Cultures were incubated with 7.5 nanomoles [^{3}H]MPTP and the accumulation of the metabolite was measured in the feeding medium and in the cells, during a 4 day exposure. The metabolism of MPTP increased linearly over time, and 24.1% of the initial concentration of MPTP (1.81 nanomoles) was oxidized in 4 days. The oxidation of MPTP was inhibited by pretreatment with 1 µM deprenyl. The metabolite of MPTP diffused readily into the feeding medium. Less than 1% of the metabolite was retained by the cells. The rate of metabolism of MPTP could be enhanced by increasing the number of cells plated into the tissue culture dish. Thus, adding cortical cells to cultures containing rhombencephalic cells, resulted in an increase of MPTP metabolism, which was proportional to the amount of protein added to the dish. Addition of cortical cells produced a 94% increase of the metabolic oxidation of MPTP, but, when expressed per mg of protein, both sets of cultures metabolized MPTP at the same rate (2.439 vs. 2.425 nanomoles/mg of protein). In the same sets of cultures, MPTP was more toxic to catecholamine neurons in the dishes that had cortical cells added. After exposure to 10 µM MPTP for 7 days, the uptake of [3H]NE was reduced to 50% of control levels in the rhombencephalic cultures; addition of cortical cell accentuated the toxicity and reduced the [^{3}H]NE uptake to 10% of control. In order to examine the involvement of glial cells in MPTP metabolism, glial proliferation was inhibited by treatment of cultures with the mitotic inhibitor fluorodeoxyuridine for 7 days (days 8 to 15 in vitro). Inhibition of glial proliferation resulted in 29.7% reduction of MPTP metabolism by the cultures. Taken together the above results indicate that MPTP is metabolized by rat embryonic brain cells in culture; the MPTP metabolite diffuses readily into the culture medium; an increase in the amount of metabolite accumulated in the feeding medium parallels an increase in the toxicity of MPTP to catecholamine neurons; and reduction in the number of glial cells suppresses the metabolic oxidation of MPTP. Supported by NIH grants NS-11631, NS-18979 and NS-23017.

220.6 MONOAMINE OXIDASE B IS PRESENT IN THE SUBSTANTIA NIGRA PARS RETICULATA OF PRIMATES: AN ENZYME HISTOCHEMICAL STUDY. K.Nakai, Y.Naka*, T.Itakura*, M.Ueno*, T.Okuno*, N.Komai*. Dept. of Neurol. Surg., Wakayama Med. Col., Wakayama 640 Japan.

We have reported that the localized infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) into the substantia nigra (SN) of monkey induced degeneration of dopamine (DA) containing neurons in the unilateral nigrostriatal (SN-ST) system(Nakai et al.Soc.Neurosci.Abst.12:968,1986), in contrast to the result of similar study in rodents which failed to demonstrate the depletion of DA contents in SN-ST system(Bradbury et al.Nature 319:56,1986). Because the oxidative conversion of MPTP into MPP^+ by monoamine oxidase (MAO) B is an important enzymatic reaction for the neurotoxicity of MPTP, we ,as a next step, investigated the precise morphlogical distribution of MAOB in primates and rodents by using the newly modified histochemical procedure (Arai et al.Neurosci. 19:905,1986).

Macaca fascicularis and Sprague-Dawley rats were transcardially perfused with cold 2% glutaraldehyde and 2% paraformaldehyde mixture(PH 7.4, 400ml/kg over 15 min.) under deep anesthesia with Nembutal. Without postfixation the brain blocks were cut into 50-micron-thick coronal sections. After the preincubation period with Clorgyrine(10^{-5},10^{-6},10^{-7}M) for 15 min. at room temperature, the sections were incubated with the mixture of tyramine hydrochloride(5mM),nickel ammonium sulfate(0.6%), diaminobenzidine(0.005%),horseradish peroxidase(0.1%), and sodium azide(20mM) in Tris-HCl buffer (PH 7.6) at 4°C for 12-18 hours. Thus the site of specific MAO activity is demonstrated as a dark blue staining. As reported by the immunohistochemical study (Westlund et al.Science 230:181,1985), we observed strong MAOB activity in the nucleus raphe dorsalis and some of the hypothalamic nuclei in both species. In addition, we observed a prominent histochemical MAOB activity in the substantia nigra pars reticulata(SNPr) of the monkey where proximal axons of DA neurons are running toward the striatum. No significant MAOB activity in SN pars compacta was observed. These MAOB activity in SNPr were dose-dependently blocked by the addition of Deprenyl into the preincubation medium. By examining the rat brain we failed to demonstrate any significant MAOB activity in SNPr.

In conclusion, the observed interspecies difference in the distribution of MAOB activity in SN-ST system may explain the different change in behavior and DA neurons between primates and rodents following MPTP treatment. Furthermore, the present result suggests a possibility ,in combination with the histofluorescence study, that the initial degeneration of DA neurons in SN-ST system following MPTP treatment starts at the proximal axons of DA neurons followed by the retro- and anterograde degeneration toward the cell body and the striatum, respectively.

220.7 FLUNITRAZEPAM CO-TREATMENT ALTERS THE METABOLIC EFFECTS OF MPTP IN THE MOUSE BRAIN. G. Pizzolato*, M. Dam*, U. Freo*, M.R. Leotta* and L. Battistin*. (SPON: S. Squatrito). Dept. of Neurology, Univ. of Padua Sch. of Med., 35100 Padua, Italy.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) produces behavioral, neurochemical, and histopathological changes in several mammalian species, including mice, which closely resemble those of Parkinson's disease. Based on the known mechanisms of MPTP neurotoxicity, a protective action of MAO-B or catecholamine reuptake inhibitors has been demonstrated. Furthermore, CNS depressant drugs, such as phenobarbital, suppress MPTP neurochemical effects and counteract neurotoxicity in other experimental conditions. In order to identify other potentially protective compounds, we investigated whether flunitrazepam (FLU) possesses antidotal effects against MPTP neurotoxicity.

A semiquantitative modification of the (^{14}C)deoxyglucose (DG) method of Sokoloff et al. (J. Neurochem. 28, 897, 1977) has been used. Two groups of six C57 black mice were injected s.c. twice with MPTP (30 mg/kg) or saline, 16 h apart. Other two groups of animals received i.p. FLU (2 mg/kg) 15 min before MPTP or saline administration. DG was injected i.p. 4 h after the last treatment, and the mice were killed 45 min thereafter.

No changes or sligth decreases in DG uptake were observed 4 h after FLU, whereas MPTP alone increased metabolism in regions containing dopamine (substantia nigra and ventral tegmental area), norepinephrine (locus coeruleus), and serotonin (dorsal raphe) cell bodies. When animals were pretreated with FLU, these MPTP-related changes were less but still evident. In addition, MPTP and FLU co-treatment increased DG uptake in several thalamic (midline and intralaminar), hypothalamic (para- and peri-ventricular), and reticular (periacqueductal gray) nuclei.

These results indicate that FLU was unable to block the effects of the neurotoxin on monoaminergic regions. Furthermore, the effects produced by MPTP and FLU co-treatment extend beyond the regional pattern of metabolic changes secondary to MPTP or FLU alone. They give, therefore, anatomo-functional evidence supporting the concept that a drug may cause different effects depending upon the preexisting state of brain neurotrasmission.

220.8 **ULTRASTRUCTURAL ALTERATIONS INDUCED BY 1-METHYL-4-PHENYLPYRIDINE (MMP+) IN ORGANOTYPIC CULTURES OF CANINE SUBSTANTIA NIGRA AND RAT MESENCEPHALON.** B. Christie-Pope*, R.S. Burns*, and W.O. Whetsell, Jr., Vanderbilt University School of Medicine, Nashville, TN 37232.

The mechanism by which MPTP and its metabolite, MPP+, destroy dopaminergic neurons in the substantia nigra is unknown. A free-radical mechanism (Cohen & Mytilineou, Life Sci. 35:237, 1985) and a mechanism of inhibition of mitochondrial oxidation (Nicklas et al., Life Sci. 36:2503, 1985) have been postulated. This study was undertaken to examine the evolution of ultrastructural changes induced by MPP+ in organotypic cultures of canine substantia nigra (SN) neurons and neurons of rat mesencephalon (MES). Canine SN cultures were prepared from newborn mongrel pups (<24 hours old); rat MES cultures, from 14-15 gestational day rat fetuses. All cultures were maintained under identical conditions for at least 21 days in vitro (DIV) prior to exposure to MPP+. The presence of metabolically-active dopaminergic nerve cells in both types of cultures was detected by measuring, with HPLC techniques, levels of homovanilic acid (HVA) in feeding medium bathing each culture after each three-day feeding period. Selected canine SN cultures, at 21 DIV and demonstrated to elaborate HVA, were incubated with 0.1 nM MPP+ (in feeding medium) for 1, 3, 6, 8, 10, 12, and 15 hours. After each of these incubation periods, some cultures were fixed and studied by electron microscopy. The earliest ultrastructural change observed was swelling of mitochondria in large nerve cells by 3 hours of incubation. At 8 hours, extensive mitochondrial swelling as observed in all cells; by 12 hours, swelling of Golgi apparatus was evident; by 15 hours a swelling of mitochondria and cisternal system had become so severe that cell types were indistinguishable. Selected rat MES cultures, at 21 DIV and shown to elaborate HVA, were similarly incubated in MPP+ but at higher levels and for longer periods (see Christie-Pope, Neurosci. Abstr. 12:982, 1986). In contrast to studies in canine SN cultures, only rat MES cultures with pre-MPP+ incubation HVA values greater than 6 pmoles/µl (range:6 to 16 pmoles/µl) exhibited ultrastructural alteration in response to MPP+ treatment. Those changes, including progressive involvement of mitochondria and cisternal system, were seen in large neurons after approximately 72 hours of MPP+ exposure. Different from the canine cultures, a generalized disruption of the rat MES cultures was not observed even after 8 days' MPP+ exposure, however, the general pattern of ultrastructural change within large nerve cells was similar to canine SN. These studies indicate that while there seem to be species differences in susceptibility to MPP+ toxicity (ibid.), the sequence of ultrastructural change occurring in dopaminergic neurons in these two kinds of cultures appear identical. The mitochondrial changes in particular, may reflect an alteration of mitochondrial function. [Supported by USPHS grant NS-10509 and a Pharmaceutical Manufacturers Association Foundation Fellowship (BCP).]

220.9 **AN ULTRASTRUCTURAL STUDY OF MPTP BODIES IN AN AGED SQUIRREL MONKEY.** L.S. Forno, J.W. Langston, L.E. DeLanney, and I. Irwin*. Departments of Pathology, VA Medical Center, Palo Alto, CA 94304 and Stanford University, Stanford, CA 94305, and Institute for Medical Research, San Jose, CA 95128.

Eosinophilic neuronal inclusions, known as Lewy bodies, are characteristically present in Parkinson's disease. We have recently reported eosinophilic inclusion bodies in an experimental model for Parkinson's disease (Forno et al., 1986). The pink bodies were found in nerve cells of three aged MPTP-treated monkeys in the substantia nigra (SN) and other predilection sites for Lewy bodies. The inclusions, here referred to as MPTP bodies, lacked the central core and peripheral halo most often found in Lewy bodies in the SN, but resembled the pale, depigmented foci in nerve cells that may represent an early stage in the formation of these bodies, a pre-Lewy body.

On electron microscopic examination of the MPTP bodies in the SN in one monkey, the inclusions were less well defined than by light microscopy. They were almost completely devoid of Nissl substance, lacked a well formed Golgi apparatus, but contained small numbers of mitochondria and pigment granules, and displayed a striking increase in rather thick filaments. The filaments measured 20 to 25 nm in diameter and resembled neurotubules. They were compared to filaments from Lewy bodies and from Pick bodies, another type of intraneuronal inclusion bodies, present in Pick's disease. The filaments in the MPTP bodies had a larger diameter than filaments in Lewy bodies and Pick bodies, but the Pick body filaments had a smooth contour. In contrast the filaments in the Lewy bodies had fuzzy deposits of electron dense material along their course. Lesser amounts of fuzzy deposits or sidearms were present on the filaments of the MPTP bodies.

At the present time it can not be established whether or not MPTP bodies are also pre-Lewy bodies. Whatever the relation of the MPTP bodies to Lewy bodies and to Parkinson's disease turns out to be, their study promises to yield important insights into the nerve cell degeneration in this form of experimental parkinsonism, and perhaps also into the nerve cell degeneration in Parkinson's disease and in other neurodegenerative disorders. Whether or not the neurotoxin gains access to the nerve cell through uptake by its terminals, or at the level of the cell body and its dendrites remains to be determined.

220.10 SACCADIC EYE MOVEMENT DEFICITS IN MPTP TREATED MONKEYS W.M. King, C. Smith*, K. Bocchiaro*, and D.M. Gash, (Spon: P. Shrager). Depts. of Physiology and Neurobiology and Anatomy, Univ. Rochester Medical Center, Rochester, New York 14642.

MPTP (1-methyl-4-phenyl- 1, 2, 5, 6-tetrahydropyridine) administered systemically to nonhuman primates produces motor deficits and specific morphological lesions virtually identical to those of human Parkinson's disease. In particular, saccadic eye movement deficits such as increased latency, decreased peak velocity, and hypometria were reported in human Parkinson patients (White, O.B., et al., Brain, 106:571, 1983). Similar deficits were reported in a severely ill monkey acutely treated with MPTP (Brooks, B.A., et al., Brain Res., 383:402, 1986). In this study, we have created an animal model of the onset of Parkinson's disease by injecting small doses of MPTP over periods of months. Monkeys were trained to fixate small light emitting diodes arranged in an array. Eye movements were monitered with an accuracy of 0.2 deg using the scleral search coil technique. Monkeys were tested with 4 paradigms designed to examine distractability, and saccades made to predictable, remembered, and visual target locations. To date, 2 monkeys have received MPTP injections: monkey 1 has received a cumulative dose of 12.4 mg/kg over 11 months and monkey 2 has received a cumulative dose of 7.0 mg/kg over 7 weeks.

Both monkeys present occasionally with mild bradykinesthesia and tremor, especially within 2 days of receiving an injection of MPTP. However, these symptoms are not severe, and the monkeys continue to perform the operant tasks, and to groom and feed themselves routinely. Both monkeys do exhibit mild oculomotor deficits. Monkey 1 (with the larger cumulative dose of MPTP) achieves slower peak velocities in all paradigms than does monkey 2. Over the past 8 weeks of testing and dosing, this animal's mean eye movement latency has progressively increased from 171 ms to 270 ms. The mean latency of monkey 2 has not increased in the same paradigm. Eye movement latencies of both monkeys have increased in a predictive tracking paradigm, from 165 to 222 ms in monkey 1 and from 177 ms to 208 ms in monkey 2. Neither monkey shows progressive increases in distractability or in making saccades to remembered target locations. Our data show that the earliest saccadic eye movement deficit associated with the onset of MPTP induced Parkinsonism is an increased latency of visually guided saccades and a modest decrease in peak saccadic velocity. It is notable that these animals usually exhibit more severe deficits within 2 days of receiving MPTP than when tested 1 week later.

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220.11 PROBENECID POTENTIATES THE NEUROTOXICITY OF MPTP BY INHIBITING ITS URINARY EXCRETION IN MICE. Y. S. Lau, J. M. Crampton* and J. A. Wilson. Depts. of Pharmacology and Physiology, Creighton University, Omaha, NE 68178.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) has been widely used in developing an animal model for Parkinson's disease. Multiple injections of high doses of MPTP are required to produce a persistent nigrostriatal toxicity in mice. We observed large amounts of radioactivity appear rapidly in the urine of ^{3}H-MPTP-treated mice following subcutaneous injection of the drug in a study of the safety of using MPTP in animal research (FASEB 46: abs.3734, 1987). The radioactive substances excreted are therefore likely to be the source of cross contamination. In this study we examined the rate of excretion and chemical species in the urine of MPTP-treated mice. We further investigated the effects of probenecid on urinary excretion of the drug and on striatal dopamine levels.

Mice (C57 BL) were treated with one dose of MPTP (15 mg/kg containing 1 uCi of ^{3}H-MPTP, s.c.). Spontaneously excreted urine samples were collected for 48 hours. The radioactivity in the urine was counted and the chemical species excreted in the urine were analyzed by using an HPLC. We observed high levels of radioactivity in early (2-4 hours) samples of urine and the excretion of radioactive substances normally reached a nadir about 12 hours after drug injection. The radioactive substances excreted were chromatographically coeluted with MPTP and MPP^{+} in a concentration ratio of 4:1.

When the mice were injected with probenecid (250 mg/kg, i.p.) prior to the MPTP treatment, the volume and rate of urinary output, and the amount of MPTP excreted were all reduced. The striatal dopamine content in control (probenecid-treated), MPTP-treated, and probenecid plus MPTP-treated animals were assayed by using HPLC electrochemical detection. Striatal dopamine levels remained unchanged in these three groups of animals during the first 24 hours after the respective drug treatment. However, significant reduction of dopamine content was detected in MPTP-treated mice 96 hours following the drug treatment. At this time, a potentiation of dopamine depletion was observed in the striatum of probenecid plus MPTP-treated mice.

In summary, within the first few hours large amounts of MPTP are excreted unchanged as the parent drug in the urine. Probenecid reduces the urinary excretion of MPTP, and potentiates the dopamine depleting effect of MPTP in the nigrostriatal region. Thus by permitting the use of smaller doses of MPTP, probenecid improves the safety and efficacy of the rodent model of parkinsonism.

Supported by a grant from the Health Future Foundation.

220.12 RETROVIRAL INFECTION IN PC12 PRODUCES MPTP RESISTANT MUTANTS. M.M.S. Lo, C.M. Dersch*, C. Mamalaki. National Institute on Drug Abuse, Addiction Research Center, Baltimore Md 21224.

N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a substance of abuse, is highly toxic and elicits neuropathologic changes in man, often resulting in Parkinson's disease. MPTP is also cytotoxic to pheochromocytoma (PC12) cells. This toxicity is mediated by uptake of MPP+, the active metabolite of MPTP, into the cell via the catecholamine uptake site. Infection of PC12 cells by retroviruses creates mutants which resist MPP+ toxicity. Retrovirus integrates into the chromosomes of infected cells and disrupts certain genes, normally coding for proteins which are involved in MPTP toxicity. Specific biological functions, normally present in PC12 cells, are lost in these mutants.

The number of MPP+ resistant mutants infected with virus is 1 per 10^{5} cells; this being 1000 fold greater than the number of resistant cells obtained by spontaneous mutation. This suggests that MPP+ resistance is caused by viral infection and inactivation of normal cellular genes. Three distinct categories revealed by neurochemical analysis were mutants 1) lacking any dopamine uptake, 2) lacking dopamine uptake specifically inhibited by mazindol or sodium, and 3) with normal dopamine uptake. In most cases, other neurochemical markers such as tyrosine hydroxylase, choline acetyl transferase, glucose uptake, dopamine content and dopamine release appear to be normal or slightly reduced Many of these MPP+ resistant mutants are responsive to NGF.

SDS gel analysis shows specific and distinct differences in the protein composition of the membrane fraction of different mutants. Two proteins were completely absent in some of the mutants

The chromosomal positions of the virus in the MPP+ resistant mutants were mapped by Southern blot analysis using viral probes. Most of the mutants contained only a single copy of the retroviral sequence. Restriction map analysis of 10 different cell lines showed viral integration occuring in three distinct chromosomal regions. This suggests that at least 3 gene targets (any of which) can confer resistance to MPP+. Using DNA recombinant techniques we have isolated flanking genomic sequences from two of these gene targets. Cloning of putative DNA sequences involved in MPP+ toxicity was achieved by insertional mutation of PC12 cells and rescue of flanking genomic sequences. Identification, purification and characterization of proteins, traditionally used in gene cloning, was avoided by this method of isolation of cDNA clones.

MODULATORS I

221.1 SWITCHING OF SECOND MESSENGER AND ELECTROPHYSIOLOGICAL FUNCTION OF CHOLECYSTOKININ RECEPTOR IN XENOPUS OOCYTES. T.M. Moriarty*, B. Gillo*, S. Sealfon*, and E.M. Landau (SPON: R. Zappulla). Dept. of Psychiatry and Fishberg Center in Neurobiology, Mt. Sinai School of Medicine and Bronx V.A. Hospital, New York, N.Y.

Oocytes of the African frog Zenopus laevis are a convenient system for studying receptor modulation. We investigated the role of second messengers in mediating the electrophysiological effect of cholecystokinin (CCK) in native oocytes. Oocytes with the follicular cell layer intact (follicles) and oocytes with the follicular cell layer stripped away by treatment with collagenase (denuded) were studied. Single cells were voltage clamped in a superfusion apparatus in standard fashion.

Application of 2 uM CCK induced a depolarizing inward current which is similar in shape, time course, and reveresal potential to the Cl^{-} current produced by intracellular injection of IP_3 and that which is seen with other transmitters known to act through the phosphatidylinositol pathway. Application of CCK after a five minute pretreatment of follicles with 0.2 uM forskolin or 1 mM 8-Br-cAMP (both in concentrations too low to induce appreciable currents alone) resulted in a reversal of this inward current to an outward hyperpolarizing current. This outward current is similar in shape, time course and reversal potential to the K^{+} current produced by intracellular injection of cAMP and that which is seen with other transmitters known to be linked to cAMP. Pretreatment of denuded oocytes (which cannot generate cAMP dependent K^{+} currents) with forskolin or 8-Br-cAMP resulted in an abolition of the inward current.

The CCK receptor appears to be linked to both the cAMP and the phosphatidylinositol second messenger systems. The cell's electrophysiological response to the transmitter depends on the present intracellular level of cAMP. In the resting state, the cell will respond to CCK through the phosphatidylinositol system and depolarize. If the adenylate cyclase is activated or the intracellular concentration of cAMP is increased, the cell will respond to CCK by switching to the cAMP system and hyperpolarizing. This suggests that the second messenger system activated by a transmitter may depend on the level of phosphorylation of some critical intracellular component.

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221.2 INTERACTIONS OF CAPSAICIN AND RELATED COMPOUNDS WITH SECOND MESSENGER PATHWAYS IN A NEURAL CELL LINE. S.I.Patterson*, T.R.Jackson* M.Dreher* and M.R.Hanley, MRC Molecular Neurobiology Unit, University of Cambridge Medical School, Cambridge, CB2 2QH, U.K.

Capsaicin and related pungent agents share a spectrum of biological actions on sensory neurones, including acute stimulation, long lived desensitization to readministration, and neonatal toxicity. The molecular basis of these effects is unknown, but a specific recognition site or "capsaicin receptor" is an attractive possibility. We have examined the ability of capsaicinoids to regulate identified second messenger events in a model system, the NG115-401L hybrid cell line, which exhibits many of the differentiated properties of the physiological target of capsaicin actions, the primary afferent neurone. At a concentration of 1 μM, capsaicin does not stimulate a production of cAMP, inhibit the production of cAMP stimulated by PGE1, mobilise intracellular calcium, or stimulate inositol lipid metabolism. However, 1 μM capsaicin is able to amplify the dose-dependent production of inositol phosphates elicited by bradykinin. This action is the opposite observed upon phorbol diester treatment, which causes an attenuation of the bradykinin response. Thus, capsaicinoids may interact with the signalling pathway modulated by phorbol diesters. Accordingly we have examined several non-invasive indicators of phorbol stimulation for sensitivity to capsaicinoids. Biologically active phorbols and related tumor promoters cause a dose dependent stimulation of phosphatidylcholine breakdown, releasing free extracellular choline. Capsaicin stimulates a similar response directly and moreover, alters the response to phorbol-12, 13-dibutyrate (PDBu). However, capsaicin does not compete for ^{3}H-PDBu binding. Capsaicin also exhibits dose dependent cytostatic and cytotoxic effects on the cell line. The structural requirements and potential relationships between these different effects will be discussed. The results suggest a close relationship between phorbol mediated actions and capsaicinoids, implying that protein kinase C may be a molecular target for the capsaicin family.

221.3 FORSKOLIN PROLONGS CALCIUM ACTION POTENTIALS IN LAMPREY SENSORY NEURONS. M.D. Womble* and W.O. Wickelgren. Dept. of Physiology, Univ. Colorado Health Sci. Ctr., Denver, CO. 80262.

Primary sensory neurons (dorsal cells) of the lamprey spinal cord, in the presence of Na^+ channel (TTX) and K^+ channel (TEA and 3,4 DAP) blockers, produce long-lasting Ca^{+2} action potentials (Ca APs) in response to brief depolarization. The Ca AP is followed by a prolonged afterhyperpolarization produced by a calcium-activated potassium conductance ($G_{K(Ca)}$). Previous work from this laboratory (Leonard and Wickelgren, J. Physiol. 375: 481, 1986) has shown that the application of gamma-aminobutyric acid (GABA) to dorsal cells prolongs the duration of Ca APs by specifically reducing $G_{K(Ca)}$. Here, we report that the action of GABA can be mimicked by the application of forskolin, a specific activator of adenylate cyclase.

Intracellular recordings were made from dorsal cells of isolated lamprey (Lampetra lamottenii) spinal cord segments. In normal saline, injected depolarizing pulses produced brief Na^+ action potentials (Na APs), without a detectable Ca^{+2} component. The addition of 50 uM forskolin to normal saline did not affect Na AP durations, resting potentials, or passive current-voltage characteristics. On the other hand, forskolin increased the duration of both mixed Na-Ca APs, produced by the addition of K^+ channel blockers to normal saline, and pure Ca APs, generated in saline containing both TTX and K^+ channel blockers. However, forskolin did not effect the resting membrane potentials or passive current-voltage characteristics of these cells. The broadening effect of forskolin on Ca APs was shown to be dose-dependent in the range of 25-200 uM, with the highest dose producing approximately a 3-fold increase in AP duration. Since forskolin had no effect on Na APs or on passive membrane properties of dorsal cells, we conclude that it does not act upon either passive membrane channels or voltage-dependent Na^+ or K^+ channels. Rather, forskolin's action appears to require the presence of a detectable Ca^{+2} current. Therefore, forskolin could act to increase the duration Ca APs either by affecting Ca^{+2} channels, to increase Ca^{+2} influx, or by decreasing $G_{K(Ca)}$, a repolarizing conductance change. The latter appears to be the case. The input resistance of dorsal cells during the plateau phase of the Ca AP was increased by forskolin, indicating that during this time a repolarizing current was decreased. Thus, the broadening of the Ca AP produced by forskolin appears to be due to the specific inhibition of $G_{K(Ca)}$, mimicking the membrane effects of GABA on these cells. This suggests that in lamprey dorsal cells, the modulation of $G_{K(Ca)}$ by GABA may be mediated by an increase in intracellular cyclic AMP.

221.4 MULTIPLE ACTIONS OF ATP ON CHICK SKELETAL MUSCLE S.A. Thomas* and R.I. Hume. (SPON: B. Pfingst). Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109.

ATP (1-10μM) has a potent depolarizing effect on chick myotubes in culture and in vivo. In the studies reported here we examined the ATP responsiveness of myotubes in culture exposed to extracellular solutions containing blockers of several voltage dependent channels over a wide range of membrane potentials. These studies indicate that ATP activates at least two distinct currents, and suggest that both are activated by the same ATP receptor.

Intracellular recordings were made in an external solution containing TTX (10^{-7}M), TEA (20mM), and Co^{++} (4mM) to block voltage dependent Na^+, K^+ and Ca^{++} currents respectively. The membrane potential (Vm) of the myotubes could be varied between +20 and -100mV with little if any sign of voltage dependent responses.

At rest (Vm = -40 mV), a brief (1 sec.) application of 10μM ATP elicited a net depolarization, but the waveform was complex. At -10 mV and potentials more positive, ATP produced a large hyperpolarization without any apparent depolarization. At intermediate potentials (for instance -20 mV) ATP produced an initial depolarization followed by a hyperpolarization, then a late depolarization. At all potentials other than -10 mV the latency to response was about 300 msec, but at -10 mV the latency was 1.1 sec. Responses at all potentials can be explained by the superimposition of a slowly activating, hyperpolarizing reponse upon a more rapidly activating, depolarizing response with a reversal potential of -10 mV. In other experiments, ATP-specific conductance was monitored with repetitive current pulses. At early time points, where only depolarization occurs, a conductance increase was seen, and an even greater conductance increase was seen during the interval corresponding to activation of the hyperpolarizing current. Thus both responses are likely due to conductance increases. Substitution of most of the Na^+ in the external solution with choline indicated a partial dependence of the depolarization on Na^+, but there was no effect on the hyperpolarization. Recordings made in an external solution with a high K^+ concentration markedly affected the hyperpolarization. In a third test solution, replacing most of the chloride with bicarbonate had no apparent effect on either current. These results suggest that a nonspecific cation channel and a K^+ channel are responsible for ATP-activated depolarization and hyperpolarization respectively.

The possibility that both of these currents are activated by the same receptor is suggested by several observations. Both currents show profound desensitization to ATP subsequent to ATP activation, with no apparent recovery after 15 minutes at room temperature. In addition, the same concentrations of ATP are required to activate each current. And finally, all the putative ATP receptor agonists which we have tested either evoke both currents or show no activity.

221.5 ACH RELEASE IN THE AVIAN CHOROID COAT IS INHIBITED VIA A G PROTEIN-MEDIATED MECHANISM WHILE RELEASE IN AVIAN IRIS IS NOT. D. Bruce Gray and G. Pilar. Department of Physiology and Neurobiology, The University of Connecticut, Storrs, CT.

It has been shown that morphine delays motoneuron death in the developing chick ciliary ganglion and inhibits ACh release from ciliary ganglion neurons in co-culture with striated muscle (Meriney et al., Science 228: 1451 (1985)).

To determine whether morphine affects transmitter release at intact neuromuscular junctions between the chick ciliary ganglion and its target musculature, ACh release from neuronal terminals in the isolated smooth muscle layer of the choroid coat and the striated iris of the chick eye was measured after stimulation by incubation in high-K+ Tyrode's. At hatching, both tissues exhibit high-affinity sodium-dependent choline uptake-mediated ACh synthesis. To measure transmitter release, excised tissues were preincubated with ^3H choline (specific activity, .5 mCi/mmole; choline concentration, 1 μM) for 1 hr at 37 C and washed repeatedly in zero-Ca++ Tyrode's until release of extrinsic labeled choline declined to insignificant levels. Labeled ACh was separated from choline by high-voltage paper electrophoresis and quantified by scintillation counting. Both tissues exhibit a basal release of labeled ACh that is potentiated severalfold during a 5-minute incubation in high-K+ Tyrode's (55mM KCl). 50% of the basal release and 100% of the stimulated release are calcium dependent.

Co-incubation of the choroid with 1μM morphine sulfate blocks approximately 90% of the stimulated release. The same effect is seen with 100nM somatostatin (1-14). Preincubation of the excised choroid in oxygenated Tyrode's containing pertussis toxin (200 ng/ml) reverses the inhibitory effect of both morphine and somatostatin.

However, ACh release from terminals in the striated iris is not affected by either morphine or somatostatin at μM levels. Thus, earlier results demonstrating morphine sensitivity of ACh release in cultured ciliary ganglion neurons may be explained by selective participation of choroid terminals in the measured ACh release or by acquisition of sensitivity to opiate modulation in vitro by ciliary neurons.

Furthermore, these results suggest that both opiate and somatostatin receptors are present in the choroid target and may act through a final common pathway to modulate ACh release via a G protein. It is unclear at this time whether the appropriate endogenous peptides are present in this system, or what is the nature of the molecular events underlying the modulation.

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221.6 **FOLATE INTERACTIONS WITH CEREBRAL G·PROTEINS,** **Dean M. Hartley*and S. Robert Snodgrass, Neurology Division, Childrens Hospital of Los Angeles, and Dept of Neurology, University of Southern California School of Medicine, Los Angeles, CA 90027.**

Large doses of folates produce seizures and excitotoxic brain damage, which is reportedly blocked by NMDA receptor blockers. Various authors have speculated on a possible transmitter or modulator role for folates in the CNS. The slime mold, Dictyostelium discoideum (Devreotes, Dev. Biol. 95: 154, 1983), has a guanine nucleotide binding protein (G proteins) which is stimulated by folic acid, increasing both GTP binding and GTPase activity. We postulated that folates interact directly with one of the cerebral G proteins to increase the excitability of some but not all transmitter receptors. This relationship might resemble that of PCP with NMDA receptors.

We prepared rat brain membranes (20,000xg pellet) by homogenizing with 50 mM Tris-HCl, pH 7.5 and incubating them at 30°C for 45 min in 50 mM Tris-HCl + 50 mM MgCl, pH 7.5. Saturable binding of ^{35}S-guanosine 5-(γ-thio)triphosphate was observed. More than 75% of binding was displaceable with the GTP analog, 5'-guanylylimidodiphosphate. Binding was sensitive to divalent cations and increased by 220% after preincubation of membranes with NaF. A study of 6 brain regions showed significant variations between regions, with greatest GTP binding found in cerebellar membranes. When membranes were preincubated with various folates (20 μM), the binding of ^{35}S-GTP-γ-S was stimulated in some but not all regions. The greatest folate stimulation (45%) was seen in cerebellar membranes, this effect was statistically significant (Bonferroni t-test). Significant folate stimulation (30%) was also observed in hippocampal membranes. Folic acid, dihydrofolate and tetrahydrofolate were all similar in potency as stimulators of GTP binding. Glutamate used at the same concentration as the folates, had no effect on ^{35}S-GTP-γ-S. Other excitotoxins (kainate, quisqualate and quinolinate) failed to duplicate the folate effect.

The effect of pertussis and cholera toxins, known to catalyze the ADP-ribosylation of the inhibitory and stimulatory α-subunit of G proteins, were tested on both hippocampal and cerebellar membranes. Cerebellar membranes were treated with pertussis toxin and preincubated with 20 μM folic acid. Pertussis treatment significantly increased the total GTP bound above the control levels and those values obtained with folic acid treated membranes (non-pertussis treated). This effect was not seen in either cholera treated cerebellar membranes or in hippocampal treated membranes.

221.7 LEUKOTRIENE C_4-BINDING SITES IN THE MOUSE FOREBRAIN. A.M. Goffinet Positron Tomography Lab., Univ. Louvain, B-1348 Louvain-la-Neuve, Belgium.

Leukotriene C_4 (LTC) is a mediator of the inflammatory response for which specific binding sites have been described in various tissues.

Binding sites for $[^3H]LTC_4$ were demonstrated in the mouse brain, by using binding to brain membranes and in vitro autoradiography. Isotherm binding analysis revealed one binding site with a dissociation constant (K_d) of 10.39 ± 1.97 nM (Scatchard) or 10.15 ± 1.00 nM (non linear analysis). The maximal binding capacity (B_{max}) was 49.20 ± 3.80 (Scatchard) or 48.50 ± 2.63 pmols/mg. prot. (non linear). Specific binding represented more than 90% of total binding. Displacement reactions on brain membranes showed that LTC_4 was 100 times more potent than LTD_4, which differs from LTC_4 by the absence of a glutamic acid residue. Glutathione was ten times less potent than LTD_4. Glutamic acid, bilirubin, hematin, azaserine and 6-diazo-5-oxonorleucine were inactive.

On brain sections, the binding of $[^3H]LTC_4$ was inhibited by cold LTC_4, but unaffected by LTD_4. The density of binding sites was minimal on fiber bundles and on choroid plexuses, high in the cerebral cortex, thalamic relay nuclei and caudoputamen, and maximal at the level of granule cell-rich structures such as the dentate gyrus, entorhinal area, indusium griseum.

these results support the hypothesis, first proposed by Lindgren et al. (PNAS 81: 6212; 1984), that leukotrienes may have a modulatory function in the central nervous system.

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221.8 AN ENDOGENOUS MODULATOR FOR CA^{2+} CHANNELS IN BRAIN TISSUE: ISOLATION AND CHARACTERIZATION
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Several classes of drugs have been shown to increase or decrease the activity of receptor-linked and "voltage-dependent" Ca^{2+} channels. These drugs have as a common feature the ability to bind with high affinity to membrane proteins that are associated with the Ca^{2+} channel. In addition, dihydropyridine binding sites were found up-regulated in sympathetic-denervated rat heart and in brain of morphine-tolerant mice suggesting that these binding sites may play a physiological role as possible recognition sites for an endogenous ligand. Recent work from this laboratory demonstrated the existence in rat brain of a substance that decreases ^3H-nitrendipine binding. In an effort to purify and characterize this endogenous ligand present in brain tissue, we studied the material obtained in various purification steps for its displacement activity of ^3H-nitrendipine binding to hippocampal membranes, and its effect on veratridine- or glutamate-stimulated $^{45}Ca^{2+}$ -uptake in cultured cerebellar granule cells. The purification of the ^3H-nitrendipine-displacing material involved: (1) Precipitation of bulk proteins with TCA (3% final concentration); (2) Methanol-extraction of lyophilized supernatant fraction; (3) Fractionation on Silica-SEPPAK cartridge by a stepwise gradient of chloroform-methanol; and, (4) Fractionation by HPLC on Partisil-10SCX column. The purified, ^3H-nitrendipine-displacing material migrates on TLC plates as one spot that is stained intensively by ninhydrin. The material eluted from Partisil-10SCX decreased reversibly and noncompetitively the specific binding of ^3H-nitrendipine to hippocampal membranes. In addition, it inhibited the veratridine- and glutamate-elicited increase of $^{45}Ca^{2+}$ -uptake as well as the increase of cyclic GMP formation in primary cultures of cerebellar granule cells. Acid hydrolysis of the material eluted from Partisil-10SCX column (5.7 N HCl, 155°C for 30 min.) abolished the ^3H-nitrendipine-displacing and Ca^{2+} uptake-inhibiting activity. The present data suggest that the nitrendipine-displacing material may be an endogenous ligand whose interaction with Ca^{2+} channels is comparable with that of organic Ca^{2+} channel antagonists.
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221.9 PURIFICATION OF THE PUTATIVE ENDOGENOUS LIGAND FOR THE HIGH AFFINITY ^3H-IMIPRAMINE RECOGNITION SITE. M.L. Barbaccia and E. Costa. FIDIA-Georgetown Institute for the Neurosciences, Georgetown University School of Medicine, Washington, DC 20007.

Previous reports from our group and other groups have suggested that human plasma and rat brain may contain at least one endogenous substance, different from serotonin, that specifically binds to the high affinity recognition site for ^3H-imipramine and modulates in an inhibitory fashion the ^3H-serotonin (5HT) reuptake by serotonergic axon terminals. Though the complete purification and therefore the identification of the chemical nature of this putative endacoid is still among our goals, we report on the progress achieved in our laboratory towards its purification. The major steps in the latest purification procedure include: 1) homogenization of the whole rat brain in 1 M acetic acid (at 90°C for 10 min); 2) methanol washing of the supernatant after lyophilization; 3) dialysis through a membrane with a m.w. cut-off of 3,500 daltons; 4) application of the small (<3,500 daltons) material to a high voltage electrophoresis on Agarose C; and 5) chromatography of the high voltage electrophoresis fractions that were active in inhibiting ^3H-imipramine specific binding on a strong cation exchange column for HPLC. The HPLC system we used allows to locate the biological activity in a major peak that displaces ^3H-imipramine binding and inhibits ^3H-5HT uptake. These biologically active HPLC fractions were then spotted on a silica gel plate for TLC and allowed to run for 3 hrs in a buffer containing ethanol:acetic acid:water (8:1:1). Three Iodine-sensitive spots were clearly separated. When tested for their activity in inhibiting ^3H-imipramine binding after scraping off each spot from the TLC plate, the major part of the activity was in the spot with the highest R_f value. The compound contained in this spot showed a U.V. absorbance spectrum with a peak at 260 nM and was not sensitive to ninhydrine. This material is now being subjected to mass fragmentographic analysis in order to gain some understanding on its molecular nature. Since its biological activity is not sensitive to proteases pretreatment it seems reasonable to conclude that it may not be peptidergic in nature. It is also interesting that this putative endogenous substance inhibits on a equimolar basis ^3H-imipramine binding and ^3H-5HT uptake into synaptosomes, but appears not to inhibit ^3H paroxetine binding and it is 10 times less potent in blocking ^3H-norepinephrine uptake. We conclude that the putative endogenous ligand effect described above cannot be ascribed to a hyperosmolarity effect as inferred by Lee et al. (Biochemical Pharmacology 36(6): 945-949, 1987).

221.10 PROTEIN PHOSPHORYLATION IN THE BRAIN AND ANTERIOR PITUITARY: EFFECTS OF NEUROPEPTIDES AND STIMULATION BY cAMP-, CALMODULIN-, AND PHOSPHOLIPID-DEPENDENT PROTEIN KINASES. S.T. Cain, M. Abramson*, J.C. Pryor* and C.B. Nemeroff. Departments of Psychiatry and Pharmacology, Duke University Medical Center, Durham, NC 27710.

The cyclic phosphorylation/dephosphorylation of specific protein substrates is likely an integral mechanism in the regulation of synaptic excitability in the central nervous system (Browning et al., 1985, J. Neurochem. 45:11-23). In addition, there is an accumulating body of evidence which suggests that protein phosphorylation changes are involved in the mediation of hormonal secretion from the adenohypophysis (Aguilera et al., 1986, Neuroendocrinology 43:79-88). Our laboratory is interested in the mechanisms of action of brain neuropeptides, in particular the brain-gut tridecapeptide neurotensin (NT) and the hypothalamic hypophysiotropic hormone, corticotropin-releasing factor (CRF). Prior reports have demonstrated that NT receptors in brain are coupled to phosphatidylinositol hydrolysis, and presumably activation of the calcium/phospholipid-dependent protein kinase (protein kinase C, Goedert et al., 1984, Brain Res. 323:193-197). Conversely, a population of CRF receptors in both anterior pituitary and brain is coupled to cAMP formation and activation of cAMP-dependent protein kinases. We have examined specific phosphoprotein substrates which are sensitive to NT and CRF and have begun to identify the types of protein kinases involved in the phosphorylation of these proteins.

Adult, male rats were decapitated and the caudate nucleus or anterior pituitary dissected and homogenized. A lysed synaptosomal fraction was prepared from the caudate nucleus homogenates and used for in vitro phosphorylation. The anterior pituitary homogenate was used for in vitro phosphorylation without further processing. Phosphorylation was accomplished by incubation for 1 minute with 10 μM ATP (containing $[\gamma^{32}P]ATP$) in the presence or absence of various cofactors.

In view of the coupling of CRF receptors to activation of adenylate cyclase, we have compared cAMP-dependent protein phosphorylation in the caudate nucleus and the anterior pituitary. In the caudate nucleus, the primary cAMP-dependent substrates had molecular weights of 74, 72.5, 68.5, 57.5, 51 and 37 Kd. In the pituitary, the cAMP-dependent substrates had molecular weights of 52, 35, 32, 31, 26 and 22 Kd. We are currently in the process of evaluating the effects of CRF on the phosphorylation of cAMP-dependent substrates in brain and pituitary. In contrast, NT stimulated the phosphorylation of a group of caudate nucleus synaptosomal proteins (76, 71 and 49.5 Kd) which appear to be substrates for the calcium/phospholipid-dependent protein kinase.
(Supported by NIMH MH-39415, MH-42088 and MH-15177.)

221.11 PLATELET-ACTIVATING-FACTOR (PAF) INHIBITS LUTEINIZING-HORMONE-RELEASING-HORMONE (LHRH) AND SOMATOSTATIN (SRIF) RELEASE FROM MALE RAT MEDIAN EMINENCE (ME). M.P. Junier*, C. Tiberghien*, V. Fafeur* and F. Dray INSERM U.207, URIA, INSTITUT PASTEUR PARIS

PAF, originally described as a platelet activating factor is effective, among various parameters, on secretory processes. It seemed then interesting to test it on LHRH, SRIF and Growth-Hormone-Releasing-Factor (GRF) release from rat ME,a hypothalamic fragment rich in nerve endings containing these neuropeptides. Additionnally an effect of PAF at the rat Medio-Basal-Hypothalamus lacking the ME (MBH) and at the pituitary level was explored.

Static incubations of ME and MBH (2ME/400 µl Krebs Ringer Bicarbonate Buffer containing 2.2 U/ml Bacitracine and BSA 0.025 %, KRBG, 2MBH/600 µl KRBG)were carried out either 45 min for ME (15 min preincubation, PI, 30 min incubation, I) or 60 min for MBH (30 min PI, 30 min I) at 37°C under an atmosphere of 95 % O_2 5 % CO_2 in the presence of PAF (10^{-17} to 10^{-8} M). LHRH and SRIF release were decreased by PAF (10^{-14} to 10^{-13} M and 10^{-14} to 10^{-11} M respectively) with a maximal inhibition at 10^{-14} M for both neuropeptides (LHRH 78±15 pg/mg protein, n=6 vs control 166±10 pg/mg protein, n=11, SRIF 447±129 pg/mg protein, n=6 vs control 1077±119 pg/mg protein, n=11), whereas GRF release was not affected. [^{3}H]PAF added to the incubation medium containing the ME was extracted and purified on HPLC. It was recovered as authentic PAF in both supernatant and ME where a second compound with a shorter carbonyl chain was present. Stimulated release of LHRH and SRIF by the ionophore A23187 5 µM was decreased by PAF 10^{-14} and 10^{-8} M (at 10^{-14} M 44% and 36% inhibition of A23187-stimulated-LHRH and SRIF release respectively). LHRH and SRIF release from MBH was not altered by PAF (10^{-14} and 10^{-8} M). Dispersed rat antepituitary cells were incubated 3 hours in the presence of PAF (10^{-14} to 10^{-8} M) which did not affect Luteinizing-Hormone neither Growth-Hormone nor Prolactin release.

PAF is the first lipidic compound described as an inhibitor of LHRH and SRIF release from ME while, on the contrary, it is ineffective on GRF release. It acts specifically at the hypothalamic neuronal endings level and counteracts the stimulatory effect of a massive Ca^{++} entry induced by the ionophore A23187 on LHRH and SRIF release. These facts, correlated with the recent finding, in our laboratory, of PAF specific binding sites in rat hypothalamus suggest that PAF is a potential physiological regulator of LHRH and SRIF release.

M.P. Junier is the recipient of a fellowship from ROUSSEL-UCLAF FRANCE and V. Fafeur from SANOFI FRANCE

221.12 CHARACTERIZATION OF PLATELET ACTIVATING FACTOR (PAF) SPECIFIC BINDING IN MALE RAT BRAIN. C. Tiberghien*, M.P. Junier*, M.T. Domingo* and F. Dray (SPON: G. Fillion). INSERM U 207, URIA, INSTITUT PASTEUR, PARIS

Following the recent discovery of a modulatory effect of PAF on neurosecretion, a specific binding of Platelet Activating Factor (PAF) was investigated on male rat brain membrane preparations.

The binding of synthetic [^{3}H]PAF, 1-O[alkyl-1',2'-^{3}H]-alkyl-2-O-acetyl-sn-glycero-3-phosphorylcholine (NEN, England), was achieved in 50 mM Tris buffer containing 0.25% BSA, 10 mM $MgCl_2$, pH 7.4 and at 0°C, with membrane aliquots of about 250 µg in a final volume of 200 µl. The non specific binding was determined by an excess (x1000) of unlabeled PAF or compounds, well known as potent antagonists of PAF on its platelet binding sites (L 652-731 and Kadsurenone, from Merck Sharp and DHOME Research Lab., NJ, USA, BN 52021 from Beaufour, France).

Under these conditions the association kinetic reached equilibrium with a $t_{1/2}$ of about 100 min. Saturation and competition experiments showed a saturable specific binding. Data analysis indicate the presence of two classes of binding sites. The Kd_1 of the high affinity binding component was 0.45 ± 0.16 nM (n_1=10,0 ± 3.5 fmol/mg protein). The Kd_2 of the lower affinity component was 15.5 ± 9 nM (n_2=70 ± 25 fmol/mg protein). The specificity of the binding was investigated in competitive experiments at 1 nM of [^{3}H]PAF. The order of displacement was PAF (ED_{50}=1.0 ± 0.33 nM, n=4) > L652-731 (ED_{50}=46 ± 13 nM, n=2) > Kadsurenone (ED_{50}=132 ± 35 nM, n=2) > BN 52021 (ED_{50}=890 nM, n=1). No competitive effect was observed with two PAF structural derivatives, lyso-PAF and 1-O-hexadecyl-sn-glycerol. Specific binding of [^{3}H]PAF at 1 nM expressed in % of specific binding ((specific bound/total bound)x100) in various brains areas was more important in cortex (33%), quite followed by hypothalamus (20%), lower in cerebellum (12%) and not detectable in pituitary.

These results demonstrate the existence of high affinity and low capacity PAF binding sites in rat brain. The recent finding in our laboratory of an inhibitory effect of PAF on Luteinizing-Hormone-Releasing-Hormone (LHRH) and Somatostatin (SRIF) release, but none on that of Growth-Hormone-Releasing-Factor (GRF), from male rat median eminence suggests that PAF could be a modulator of endocrine function, at the neurosecretory level, through a receptor.

M.P. Junier is the recipient of a fellowship from ROUSSEL-UCLAF France and M.T. Domingo from BEAUFOUR FRANCE.

ACTION POTENTIALS AND ION CHANNELS IX

222.1 ***CONUS* VENOM ALTERS IONIC CURRENTS IN IDENTIFIED *APLYSIA* NEURONS.** Varda Lev-Ram*, Baldomero Olivera* and Irwin B. Levitan (SPON: Herman T. Epstein). Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254 and Department of Biology, University of Utah, Salt Lake City, UT 84112.

Crude venom extracted from the predator snail *Conus textile* alters membrane excitability in identified *Aplysia* neurons. When venom was applied to the medium bathing an *Aplysia* abdominal ganglion, the electrical activity of the bursting pacemaker neuron R15 changed dramatically. First there was an increase in membrane excitability which resulted in enhancement of bursting, and this was followed by a long lasting hyperpolarization. The hyperpolarization appeared to be a long lasting synaptic inhibition, resulting from an increase in excitability of some unidentified presynaptic cell. The increase in synaptic input to R15 persisted even when cobalt was substituted for calcium in the bathing medium. *Conus* venom also caused an increase in excitability followed by an enhancement of synaptic input in left upper quadrant (LUQ) bursting neurons. Voltage clamp experiments demonstrated that the long lasting synaptic hyperpolarization induced in R15 was due to a decrease in a subthreshold calcium current. Dopamine application also decreases calcium current in R15, and the synaptic component of the venom effect was occluded by dopamine.

The synaptic component was eliminated by axotomizing R15 and LUQ neurons and "puffing" venom from an electrode in the immediate vicinity of the target cell bodies. Under these conditions we observed a decrease in outward current evoked by depolarizing voltage clamp pulses; this could be due either to a decrease in potassium current or an increase in calcium current (or both). In addition the venom caused a decrease in an inwardly rectifying potassium current evoked by hyperpolarizing current pulses. These changes are consistent with the observed increase in membrane excitability, and might also account for increased release of transmitter from the unidentified presynaptic cells. It will be of interest to determine whether individual purified components of the venom specifically affect individual membrane ion currents in these neurons.

Supported by NIH Grant NS17910 to IBL and a Weizmann Fellowship to VL-R.

222.2 A COMPONENT OF CALCIUM CHANNEL CURRENT IN APLYSIA SENSORY NEURONS IS BLOCKED BY A DIHYDROPYRIDINE. B. W. Edmonds, M. Klein* and E. R. Kandel. Howard Hughes Medical Institute, Center for Neurobiology & Behavior, Columbia University, College of P & S, and NYS Psychiatric Institute, New York, NY 10032.

Recent introduction of pharmacological agents which selectively alter the function of distinct classes of calcium channels has facilitated the examination of multiple types of voltage-sensitive calcium channels in neurons (Nowycky et al., Science, 316, 1985). These channel-specific agents also allow assay of the functional roles of different types of channels. We have begun an investigation of the properties and roles of various calcium channels in the sensory neurons of Aplysia utilizing nifedipine, a dihydropyridine thought to interact with a specific class of calcium channels.

Pleural sensory neurons were voltage-clamped with two micro-electrodes in the presence of (in mM) 460 TEA, 10 KCl, 44 $MgCl_2$, 22 $BaCl_2$ and 10 HEPES, and the effect of nifedipine (1-15 uM in 0.1% ethanol) was examined on inward barium current. Currents were elicited once every 30 sec by 100 ms steps from a holding potential of -60 mV to a test potential of +10 mV, the potential at which the inward current was largest. Nifedipine at concentrations above 2 uM produced a dose-dependent, reversible block of a component of the inward current. Subtraction of currents in the presence of nifedipine from control currents suggests that nifedipine is blocking a non-inactivating inward current similar to the L-type current previously described by Nowycky et al. The observation that a saturating concentration of nifedipine (~ 10 uM) produces only a 50% reduction (SEM 1.7, N = 5) in the peak of the inward current suggests that the barium current in Aplysia sensory neurons comprises at least two components: a dihydropyridine-sensitive and a dihydropyridine-insensitive component. We do not know, however, whether these components are kinetically distinct.

In addition to a more thorough characterization of the currents, we plan to assess the functional roles of the different types of channels by defining the role of these channels in neurotransmitter release and in the modulation of this release.

222.3 Phosphoinositide turnover and modulation of Ca current in dorsal root ganglion neurons are mediated by norepinephrine.

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In dorsal root ganglion neurons, norepinephrine (NE) inhibits the voltage dependent Ca conductance (Dunlap & Fischbach J.Physiol. 317, 1981). Recently, Rane and Dunlap (PNAS 83: 184, 1986) have demonstrated that agents which activate protein kinase C such as 1,2-oleoyl acetylglycerol mimic the effects of norepinephrine (NE) in reducing the voltage activated Ca current in embryonic sensory neurons. Since C kinase has been shown to be activated as the result of phosphoinositide (PI) hydrolysis we examined the possibility that NE might enhance PI metabolism and thereby activate protein kinase C. We have measured the production of inositol phosphates (IP's) in sensory neurons, as an indication of the degree of activation of PI turnover. Dispersed sensory neurons in culture were prelabeled with ^{3}H-inositol and incubated with test agents. The cells were then extracted and the labeled sugars were separated by column chromatography (Downes & Michell, Biochem.J. 198:133, 1981). A significant increase in the production of pooled ^{3}H-IP's was observed at the shortest time that we can measure (~15 sec.). The maximum response (3 fold) was obtained after 1.5 minutes. By 10 minutes the IP's production has returned to the control level. This rapid and transient feature of the NE induced IP's production would predict a similar time course for NE modulation of the Ca current if these two events are *causally* related. Analysis of the time course of the NE modulation of Ca current revealed a steady decline of the response with the continued presence of NE in a manner that matches the time course of inositol phosphates production. Both responses to NE are rapid in onset with significant changes detected immediately. Furthermore, the two responses to NE have similar pharmacology in that they both are inhibited by yohimbine (an α_2 antagonist) and are insensitive to isoproterenol (a β agonist). Finally, Dunlap and collaborators (Holz, Rane & Dunlap Nature 319:20, 1986) have shown that pertussis toxin, which interferes with G-protein activation, decreases the NE modulation of the Ca current. We report here a similar decrease in the NE induced PI hydrolysis after pretreatment with pertussis toxin. This indicates that the two responses are mediated via a G-protein, and further support the idea that these two phenomena are closely linked. We suggest that the production of the inositol phosphates is closely linked to the effect of NE on the modulation of the Ca current in the sensory neurons, and may in fact display the first step in the mechanism for the Ca channel modulation by NE, in the sensory neurons of chick embryos. Supported by awards to LWR from the Klingenstein & Sloan Foundations & by an award to OEH from the Benin Foundation.

222.4 **NOREPINEPHRINE INHIBITION OF SENSORY NEURON CALCIUM CURRENT IS BLOCKED BY A SPECIFIC PROTEIN KINASE C INHIBITOR.**

S.G. Rane, M.P. Walsh* and K. Dunlap. Dept. of Physiology, Tufts Univ. Sch. of Med., Boston, MA 02111 and Dept. of Medical Biochemistry, Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1.

The voltage-dependent, slowly inactivating calcium (Ca) current of chick sensory neurons can be inhibited by norepinephrine (NE) via a mechanism which does not appear to involve either cAMP, cGMP or calmodulin-dependent signal transduction systems. Because phorbol ester and 1,2-oleoylacetyl-glycerol (OAG), compounds which activate the phospholipid/Ca-dependent protein kinase C (PKC), mimic the action of NE, it has been suggested that PKC may mediate this inhibition of Ca current (Rane & Dunlap, 1986, PNAS 83, 184-188). To more directly test this hypothesis, we used the whole cell configuration of the patch clamp technique to introduce into sensory neurons a potent and highly specific 17 kDa protein kinase C inhibitor (PKCI) isolated from bovine brain (McDonald & Walsh, 1985, BBRC 129, 603-610). To promote dialysis of the cell interior, large diameter (3-5 μm ID) borosilicate glass (WPI 1B150F-4) pipettes were used. Cells were dialyzed with an internal solution (containing, in mM, 150 CsCl, 10 HEPES, 5 MgATP, and 5 BAPTA), with or without PKCI, and the responses of the two populations of cells to NE or OAG were compared. Electrophysiological and visual criteria were used to judge that both control and PKCI-treated cells were well dialyzed with patch pipette solution for at least 10 minutes before application of NE (10 μM) or OAG (60 μM). Concentrations of PKCI from 25 to 150 μg/ml were tested and found to attenuate the NE- and OAG-induced inhibition of Ca current in a dose-dependent fashion. At 75 μg/ml PKCI, NE inhibition of Ca current was half of that observed for control cells, and at 150 μg/ml PKCI, NE's effect was reduced by more than 90%. PKCI was somewhat less effective on OAG-mediated responses, producing a 70% attenuation at 150 μg/ml. As a control, a preparation of 150 μg/ml PKCI was exposed to trypsin digestion; dialysis of neurons with this preparation reduced NE's effect by only 30%. Half maximal and maximal inhibition of PKC-dependent phosphorylation in vitro occurred at 37 and 50 μg/ml, respectively (McDonald & Walsh, 1985). Thus, the dose-response curve for PKCI inhibition of NE action on Ca current is right-shifted by a factor of 2 to 3 relative to that describing its inhibition of PKC, in vitro. This discrepancy probably reflects differences between in vivo and in vitro conditions, and the fact that PKCI concentrations in the dialyzed neurons are likely to be less than those in the pipette solution.

The ability of neurotransmitters and PKC activators (OAG and phorbols) to similarly affect certain ion channels has been used as inferential evidence that PKC may be involved in channel modulation. We have used a specific PKCI to show that activation of PKC is an essentail step in the process by which NE down modulates voltage-dependent Ca current in chick sensory neurons. [Supported by NS16482 (KD) and NS07756 (SGR).]

222.5 β-ADRENERGIC AGONISTS INCREASE CALCIUM CHANNEL ACTIVITY IN SECRETORY CELLS OF THE MOUSE ANTERIOR PITUITARY.

Martha C. Nowycky, Dept. Anatomy, Med. Coll. Penn., Philadelphia, PA, 19129.

AtT-20 cells are a clonal line derived from mouse anterior pituitary which secrete adrenocorticotrophic hormone (ACTH) and β-endorphin *in vitro*. β-adrenergic agents, such as isoproterenol, increase secretion via a mechanism which includes an increase in action potential (AP) frequency and consequent increase in Ca^{2+} influx (Surprenant, J. Cell Biol., 95, 559, 1982). Previously, I have shown that β-adrenergic agents act in part by decreasing the activity of a voltage-gated K channel (FK channel) which helps terminate APs (Nowycky, Bphys. J. 51, 57a, 1987).

Using single channel, cell-attached patch clamp techniques, I have studied calcium channel activity in this cell line. The recording pipette contained 110 mM $BaCl_2$ as the charge carrier and 10 mM tetraethylammonium ion to block the FK channel. The cell resting potential was zeroed with isotonic K aspartate solution. Channel activity was elicited by test pulses to +20 or +30 mV from a holding potential of -80 mV, administered at 0.33 Hz.

Under these conditions, the most frequently observed calcium channel generally resembles "L"-type channels of chick dorsal root ganglion (DRG) cells (Nowycky et al., Nature 316, 440, 1985). The single channel conductance is ~23 pS, somewhat smaller than in DRG cells. Channel openings are seen throughout a 70 msec pulse, and the averaged current reconstructed from single channel recordings is flat. In addition, in whole cell recordings with 3 mM Ca^{2+} as the charge carrier, the inward current does not inactivate during a 200 msec pulse. The channels possess dihydropyridine receptors, since the mean open time is greatly increased by the dihydropyridine agonist, Bay K 8644.

Application of isoproterenol (1 μM) to the bath, caused an increase of calcium channel activity in 14 of 21 patches. In responsive cells, the average increase is about 2-fold, while 5 patches exhibit more than a 5-fold increase. The increased activity results from both a decrease in the number of null sweeps and a large increase in the probability of opening in a given sweep. In contrast to the isoproterenol-enhanced increase of calcium current in frog heart cells, which subsided after approximately 10 min.(Bean et al., Nature 307, 371, 1984), the enhanced activity in AtT-20 cells persists for the duration of the experiment (maximum thus far, 30 min.). It is rapidly reversed by addition of propranalol (1 μM).

These results suggest that in AtT-20 cells, β-adrenergic agents modify action potential frequency and secretion by combined effects on calcium and potassium channels. It will be interesting to see if other hormones which regulate ACTH and β-endorphin secretion also interact with these channels, either through second messenger or G protein systems.

222.6 ONE TYPE OF CALCIUM CHANNEL IN NERVE TERMINALS OF THE RAT NEUROHYPOPHYSIS IS SENSITIVE TO DIHYDROPYRIDINES.

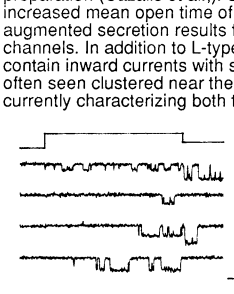
José R. Lemos and Martha C. Nowycky. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545 and Dept. Anatomy, Medical College of Pennsylvania, Philadelphia, PA 19129.

Calcium influx through voltage-gated calcium channels is an essential component of transmitter release and hormone secretion. In a variety of neuronal preparations, multiple types of calcium channels have been described, which can be distinguished on the basis of kinetic properties, ionic selectivities, and pharmacology. Thus far, most of the characterizations have been performed at the cell soma. It is not known if the same types of calcium channels are also transported to the nerve terminal and are associated with the site of release.

Using the cell-attached patch clamp technique, we have studied single calcium channel activity in a preparation of freshly dissociated nerve terminals. "Neurosecretosomes" were prepared from the posterior pituitary of adult male rats (Cazalis et al., J. Physiol. 390, 1987). Patch clamp recordings were obtained from isolated terminals of ~ 2 to 5 μ diameter. Patch pipettes were filled with 110 mM $BaCl_2$, 10 mM HEPES, and 0.2 μM TTX. Recordings were performed in either a low Ca^{2+} (3 μM) saline solution or in Ca^{2+}-free isotonic K glutamate.

In the presence of the dihydropyridine calcium channel agonist, Bay K 8644 (1 μM), the predominant inward currents correspond to the L-type calcium channel activity described in dorsal root ganglion cells (Nowycky et al., Nature 316, 440, 1985). Channel openings are characteristically long, often outlasting the pulse and deactivate slowly during repolarizing pulses. The slope conductance is ~28 pS. In the absence of Bay K 8644, channel openings of similar amplitude are brief (~1 msec), as is typical of L-type channels in other preparations.

It has been shown previously that Bay K 8644 potentiates depolarization-induced release of oxytocin and vasopressin in this preparation (Cazalis et al.,). Since this compound causes a dramatically increased mean open time of the L-type Ca channels, it is likely that the augmented secretion results from increased Ca^{2+} influx through these channels. In addition to L-type Ca channels, neurosecretosomes also contain inward currents with smaller conductances. These openings are often seen clustered near the beginning of the test pulse. We are currently characterizing both the large and small conductance channels.



Single sweeps with L-type Ca channel activity in the presence of Bay K 8644, recorded in low Ca^{2+} saline. The holding potential for all sweeps is RP-20 mV, and the patch is repolarized to RP+20 mV. The test potentials are (from top to bottom): RP+70, +60, +50 and +40 mV. Scale bars represent 2 pA and 20 msec.

222.7 LONG TERM STIMULATION OF DOPAMINE RECEPTORS INHIBITS CALCIUM CHANNEL ACTIVITY IN CULTURED PITUITARY CELLS. G. Cota* and C. M. Armstrong* (SPON: B. Ehrlich). Dept. of Physiol., Univ. of Penn., Phila., PA 19104.

The innervation of the pars intermedia (PI) of the rat pituitary gland includes dopaminergic nerve fibres with origin in the hypothalamus. It is well established that dopamine, acting through D-2 dopamine receptors on the PI cells, plays a predominant role in the regulation of the synthesis and release of pro-opiomelanocortin-derived peptides. Previous experiments have shown that the voltage-dependent Ca channel activity of denervated PI cells kept in primary culture increases markedly with time in culture. This may depend on the removal of dopaminergic innervation from the PI cells. We have tested this possibility by culturing the denervated PI cells in the presence of bromocriptine, a potent D-2 agonist. Ca tail currents were recorded in whole-cell mode at the holding potential (-80 mV) after 15-ms activating steps to +60 mV. The tails were fit with two exponentials, thus separating the contribution of SD and FD Ca channels. Individual traces were normalized by cell capacitance. Table I shows the increase in Ca channel activity with time in culture (mean±SEM, n=8-11):

	Time in culture (days) 0.7	2	5	10-11	15-16
FD current (%)	100±14	177±16	321±15	393±40	414±60
SD current (%)	100±9	90±10	115±18	139±18	135±15

Addition of 1 μM bromocriptine to the culture medium prevents or even reverses the increase in Ca channel activity, as illustrated in Table II. All recordings were from 10-11 day cells. Bromocriptine was added 1 hr to 4.5 days before the recordings, but was not present in the recording medium.

	Time of preincubation in bromocriptine 0	1 hr	2 days	4.5 days
FD current (%)	393±40	378±35	190±24	117±17
SD current (%)	139±18	148±24	139±24	103±18

The results indicate that long term stimulation of dopamine receptors reduces the Ca channel activity of PI cells, and raise the possibility that dopaminergic neurons may exert a tonic inhibition on Ca channel expression in PI cells in vivo.

We have also observed acute effects of dopamine on the ionic channel activity of cultured PI cells. Dopamine increases the amplitude of a voltage-dependent, slowly inactivating K current (this effect is blocked by prolonged intracellular dialysis) and speeds the "washout" of FD Ca channels.

222.8 INVOLVEMENT OF C-KINASE AND G PROTEINS IN INHIBITION OF Ca^{2+} CURRENTS BY NEUROPEPTIDE Y IN RAT DORSAL ROOT GANGLION NEURONS D. A. Ewald[1], R. J. Miller[1] and P. C. Sternweis[2]
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[2] Dept. of Pharm., U. of Texas Health Sci. Cntr., Dallas TX 75234

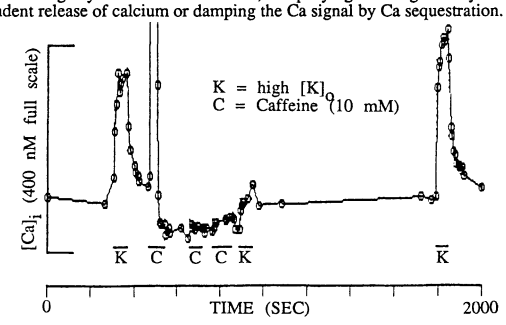
A variety of modulatory neurotransmitters endogenous to the vertebrate spinal cord can inhibit Ca^{2+} currents in cultured dorsal root ganglion (DRG) neurons. Neuropeptide Y (NPY), the most recently described member of this family of neuromodulators, inhibits both the sustained Ca^{2+} current evoked at 0 mV from a holding potential of -40 mV and the transient Ca^{2+} currents which are additionally evoked at 0 mV from a holding potential of -80 mV. (Whole-cell patch clamp with (in mM) 140 Cs, 10 EGTA, 1 Mg, 2 ATP and an ATP regenerating system in pipette and 10 Ca, 140 TEA and 1 Mg bathing the neurons.) Maximal effect of 10^{-7} M NPY ranged from 40-70% and half-maximally effective dose was 10^{-9} M (n=15). We tested the possibility that activation of C-kinase by the second messenger diacylglycerol (DAG) is a necessary step in this inhibition by "down-regulating" DRG neurons for C-kinase by long-term pretreatment with phorbol esters (Matthies *et al.* J. Neurosci. 7 1198 '87). The inhibitory effect of 10^{-9} M NPY was completely prevented in down-regulated neurons (n=4). At higher concentrations of NPY (e.g. 10^{-7} M) inhibition was not prevented (n=6). It was, however, qualitatively different from inhibition on non-down-regulated neurons being predominantly on the transient Ca^{2+} currents. These results suggest the existence of two types of NPY receptors: a low affinity type which is either directly coupled to Ca^{2+} channels or utilizes a second messenger other that DAG/C-kinase and a higher affinity type which inhibits Ca^{2+} currents via DAG-activated C-kinase.

Inhibition of Ca^{2+} currents by NPY is completely prevented by pretreatment with pertussis toxin (n=10). This implies that G proteins are involved in both the C-kinase mediated and the non-C-kinase mediated inhibitions. We tested the ability of G_o, a pertussis toxin sensitive G protein, to reconstitute the inhibitory effects of NPY by including its alpha subunit (α_o) in the patch pipette along with 1 mM GTP. Reconstitution of NPY-induced inhibition by α_o occured after a time delay of 5 to 15 min. With 10 nM α_o the inhibition ranged from 20-50% (5 neurons, 22 exposures) and was readily reversed after exposure. With 100 nM α_o the inhibition ranged from 50-80% (6 neurons) and was not readily reversed. The effect of NPY in control neurons is larger than that seen with 10 nM α_o and more reversible than that seen with 100 nM α_o. We are pursuing the hypothesis that 10 nM α_o reconstitutes a receptor system coupled directly to the Ca^{2+} channels and that 100 nM α_o additionally reconstitutes a receptor system which requires a second messenger such as DAG to produce inhibition.

Supported by PHS grants DA-02121, DA-02575, and MH-40165, and by grants from U. of Chicago Brain Research Inst. and Miles Pharmaceutical Inc.

222.9 **FURA-2 IMAGING DEMONSTRATES THE IMPORTANCE OF INTRACELLULAR STORES AND ION CHANNELS IN REGULATING CYTOSOLIC FREE CALCIUM IN SYMPATHETIC NEURONES.** *D.V. Madison, D. Lipscombe*, M. Poenie=*, H. Reuter*, R.W. Tsien, R.Y. Tsien=*, Dept. of Physiology, Yale School of Medicine, New Haven, CT 06510, and =Dept. of Physiology-Anatomy, U.C. Berkeley, CA. 94720.*

We have studied the levels of cytosolic free calcium in dissociated frog sympathetic neurons with the ratio imaging technique using the fluorescent calcium indicator fura-2. The resting cytosolic free calcium level in the neurons was approximately 80 nM. When depolarized with 60 mM extracellular potassium ($[K]_o$), the intracellular calcium concentration ($[Ca]_i$) rose rapidly to an average level of approximately 300 nM and decayed only slightly while the cell remained in high $[K]_o$. $[Ca]_i$ returned to baseline shortly after returning the cell to normal $[K]_o$. Focal extracellular electrical stimulation also caused a similar increase in $[Ca]_i$. High $[K]_o$-induced responses were abolished when $[Ca]_o$ was removed. Application of caffeine (10 mM) caused a very rapid rise in $[Ca]_i$ which decayed very nearly back to baseline levels within a few seconds, even in the continued presence of caffeine. Upon washing caffeine from the extracellular solution, $[Ca]_i$ undershot the baseline level for several seconds. The caffeine response was due to release of calcium from intracellular stores since it was not abolished by removal of $[Ca]_o$. This response was eventually abolished with repeated caffeine applications. Several lines of evidence suggest interactions between voltage-gated Ca entry and Ca release from stores: (1) $[Ca]_i$ continues to rise after electrical stimulation ceases; (2) response to high $[K]_o$ is diminished by prior depletion of stores with caffeine (Figure); (3) Simultaneous application of caffeine and high $[K]_o$ often evoked rhythmic oscillations of $[Ca]_i$. These findings suggest that intracellular Ca stores play a major role in controlling cytosolic Ca transients, amplifying Ca signals by calcium-dependent release of calcium or damping the Ca signal by Ca sequestration.



222.10 **PROPERTIES OF THE CALCIUM CURRENT OF PANCREATIC B-CELLS.** L.S. Satin* and D.L. Cook* (SPON. S. Fatherazi), Departments of Physiol. and Biophys. and of Med., Univ. of Wash., and VA Med. Ctr., Seattle, WA 98108

In pancreatic islet B-cells Ca channel activation leads to Ca uptake and insulin secretion. To characterize these channels we used the whole-cell patch-clamp to measure the Ca current in cultured neonatal rat B-cells. K currents were blocked with internal Cs and ATP and external TEA (10mM) while Na current was blocked with external TTX (3 μM).

Ca current (< 100 pA) activated near -40 mV (from a holding potential of -60 mV), peaked between 0 and + 10 mV, reversed below +100 mV and was abolished by Ca removal or by the addition of 2 mM Co (Satin, L.S. and Cook, D.L., Pflugers Arch 404:385-7, 1985) or very low doses of Cd ($K_D \sim 1$ μM). BAY k 8644 (5 μM) caused a small enhancement of the current.

Several minutes of hyperpolarization increased the amplitude of the Ca current while depolarization reversibly depressed it. The "steady-state" inactivation curve had a $V_{1/2}$ = -60 mV, which is more negative than the activation threshold of the current, suggesting that voltage can affect channel availability independent of Ca influx. However, the decay of the current during a 40 msec command was more marked in Ca than in Ba as expected if inactivation was mediated by a rise in $[Ca]_i$.

Some cells showed evidence of an additional inward current (when holding at -100 mV), which was much less sensitive to Cd and was abolished by removing external Ca.

These data suggest that the major Ca current of B-cells is highly Cd-sensitive and may have an inactivation mechanism that is both voltage and $[Ca]_i$-dependent. Some cells appeared to have a second inward Ca current. More work is necessary to fully isolate and characterize these two Ca conductance mechanisms and to determine their respective roles in the generation of B-cell bursting electrical activity.

L.S. was supported by NIH training grant NS07097 and the Diabetes Research Council and D.C. by NIH grant AM29816 and the V.A.IH

222.11 Ca CHANNELS INDUCED IN *XENOPUS* OOCYTES BY RAT BRAIN mRNA:SIZE FRACTIONATION AND PHYSIOLOGICAL STUDIES. J.P. Leonard, T.P. Snutch*, N. Davidson* and H.A. Lester. Divisions of Biology and Chemistry, Caltech, Pasadena CA 91125.

We have continued to study Ca channels induced by injection of rat brain mRNA into Xenopus oocytes and have begun to characterize the mRNA species responsible by size fractionation. RNA was isolated from fresh brains of 15-17 day old rats by a lithium chloride-urea procedure. Oocytes were injected with 70 ng samples of poly A mRNA and membrane currents were recorded under standard 2-microelectrode voltage clamp after allowing 2-7 days for expression. Ba currents through Ca channels exhibited the following properties: relatively slow partial inactivation (τ=650 ms), peak current activation at +15 mV, insensitivity to dihydropyridines, insensitivity to ω-CgTX, 50% inhibition at <10 uM cadmium, insensitivity to forskolin, and enhancement by phorbol esters. Many of these properties are shared by Ca channels involved in neurotransmitter release. Preliminary recordings from excised outside-out patches revealed a single channel conductance of 10-20 pS in 70 mM $BaCl_2$. Reconstruction of macroscopic currents from single channel records shows a current with little inactivation during a 60 ms pulse at +20 or +40 mV.

Size fractionation on a 6-20% sucrose density gradient has provided the initial characterization of the mRNA species encoding the induced Ca channels. Surprisingly, Ca channel activity is found in two peaks: (8.5-10 kb) and (4-7 kb) MW fractions. The I_{Ba} from high MW fractions shares many features with currents from unfractionated mRNA including relatively slow inactivation, peak at +15 mV, high sensitivity to cadmium, and insensitivity to nifedipine. Further physiological characterization of I_{Ba} induced by low and high MW mRNA is underway.

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222.12 INDUCTION OF Na CHANNELS AND Na CHANNEL mRNA BY NGF IN PC12 CELLS. B. Kirschenbaum*, T. Snutch*, H. Lester, L. A. Greene, N. Davidson* & B. Rudy (SPON: B.M.Curtis). New York Univ. Med. Ctr., N.Y. N.Y. 10016 and Divs. of Biology and Chemistry, Caltech, Pasadena, CA, 91125.

The PC12 clone is a line of rat pheochromocytoma cells that undergoes neuronal differentiation in the presence of nerve growth factor (NGF). In an effort to elucidate the mechanism by which NGF stimulates these cells to develop neuronal characteristics we have concentrated on the induction of Na action potentials. While PC12 cells grown in the absence of NGF are electrically inexcitable, exposure to NGF for periods of one week or greater results in the appearance of Na action potentials. Previously, we have demonstrated that NGF treatment brings about an increase in Na channel density that is sufficient in magnitude to account for the observed induction of electrical excitability. The mechanism by which NGF brings about this increase remain unknown. Utilizing a cDNA clone for the alpha subunit of the rat brain Na channel to probe PC12 mRNA, we now find an approximately 6 fold induction of Na channel mRNA in NGF stimulated cells. These results indicate that the NGF stimulated appearance of Na channels is a result of either an increased stability of Na channel mRNA or to an enhanced transcription of Na channel gene(s). We find that the observed enhancement of both, Na channel specific Na fluxes and Na channel mRNA levels in NGF stimulated cells occurs rapidly; within hours of NGF application. The discrepancy between the time course of Na channel increase and action potential development suggests that additional factors (e.g. a decrease in K channels), may intervene in the regulation of NGF-induced PC12 excitability. PC12 Na channel mRNA differs slightly in molecular length from that found in rat brain, therefore the possibility exists that the Na channels in PC12 cells are different. This may explain why antibodies against rat brain Na channel do not cross react with PC12 or other peripheral nervous tissue Na channels. Supported by GM26976, NS16036, and a Fellowship from Canadian NSERC.

CHARACTERIZATION OF CHOLINERGIC RECEPTORS II

223.1 PHENOTYPIC INSTABILITY OF PC12 CELL CULTURES AND APPROACHES TOWARD THE IDENTIFICATION OF NICOTINIC ACETYLCHOLINE RECEPTORS. R. J. Lukas. Division of Neurobiology, Barrow Neurological Institute, 350 West Thomas Road, Phoenix, AZ 85013.

The PC12 rat pheochromocytoma clonal line is a useful model system for the study of sympathetic neuronal differentiation, for the investigation of the mechanism(s) of action of nerve growth factor, and for the study of ganglionic-type nicotinic acetylcholine receptors (nAChR). In this laboratory, where clonal cell lines are routinely maintained in Dulbecco's modified Eagle's medium supplemented with 10% horse serum and 5% fetal bovine serum, PC12 cultures originally obtained from a variety of sources become increasingly adherent and undergo progressive changes in morphological appearance (the ratio of rounded, phase-bright cells to flattened cells decreases) with increasing cell passage.

Four lines of study have been initiated toward elucidation of the relationship(s) between functional nicotinic acetylcholine receptors (nAChR), high-affinity binding sites for ^{3}H-acetylcholine (T-ACh), and ^{125}I-labeled alpha-bungarotoxin (I-Bgt) binding sites on PC12 cells. (1) The expression of functional nAChR (measured by the use of isotopic efflux assays once the cells have achieved a threshold level of adherence to poly-L-lysine-coated substrata), high-affinity binding sites for T-ACh, and I-Bgt binding sites all decrease in parallel with increasing cell passage. (2) The ratio of I-Bgt binding sites to high-affinity T-ACh binding sites on PC12 cells is about 30 regardless of cell passage. (3) Those anti-nAChR antisera that block nAChR function on PC12 cells also block I-Bgt binding to PC12 cell sites with approximately equal efficacy. (4) None of over 100 polypeptide fractions (including kappa-bungarotoxin) purified from the venoms of a variety of snakes (krait, cobra, mamba, seasnake) blocks nAChR function on PC12 cells with an IC_{50} of 1 uM or less.

The data obtained to date are consistent with a variety of interpretations, including (i) that high passage PC12 cell cultures remain monoclonal, but undergo a global loss of neuronal phenotypic markers, (ii) that PC12 cultures of high passage become progressively dominated by non-neuronal PC12 cell mutants, (iii) that functional nAChR and toxin binding sites are distinct elements whose expression is nevertheless co-regulated, and (iv) that PC12 cells express only one class of functional nAChR, which binds neurotoxins in a functionally-impotent configuration and displays low capacity for high-affinity agonist binding. Studies on nerve growth factor effects on high and low passage PC12 cell cultures, attempts to subclone PC12 cell mutants, and nAChR affinity purification protocols are being used to challenge these provisional interpretations.

223.2 **CHARACTERIZATION OF NICOTINIC RECEPTORS IN CHICK RETINA.** R.H. Loring[1], E. Aizenman[2], S.A. Lipton[2] and R.E. Zigmond[1]. Depts. of Pharmacology[1] and Neurology[2], Harvard Med. School and Div. of Neuroscience[2], Children's Hospital, Boston MA 02115.

Toxin F (TXF), but not alpha-bungarotoxin (BGT), from Bungarus multicintus venom blocks nicotinic electrical responses in chick ciliary ganglia (CG; Neurosci. 11:989,1984), cultured neurons from rat superior cervical ganglia (Neurosci., in press) and isolated ganglion cells from rat retina (Aizenman et al., S. Neurosci. Abst., 1987). We also find that TXF (750 nM), but not BGT (10 uM), antagonizes hexamethonium-sensitive depolarizations induced by carbachol in intact chick retina. Since these data suggest that TXF blocks nicotinic receptors in chick retina, we examined the binding of ^{125}I-TXF to this tissue. ^{125}I-TXF binds to two sites in homogenates of retinas from young chicks: one site which is shared with BGT (B_{max}=270 fmol/retina, k_d=5nM) and another site which is not (B_{max}=80 fmol/retina, k_d=3nM). The electrophysiological data suggest that the specific TXF site, and not the site shared with BGT, represents neuronal nicotinic receptors. ^{125}I-TXF dissociates slowly from the TXF specific site with a $T_{1/2}$ of >5h. Nicotinic agonists such as nicotine (k_i=4X10^{-7}M), cytisine (5X10^{-7}M), dimethylphenylpiperazinium (6X10^{-7}M), and carbachol (2X10^{-5}M) inhibit binding to the TXF specific site. Antagonists such as dihydro-B-erythroidine (k_i=1X10^{-6}M) and d-tubocurarine (1X10^{-5}M) but not mecamylamine, hexamethonium or pempidine also inhibit the binding. This pharmacological profile is similar to that reported by others for high affinity nicotine binding in brain. We find by light microscopic autoradiography that ^{125}I-TXF binding localizes to the inner plexiform layer of the chick retina. Preliminary data suggest that Triton-solubilized TXF binding sites are not immunoprecipitated by a monoclonal antibody (Mab 35) that has been shown to recognize putative nicotinic receptors from chick CG (Smith et al., J. Neurosci. 5:2726,1985) and brain (Whiting and Lindstrom, J. Neurosci. 6:3061,1986). If the TXF specific site indeed represents neuronal nicotinic receptors in chick retina, these data suggest that the retinal receptors are immunologically distinct from other putative nicotinic receptors in the chick nervous system. NS22472, NS12651, EY05477.

223.3 THE BINDING OF ANTI-ACETYLCHOLINE RECEPTOR ANTIBODIES TO SYNAPTIC SITES AT INTERNEURONAL SYNAPSES IN THE FROG CARDIAC GANGLION. P. B. Sargent[1] and J. M. Lindstrom[2]. [1]Division of Biomedical Sciences, University of California, Riverside, CA 92521, and [2]Receptor Biology Laboratory, The Salk Institute for Biological Studies, San Diego, CA 92138.

Nicotinic acetylcholine receptors (AChRs) on neurons are poorly characterized in comparison to their muscle and electric organ counterparts. For example, relatively little work has been done to map quantitatively the distribution of neuronal AChRs on the cell surface or to study how receptor distribution might change in response to denervation and reinnervation. We have therefore sought to identify antibodies made against electric organ AChRs which cross-react with receptors on the neuronal surface. Such antibodies would presumably be useful as specific ligands for neuronal AChRs.

Forty-nine rat monoclonal antibodies (mAbs) made against electric organ AChRs were tested for their ability to bind to synaptic sites at interneuronal synapses in the parasympathetic cardiac ganglion of *Rana pipiens*. All the mAbs tested were found previously to cross-react with AChRs in *Rana pipiens* skeletal muscle [Sargent *et al.*, J. Cell Biol. **98**(1984)609-618]. These mAbs included those specific for each of the four electric organ subunits ($\alpha,\beta,\gamma,\delta$) as well as a number of mAbs specific for the main immunogenic region, a highly conserved and highly immunogenic region of overlapping epitopes on the extracellular surface of the α subunit.

Antibodies were tested in protease-treated ganglia (to promote access of reagents to the synaptic cleft) using a double-label fluorescence technique. Rat mAb binding was visualized using a fluorescein-conjugated rabbit anti-rat IgG, and the position of synaptic boutons in the same tissue was revealed by using a mouse anti-synaptic vesicle antibody (kindly provided by Dr. Louis Reichardt) followed by a rhodamine-conjugated horse anti-mouse IgG. Several of the mAbs tested bound to circumscribed regions of the cell surface which co-localized with synaptic boutons. Electron microscopic analysis of antibody binding using immunoperoxidase or immunogold procedures revealed that detectable binding is restricted to that part of the postsynaptic membrane lying beneath the active zone, as found at other autonomic synapses by Marshall [Proc. Natl. Acad. Sci. **78**(1981)1948-1952] and Jacob *et al.* [Proc. Natl. Acad. Sci. USA **81**(1984)3223-3227].

Only 8 of the 49 mAbs which cross-reacted with AChRs in *Rana* skeletal muscle produced detectable binding in the cardiac ganglion. All 8 cross-reacting mAbs were specific for the main immunogenic region (MIR). The likelihood that the cross-reacting mAbs are indeed binding to neuronal AChRs in the cardiac ganglion is increased by the recent finding that mAbs with a similar anti-MIR specificity have been shown to bind to neuronal AChRs in the chick ciliary ganglion [Halvorsen and Berg, J. Neurosci., in press]. These mAbs are presently being used to study the distribution of AChRs on the neuronal surface in normal and denervated cardiac ganglia.

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223.4 **EXPRESSION OF NEURONAL ACETYLCHOLINE RECEPTOR mRNA IN CHICK CILIARY GANGLIA.** R.T. Boyd[1,*], M.H. Jacob[1], M. Ballivet[2,*], J. Patrick[3], and D.K. Berg[1]. [1]Dept. of Biology, UCSD, La Jolla, CA 92093, [2]Dept. Biochem, Univ. Geneva, Geneva, Switzerland, and [3]The Salk Institute, San Diego, CA 92138,

Previous work has identified 4 distinct genes that encode homologous α-subunits for nicotinic acetylcholine receptors (AChRs) both in chick and in rat. One of these, alpha1, represents the α-subunit of the muscle AChR. The others (alpha2-4) represent AChR α-subunits expressed in parts of the nervous system. Reconstitution experiments in the *Xenopus* oocyte expression system confirm that the rat alpha2-4 genes code for functional α-subunits. We have used the equivalent chick probes to identify homologous mRNAs in chick ciliary ganglion. The ganglion presents a relatively homogeneous population of primary neurons accessible for developmental and regulatory studies of AChR gene expression.

A probe for the alpha3 gene transcript was constructed by subcloning an 800 bp region from an alpha3 genomic clone (300 bp corresponding to amino acids 96-196 of the muscle gene and 500 bp of intron) into the vector pSP65 having an SP6 promoter. Labeled alpha3 RNA probe was then synthesized and used to identify homologous message in embryonic ciliary ganglia. *In situ* hybridization under high stringency conditions revealed homologous message in ciliary ganglion sections when examined with labeled alpha3 probe. The signal was specific since no hybridizing material was apparent when a sense strand probe was used, or when liver or spinal cord sections were examined. The alpha3 probe detected homologous message in ciliary ganglia from 8- and 18-day chick embryos as well as ganglia from adult chickens. The alpha2 and alpha4 probes failed to detect homologous message in ciliary ganglion sections by *in situ* hybridization.

Northern blots of total RNA extracted from 18-day embryonic chick ciliary ganglia also revealed homologous message when hybridized with alpha3 probe at high stringency. A major component of 3.5 kb and a minor component of 2 kb were observed. A component of 3.5 kb was also observed when Northern blots of chick brain poly(A^+) RNA were hybridized with alpha3 probe. The components represented specific hybridization since no bands were obtained with sense strand probe, and since the brain component was much enriched in poly(A^+) vs. total RNA. Again, alpha2 and alpha4 probes failed to reveal homologous components in Northern blots of ciliary ganglion RNA, though they did detect hybridizing message in Northern blots of chick brain poly(A^+) RNA.

Ganglionic levels of AChRs are likely to be regulated by cell-cell interactions: previous work has shown that postganglionic axotomy of ciliary ganglia in newly-hatched chicks causes a 10-fold decline specifically in AChR levels within 5 days. We find that postganglionic axotomy results in a visible diminution of hybridizing signal when ciliary ganglion sections are examined with alpha3 probe by *in situ* hybridization.

These findings indicate that a homologous message likely to represent an AChR subunit can be detected in chick ciliary ganglia and that of the 3 known "neuronal" alpha genes only alpha3 appears to be expressed in the ganglion. The abundance of alpha3 message is sufficient to permit *in vivo* regulatory studies using *in situ* hybridization and Northern analysis. Moreover, the postganglionic axotomy studies suggest that receptor message levels may be regulated by cell-cell interactions. (Supported by NIH grant NS12601, the Muscular Dystrophy Assoc., and the American Heart Assoc.)

223.5 **A DESENSITIZED FORM OF NEURONAL ACETYLCHOLINE RECEPTOR DETECTED BY ^{3}H-NICOTINE BINDING ON BOVINE ADRENAL CHROMAFFIN CELLS.** L.S. Higgins, and D.K. Berg. Dept. of Biology, Univ. of Calif., San Diego; La Jolla, CA 92093.

Nicotinic acetylcholine receptors (AChRs) trigger catecholamine release from bovine adrenal chromaffin cells when activated by synaptic input from the splanchnic nerve. Here we report that agonists can induce a high affinity, desensitized form of the AChR on bovine adrenal chromaffin cells that can be detected by ^{3}H-nicotine binding. The receptors have 1:1 stoichiometry for binding ^{3}H-nicotine and ^{125}I-mAb 35, a monoclonal antibody previously shown to recognize the receptor. Chronic treatment of the cells with mAb 35 results in receptor modulation such that all of the high affinity ^{3}H-nicotine binding and all of the functional response to nicotine are lost from the cells.

For these experiments bovine adrenal chromaffin cells were isolated by enzymatic digestion of adrenal tissue and purification on Percoll gradients. ^{3}H-Nicotine binding was examined both with intact cells and with membrane fragments prepared from the cells after 3-9 days in culture. For membrane fragments, rapid filtration over glass fiber filters was used to separate bound and free ligand. Two classes of high affinity ^{3}H-nicotine binding sites were detected. The first class represented the α-bungarotoxin (α-Bgt) binding component in the membrane which previous studies have shown to be distinct from the functional AChR on the cells. The second class of sites had the properties expected for a desensitized, high affinity form of the functional AChR.

Scatchard analysis of the binding (in the presence of 1 μM α-Bgt to block binding to the first class of sites) revealed a single class of high affinity ^{3}H-nicotine binding sites with a K_D of 20 ± 2 nM and B_{max} of 17.3 ± 1.9 fmoles per culture of about $4x10^5$ cells (n=4). Cholinergic antagonists (d-tubocurarine, hexamethonium, decamethonium, mecamylamine, trimethaphan, and the α-neurotoxin Bgt 3.1) blocked the ^{3}H-nicotine binding with the same approximate K_i that they inhibited nicotine-induced catecholamine release from the cells. Agonists (ACh, carbachol, and nicotine), however, inhibited binding with affinities about 2 orders of magnitude greater than the affinities with which they triggered receptor activation. The discrepancy with agonists can be accounted for by an agonist-induced conversion of the AChR to a form having increased affinity for agonist but no change in affinity for antagonists. Thus, preincubation with nicotine in the presence of Substance P desensitizes the receptor (reduces nicotine-induced catecholamine release), and does so with an apparent K_D of 18 ± 5 nM. Preincubation with nM nicotine accelerates the rate of ^{3}H-nicotine binding, and kinetic analysis of this fast association rate together with the dissociation rate yields a K_D of ~20 nM. This value is in good agreement with the equilibrium binding data. Substance P increases the rate but not extent of agonist-induced AChR desensitization and it increases the rate of ^{3}H-nicotine binding. The results indicate that formation of the high affinity, desensitized AChR is rate-limiting in detecting ^{3}H-nicotine binding.

Comparing ^{3}H-nicotine binding to intact cells with that of membrane fragments indicates that about 80% of the sites are on the cell surface. Binding studies with ^{125}I-mAb 35 reveals the same distribution of surface and internal sites, and yields a constant ratio of 1:1 for nicotine and mAb 35 binding sites. Chronic treatment of cultures with mAb 35 results in a specific and complete loss of receptor function as well as loss of ^{3}H-nicotine binding sites from the cell surface, corroborating the ^{3}H-nicotine binding sites as being associated with AChRs. The results indicate that adrenal chromaffin AChRs are similar to muscle AChRs in undergoing an agonist-induced conversion to a desensitized form having increased agonist affinity and unaltered antagonist affinity. (Supported by NS12601, MDA, & AHA.)

223.6 KAPPA-BUNGAROTOXIN BLOCKS NICOTINIC ACETYLCHOLINE RECEPTORS IN BOVINE CHROMAFFIN CELLS AND INSECT CENTRAL NEURONS IN A VOLTAGE-INDEPENDENT MANNER. V.A. Chiappinelli, J.J. Lambert*, J.M. Nooney*, R.D. Pinnock* and D.B. Sattelle*. Dept. Pharmacol., St. Louis Univ. Sch. Med., St. Louis, MO 63104; Dept. Pharmacol. and Clin. Pharmacol., Univ. of Dundee, Dundee DD1 9SY, Great Britain; AFRC Unit, Dept. Zool., Univ. of Cambridge, Cambridge CB2 3EJ, Great Britain.

Kappa-Bungarotoxin (KBgT) and Kappa-Flavitoxin are structurally-related polypeptide neurotoxins isolated from crude venom of the snakes Bungarus multicinctus and B. flaviceps (Chiappinelli, V.A., et al., Brain Res. 402:21, 1987). These Kappa-Neurotoxins bind with high affinity to neuronal nicotinic acetylcholine (ACh) receptors and block nicotinic transmission in avian and murine autonomic ganglia (reviewed in Chiappinelli, V.A., Pharmacol. Therap. 31:1, 1985). Using voltage-clamp techniques, we have now examined the effects of KBgT on bovine chromaffin cells and cockroach neurons.

Bovine chromaffin cells. Whole-cell recordings were made on bovine chromaffin cells maintained in culture for 1-7 days as described (Cottrell, G.A., et al., Brit. J. Pharmac. 90:491, 1987). Local application of ACh (100 µM by pressure ejection) evoked an inward current of 371±20 pA (n=59) on cells voltage-clamped at -60 mV. Application of KBgT produced a dose-dependent blockade of the ACh-induced current. At 600 nM KBgT, the ACh response was 11±4% of control after 10 min of toxin exposure and 7±2% of control after 40 min (n=6). Following prolonged exposure to KBgT, extensive washing (>1 hr) failed to elicit any recovery of the ACh-induced current. The percentage-blockade produced by KBgT was independent of membrane potential over the range of voltages examined (-100 to -20 mV). Perfusion of cells with the competitive antagonist trimethaphan (Durant, N.N., Brit. J. Pharmac. 86:609P, 1985) at 10-30 µM protected against subsequent exposure to 600 nM KBgT.

Cockroach neurons. The cell bodies of the fast coxal depressor motorneurons (D_f) contain nicotinic ACh receptors (David, J.A. and Sattelle, D.B., J. Exp. Biol. 108:119, 1984). KBgT blocked ACh-induced inward currents in voltage-clamped D_f neurons in a dose-dependent manner (IC_{50}=100 nM KBgT). The blockade produced by KBgT was voltage-independent over the range examined (-100 to -30 mV). The pharmacology of cockroach receptors was similar to that of vertebrate autonomic nicotinic receptors. The one striking exception was alpha-bungarotoxin, which was the most potent antagonist at cockroach receptors but failed to recognize functional nicotinic receptors in avian, murine or bovine neurons at 10 µM.

Conclusions. Since Kappa-Neurotoxins bind only weakly to muscle nicotinic receptors, the results provide evidence that the neuronal-type of nicotinic receptor diverged from muscle nicotinic receptors at a very early evolutionary stage. The neuronal nicotinic receptors of species as diverse as cow and cockroach share many pharmacological features. (NIH Grants NS17574 and TW01254 to V.A.C.)

223.7 **Development of ACH and GABA Responses in Embryonic Chick Ciliary Ganglion Neurons.** Engisch,K.*, Yang,J., and Fischbach,G.D., Dept Anat. & Neurobiol., Wash.Univ.Sch.Med., St.Louis, MO, 63110.

Neurons in the chick ciliary ganglion receive cholinergic innervation from cells in the accessory oculomotor nucleus of the midbrain. Preganglionic fibers first arrive in the ganglion between embryonic day 4 and 5 (E4-E5); all ganglionic neurons are innervated by E8. Little is known about the expression of ACH receptors on the target neurons during this period of synapse formation. We have begun to investigate this issue by examining ACH currents in voltage-clamped neurons a few hours after dissociation from E6 to E18 ganglia. We assume that the ACH responses of freshly dissociated neurons are no different from those in vivo. We compared the ACH responses to GABA-induced currents and voltage-gated K+ currents over the same time course.

Neurons were held at -50 mv and ACH or GABA (100 um) was applied via pressure ejection through a micropipette positioned close to the cell soma. We found a dramatic age-dependent increase in ACH responses of neurons isolated between E6 and E10 (E6=94+/-11 pA, E10=500+/-40 pA, n=15), coincident with the establishment of functional transmission in the ganglion. There was a small decrease in ACH responses after E10 followed by a second, 2-fold increase from E14 to E18. GABA responses increased between E6 and E10 but in contrast to ACH responses, continued to increase, reaching a maximum at E12. This was followed by a rapid decline in responses between E12 and E18. Voltage gated K+ currents, on the other hand, showed a gradual but continuous increase during this developmental period.

Innervation plays a major role in regulating ACH receptors on muscle. Our results suggest that innervation may similarly influence the regulation of neuronal ACH receptors. Although the ciliary ganglion receives no known GABAergic innervation, it is interesting that GABA responses roughly follow the early developmental pattern of ACH responses. However, the lack of specific GABAergic input may account for the decrease in GABA responses after E12 and the subsequent divergence from the developmental pattern of ACH responses.

223.8 DIFFERENTIATED CHICK MYOBLASTS THAT EXPRESS ACETYLCHOLINE RECEPTORS CAN PROLIFERATE IN VITRO. M.Morgan* and G.D.Fischbach. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO. 63110

The majority of the mononucleated muscle cells isolated from embryonic day 11 (E11) chick pectoral muscle bind rhodamine-conjugated alpha-bungarotoxin (Rh-BTX) immediately after mechanical dissociation (Morgan and Fischbach 1986 NS Abstract). After 24 hours in vitro, only 45% of the myogenic cells exhibit Rh-BTX binding sites. It is possible that this decline is due to the selective proliferation of undifferentiated cells that do not express acetylcholine receptors (AChRs). However, we have found that those cells expressing AChRs are capable of proliferation in vitro.

Three different experimental approaches were used. First, dissociated mononucleated cells from E11 chick pectoral muscles were plated at clonal densities, and surface AChRs were labelled with Rh-BTX. Individual cells were identified, their location noted, and the presence or absence of AChRs recorded. The cultures were then returned to an incubator (37°, 5% CO_2, 100% humidity) for 24 hours, at which time the identified cells were located again. A total of 136 cells from 50 cultures were unambiguously identified and followed over time in culture. Ninety-one cells divided (67%), and of these, 37 expressed surface AChRs before cell division (41%). Sixteen cells with AChRs did not divide during 48 hours in vitro. These data demonstrate that differentiated cells (those expressing muscle-specific proteins) are capable of dividing in vitro. In a second set of studies, colchicine was added to the cultures in order to accumulate cells in mitosis. The observation of Rh-BTX-labelled cells in colchicine-induced metaphase arrest further demonstrated AChR expression in proliferating cells. Third, cultured muscle cells were exposed to bromodeoxyuridine (BUdR; a thymidine analog) and ^{125}Iodine-BTX. The BUdR was visualized with an anti-BUdR antibody and a fluorescein-conjugated secondary antibody. Because only proliferating cells will incorporate BUdR into their DNA, the double-labelled cells which were observed indicate that AChR-expressing, dividing myoblasts exist in dissociated cell culture.

When nerves first enter the developing chick limb bud (E4), the muscle mass is composed of proliferating, mononucleated muscle cells. The simultaneous presence of neurally-released ACh and of AChRs on proliferating myoblasts could provide an opportunity for neural influence on muscle development via these receptors. In vivo studies are underway to determine whether proliferating presumptive muscle cells express AChRs in the intact animal, and at what developmental stage this becomes evident.

This work was supported by an NSF Predoctoral Fellowship.

223.9 ACETYLCHOLINE RECEPTOR CLUSTERS ARE NOT INVARIABLY ASSOCIATED WITH 43K PROTEIN AT EARLY STAGES Of CHICK MUSCLE DEVELOPMENT. H-C. T. Tsui, C. Carr*, J.B. Cohen, & G.D. Fischbach. Dept. Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Antibodies raised against a 43,000 dalton acetylcholine receptor-associated protein purified from Torpedo electrocytes cross react with determinants at vertebrate endplates. It has been suggested that this protein, which is located on the cytoplasmic surface of the membrane, may be responsible for the formation of acetylcholine receptor (AChR) clusters. We have used cross-reacting antibodies to examine the appearance of 43K immunoreactivity in developing chick myotubes. Cultures were prepared from E11 chick pectoral muscle, labeled with rhodamine-alpha-bungarotoxin (Rd-BTX), permeabilized with 95 % ethanol at -20 degree C, and then labeled with a monoclonal antibody to 43 K followed with a FITC-secondary antibody. Fluorescence images captured with a SIT camera were digitized and analysed with a Trapix image processor-micro-Vax system. Rhodamine and fluorescein images were aligned and the intensities of the fluorochromes were compared and ploted on a pixel by pixel basis.

In mature myotubes, 4 to 6 days in vitro, 43 K was present at all AChR clusters. The correlation coefficient between rhodamine and fluorescein within individual clusters ranged between .73 to .96.(>2000 pixel per cluster) Some mononucleated cells (myoblast) examined immediately after dissociation from E11 muscles exhibited AChR clusters as intense as those of myotubes. However, 32 % (n=59) of these intense myoblast clusters were not associated with 43K. The intensity of 43K fluorescence at those myoblast clusters that did exhibit co-localization was clearly less than that at mature myotube clusters. The mean slope of rhodamine vs. fluorescein linear regression line was only 17 % of the mean slope measured at myotube clusters. It is unlikely that the low level of 43K is due to inadequate membrane permeabilization. Moreover, the same paucity of 43 K fluorescene were observed at some receptor clusters identified in frozen sections of E11 pectoral muscle. Our results suggest that 43K is not essential for receptor cluster formation during early stages of muscle development.

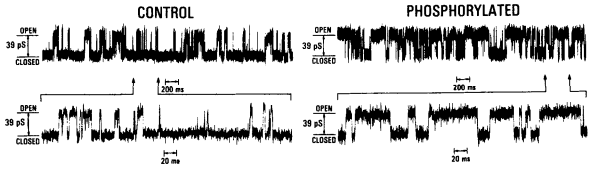
223.10 DETERMINATION OF THE SITES OF cAMP-DEPENDENT PHOSPHORYLATION ON THE γ AND δ SUBUNITS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR. Richard L. Huganir, Elizabeth Moritz* and Gene H. Yee*. The Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

The nicotinic acetylcholine receptor is a neurotransmitter-regulated ion channel that mediates the depolarization of the postsynaptic membrane at the neuromuscular junction. The receptor is an integral membrane protein which consists of four subunits in a stoichiometry of $\alpha_2\beta\gamma\delta$. The nicotinic acetylcholine receptor has been shown to be multiply phosphorylated by at least three different protein kinases. cAMP-dependent protein kinase phosphorylates the γ and δ subunits of the receptor, protein kinase C phosphorylates the δ and α subunits, while a tyrosine-specific protein kinase phosphorylates the receptor on the β,γ, and δ subunits.

Recently, it has been demonstrated that phosphorylation of the nicotinic receptor by cAMP-dependent protein kinase increases its rate of rapid desensitization. We now report the identification of the cAMP-dependent phosphorylation sites on the γ and δ subunits. Two-dimensional phosphopeptide mapping of the phosphorylated γ and δ subunits, after limit proteolysis with thermolysin, indicated that each subunit is phosphorylated on a single site. Phosphoamino acid analysis of the ^{32}P-labeled subunits demonstrates that phosphorylation had occurred exclusively on serine residues. Purified phosphorylated subunits were cleaved with cyanogen bromide and the resultant phosphopeptides were purified by reverse-phase high performance liquid chromatography. Shorter phosphopeptides, obtained by secondary digestion with trypsin, were purified and subjected to both automated gas-phase sequencing and manual Edman degradation. The results demonstrate that the γ subunit was phosphorylated at Ser-353, contained within the sequence Arg-Arg-Ser(P)-Ser-Phe-Ile and that the δ subunit was phosphorylated at Ser-361, contained within the sequence Arg-Ser-Ser(P)-Ser-Val-Gly-Tyr-Ile-Ser-Lys. Determination of the sites phosphorylated within the structure of the γ and δ subunits should contribute to the molecular characterization of the regulation of desensitization of the nicotinic acetylcholine receptor by protein phosphorylation.

223.11 PHOSPHORYLATION OF THE NICOTINIC CHOLINERGIC RECEPTOR INCREASES THE PROBABILITY OF CHANNEL OPENING. M.S. Montal*† and M. Montal* (SPON: R. Anholt). Department of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110 and †Departments of Biology and Physics, University of California, San Diego, La Jolla, California 92093

To establish the functional modification produced by phosphorylation of the nicotinic acetylcholine receptor (AChR) single channel currents activated by acetylcholine (ACh) from purified *Torpedo californica* ACh receptors reconstituted in lipid bilayers were recorded. Phosphorylation by the purified catalytic subunit of the cyclic AMP dependent protein kinase incorporates phosphate primarily into the ACh receptor γ and δ subunits (Huganir, R.L. and Greengard, P. *Proc. Natl. Acad. Sci. USA*, 80:1130, 1983). Inspection of single channel records show that receptor phosphorylation increases the channel opening probability without affecting the single channel conductance (see Figure top panel). Detailed analysis of the single channel currents indicates that the increment in the opening frequency arises primarily from a shortening of the characteristic long quiescent periods (channel closed times) that separate the bursts of channel openings (note details in expanded records displayed in corresponding lower panels). The results are consistent with the view that ACh receptor phosphorylation modifies the kinetics of its ability to enter into(Huganir, R.L. *et al. Nature*, 321:774, 1986) and exit from the desensitized state. Thus, it is plausible that such covalent modification of a postsynaptic receptor may exert a regulatory role in synaptic plasticity.



Legend: Single AChR channel currents activated by 1 μM ACh and recorded at V = 85 mV (Control) and V = 95 mV (phosphorylated), respectively. The single channel conductances were 40 pS and 41 pS, respectively (in 0.5 M NaCl, 0.5 mM $CaCl_2$, 5 mM tricine, pH 7.4). Lipid bilayers were assembled from AChR vesicles derived monolayers and formed at the tip of patch pipets (*Biochemistry* 22:2319, 1983).

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223.12 "DELTALESS" ACETYLCHOLINE RECEPTORS ARE HIGHLY VOLTAGE-DEPENDENT. Michael M. White, Dept. of Pharmacology, Univ. of Pennsylvania, Philadelphia, PA 19104.

The nicotinic acetylcholine receptor is a multisubunit protein of stoichiometry $\alpha_2\beta\gamma\delta$. The availability of cDNAs for the various subunits makes it possible to apply techniques such as site-directed mutagenesis to the study of the structural features that underlie AChR function. We have chosen to focus our initial efforts some on not-so-subtle (but easily done) "mutations":deletion of whole subunits. Using our in vitro transcription-Xenopus oocyte translation system, we have examined the effects of deletion of various Torpedo subunits. Deletion of the α, β, or γ subunit prevents the appearance of functional AChRs on the oocyte surface. In contrast, when the δ subunit is deleted, one can still record ACh-activated, curare-sensitive currents. Macroscopic currents recorded at -60 mV from oocytes with δ^- receptors are, on the average, ≈10% of those recorded from eggs with "wild-type" receptors. However, when one uses αBTX to "count" the number of receptors on the cell surface, δ^- oocytes have ≈50% of the number of receptors found on the wt oocytes, an indication that the smaller currents are not entirely due to decreased synthesis, but also reflects some difference in intrinsic properties. Both types of receptors have identical affinities for d-tubocurarine(≈50 nM), which rules out a difference in fractional activation by ACh. The I-V relationship of wt AChRs is essentially linear between -160 mV and +50 mV. However, δ^- receptors show a highly non-linear I-V relationship with large inward currents at very negative voltages, and very small outward currents at positive potentials. The current ratio (δ^-/wt) is ≈0.5 at -160 mV (consistent with the toxin-binding data), ≈0.1 at -60 mV, and ≈0 at +50 mV. Current efforts are focussed on high-resolution single-channel recording to determine the microscopic basis of the voltage dependence of the macroscopic currents, and determination of the subunit stoichiometry of δ^- receptors. Supported by NIH NS23885

223.13 SITE-DIRECTED MUTAGENESIS OF TORPEDO CALIFORNICA ACETYLCHOLINE RECEPTOR. L. PRADIER*, A. S. YEE* and M. G. McNAMEE. Dept. of Biochemistry and Biophysics, University of California, Davis CA 95616.

Purified nicotinic acetylcholine receptor (nAChR) from Torpedo californica was derivitized with N-phenylmaleimide under non-reducing conditions in the presence of cholate detergent (Yee et al., Biochemistry, 25:2110, 1986) and reconstituted into lipid vesicles using dialysis to remove the cholate. The derivitized protein exhibited normal alpha-bungarotoxin binding. In addition, activating ligands such as carbamylcholine underwent the low to high affinity binding transition characteristic of functional receptor. However, inhibition of ion-flux activity, as measured by the carbamylcholine-stimulated influx of rubidium ions into the vesicles, was well-correlated with the level of maleimide labeling. Using tritiated N-phenylmaleimide, the gamma-subunit of the nAChR was shown to be preferentially labelled. Further localization of the label by peptide mapping showed that the label was located primarily on one cyanogen bromide fragment.

In parallel experiments, an expression system using in vitro transcription of the cDNAs coding for the four subunits of T. californica and injection of the transcripts into Xenopus laevis oocytes (Mishina, M. et al., Nature, 313:364, 1985) was set up. The coding sequences for the four subunits were cloned downstrean to the SP6 promoter in the vectors pSP64, pSP65 and pX m (Krieg, P. and Melton, D. A., Nucl. Acid Res., 12:7057, 1984). Preliminary experiments have shown that certain sequences upstream to the initial AUG inhibit in vitro translation and new constructs have been made to increase translation levels. Using site-directed mutagenesis, the cysteine amino acid residues tentatively identified as the N-phenylmaleimide-labeled groups have been altered. The functional properties of these mutants are being assayed in Xenopus oocytes using voltage-clamp methods. (Supported by NIH Grants NS22941 and NS13050).

224.1 SHAKING BEHAVIOR IN THE RAT: INTERACTIONS BETWEEN BENZODIAZEPINE AND SEROTONIN 5-HT-1A AGONISTS. M.R. Pranzatelli. Depart. Neurol., Div. Pediatric Neurology, College of Physicians and Surgeons of Columbia University, New York, NY 10032.

Benzodiazepine (BDZ)-induced excitatory phenomena in rodents, such as wet-dog shakes (WDS), have not been explained pharmacologically. In studying the mechanism of action of BDZ-induced WDS, we found that select BDZ agonists evoked dose-related WDS at low doses in the adult rat with the rank order of potency clonazepam > nitrazepam> flunitrazepam> nimetazepam. WDS appeared within 3-5 minutes of injection and lasted approximately 60 minutes. Lorazepam was the only BDZ agonist without a nitrous-group on the A-ring (diazepam, fludiazepam, oxazepam) at doses up to 60 mg/kg to induce WDS. Non-BDZ agonists (CL 218,872), inverse agonists (B-CCE), peripheral type receptor agonists (Ro 5-4864), BDZ antagonists (Ro 15-1788), and vehicle (ethanol/propylene glycol) were ineffective. Representative putative 5-HT-1 and 5-HT-2 agonists (Ru 24969 and 8-OH-DPAT vs DOI) and antagonists (TVXQ7821 vs ritanserin) were tested as blockers but only the 5-HT-1A agonist 8-OH-DPAT significantly reduced WDS in a dose-dependent manner (ID_{50}= 0.86 mg/kg), whereas Ro 15-1788 increased BDZ-evoked WDS 70% at 20 mg/kg, and diazepam had no blocking effect. Intracisternal injections of 5,7-dihydroxytryptamine which significantly depleted 5-HT in multiple brain regions, did not alter frequency, latency or time course of WDS induced by nitrazepam or flunitrazepam. Only BDZ agonists (not CL 218,872) induced ataxia and sedation, which outlasted WDS and were not blocked by serotonergic drugs. These data suggest a difference in pharmacologic mechanism from 5-HT-2-dependent BDZ-evoked head-twitch in mice, and also indicates a pharmacologic dissociation between BDZ-evoked excitatory (WDS) and inhibitory (sedation, ataxia) behavior in the rat. No simple correlation between BDZ-induced WDS and BDZ radioligand binding, anti-pentylenetetrazol activity, or other BDZ property was found. BDZ-evoked WDS may relate to the unique predominance of BDZ II and 5-HT-1A sites in hippocampus, an apparent locus of WDS, but the role of the 5-HT-1A involvement at the level of the receptor remains to be elucidated. BDZ-induced WDS in the rat may be a useful model of BDZ-serotonin interactions.

224.2 BLOCKADE OF THE ELECTROPHYSIOLOGICAL EFFECT OF 5-HT AND 8-OH-DPAT BY BMY 7378, A PUTATIVE 5-HT_{1A} RECEPTOR ANTAGONIST, IN THE RAT CNS. Y. Chaput* and C. de Montigny. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Canada H3C 3J7.

Electrophysiological and radioligand binding studies suggest that the receptors mediating the inhibitory response to 5-HT in the rat hippocampus and in the nucleus raphe dorsalis are of the 5-HT_{1A} subtype. The buspirone analogue BMY 7378 potently displaces the binding of [^{3}H]8-OH-DPAT, a selective 5-HT_{1A} agonist, from rat hippocampal membranes; furthermore, BMY 7378 blocks the effect of 5-HT and 8-OH-DPAT on forskoline-stimulated cAMP formation while being devoid of intrinsic activity (Yocca et al., 1987). This suggests that BMY 7378 might be a 5-HT_{1A} receptor antagonist. The present study was undertaken to investigate the effect of BMY 7378 on the response of CA_3 dorsal hippocampus pyramidal neurons and of dorsal raphe 5-HT neurons to 5-HT and 8-OH-DPAT.

Male Sprague-Dawley rats (240-300 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.). Extracellular unitary recordings were obtained with five-barrelled glass micropipettes. The following solutions were used for microiontophoresis: 5-HT (0.5 or 2 mM in 200 mM NaCl), 8-OH-DPAT (1 mM in 100 mM NaCl), NE (100 mM in 200 mM NaCl), GABA (10 mM in 50 mM NaCl), and BMY 7378 (50 mM in 200 mM NaCl). Acetylcholine (20 mM in 200 mM NaCl) or glutamic acid (100 mM in 50 mM NaCl) were used to maintain the firing activity of the neuron recorded within its physiological range.

A 50% reduction of the suppressant effect of 5-HT on hippocampus pyramidal neuron firing activity was obtained with an average ejection current of 2 nA of BMY 7378, whereas the same degree of blockade of the effect of 8-OH-DPAT was obtained with an average ejection current of 0.5 nA. The effects of neither NE nor GABA were altered by the microiontophoretic applications of BMY 7378 with currents up to 18 nA. The intravenous administration of BMY 7378 readily antagonized the suppressant effect of microiontophoretically-applied 5-HT on these neurons, with an ED_{50} of approximately 250 g/kg, whereas that of NE was unaffected by doses up to 5 mg/kg. Interestingly, BMY 7378, applied with an average current of 3 nA, did not alter the effect of microiontophoretically-applied 5-HT on dorsal raphe 5-HT neurons whereas it reduced by 30% that of 8-OH-DPAT.

The present results provide in vivo evidence that 5-HT_{1A} receptor blockade by BMY 7378 markedly reduces the inhibitory effect of 5-HT and 8-OH-DPAT in the rat hippocampus. The differential effect of BMY 7378 on 5-HT neuron responses to 5-HT and 8-OH-DPAT might suggest that these agonists act at different receptors. However, given the existing evidence that the somatodendritic 5-HT autoreceptor is of the 5-HT_{1A} subtype, it is more likely that both agonists activate the same receptor via distinct recognition sites.

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224.3 FAILURE OF SUPRAMAXIMAL ELECTRICAL STIMULATION OF THE DRN TO OVERCOME NERVE TERMINAL SEROTONIN AUTORECEPTOR ACTIVATION. K.F. Martin*, and C.A. Marsden (SPON: C.H. Page). Dept. of Physiol. & Pharmacol., Univ of Nottm. Med. Sch., Nottingham NG7 2UH, U.K.

Neuronal release of serotonin (5HT) is under the control of a 5HT nerve terminal autoreceptor which in the rat suprachiasmatic nucleus (SCN) is of the $5HT_{1B}$ sub-type (Marsden, C.A. & Martin, K.F., Br.J.Pharmac., 85:219P, 1985). Infusion of the $5HT_{1A}$ receptor agonist DPAT into the dorsal raphe (DRN) which inhibits 5HT unit activity (Wilkinson, L.O. et al, Eur.J.Pharmac., in press) does not affect extracellular 5-hydroxyindoleacetic acid (5HIAA) levels in the SCN (Marsden, C.A. & Martin, K.F., Br.J.Pharmac., 86:445P, 1985). These data suggested that 5HIAA levels may not reflect 5HT unit activity. We have therefore examined the possibility that 5HT autoreceptor activity may have a greater influence than 5HT neurone firing rate on 5HT release and metabolism.

Male Wistar rats (295-305 g), anaesthetised with chloral hydrate (600 mg/kg i.p.) had carbon fibre electrodes (0=20 µm) stereotaxically implanted in the left SCN. A 30 g stainless steel injection cannula was implanted into the contralateral SCN so that its tip was 100-150 µm from the working electrode. A bipolar SNE 100 stimulating electrode was implanted into the DRN. Differential pulse voltammograms were obtained every 5 min and the height of the oxidation peak at +300 mV (peak 3) taken as an index of extracellular 5HIAA concentration (Crespi, F. et al, Neurosci.Lett., 43:203, 1983).

Electrical stimulation (square pulses, 0.5 msec duration, constant current) of the DRN for 10 min resulted in a current (50-200 µA) and frequency (5-20 Hz) dependent increase in the height of peak 3 recorded in the SCN. The maximum increase (60±14%, n=4) occurred at 200 µA and 20 Hz approx. 20 min post stimulation. Administration of 0.9% saline (1 µl over 1 min) into the SCN 10 min before stimulation did not affect the response (peak 3 height=150±15%, of control 30 min from start of stimulation, n=5). Infusion of RU 24969 (10 µg in 1 µl over 1 min) into the SCN resulted in a rapid, marked decrease in peak 3 height in the SCN (-80±18% 10 min post infusion, n=5, -95±5%, 40 min post infusion, n=5). Infusion of RU 24969 (10 µg) into the SCN prevented the DRN stimulation induced rise in peak 3 height observed previously. (Peak 3 height 20 min post stimulation and 40 min post RU 24969 = 10±5% of control, n=4.)

These data demonstrate that stimulation of the nerve terminal 5HT autoreceptor in the SCN exerts a more powerful influence over 5HT release and metabolism than 5HT neurone firing rate.

These studies were supported by the Wellcome Trust.

224.4 STIMULATION OF BRAIN SEROTONIN_{1A} RECEPTORS BY THE URAPIDIL ANALOGUE, B695-40, LOWERS ARTERIAL BLOOD PRESSURE. R.A. Gillis*, J.A. Quest*, I.J. Namath*, A.M. Martino-Barrows* and K.J. Kellar (SPON: R. McGee). Dept. Pharmacol., Georgetown Univ., Schools of Medicine and Dentistry, Washington, DC 20007.

We previously demonstrated that the new antihypertensive agent, urapidil, lowers arterial blood pressure by central sympathoinhibition and peripheral blockade of alpha_1-adrenoceptors (Gillis et al., J. Cardiovasc. Pharmacol. 9: 103, 1987). To further investigate the mechanism of the central effect of urapidil, the analogue of urapidil, B695-40, was studied on the ventral surface of the medulla of chloralose-anesthetized cats. Topical application of B695-40 (5 ug/side) produced a marked hypotensive response averaging -64 + 9 mm Hg. This response was much greater in magnitude than that previously described for urapidil on the ventral surface of the medulla. The hypotensive response evoked by B695-40 was mimicked by 8-OH-DPAT, a selective agonist for serotonin_{1A} receptors (Middlemiss and Fozard, Eur. J. Pharmacol. 90: 151, 1983). Pretreatment with WB-4101, an antagonist at the serotonin_{1A} receptor (Norman et al., Mol. Pharmacol. 28: 487, 1985) prevented the hypotension produced by both B695-40 and 8-OH-DPAT. These results suggest that both urapidil and the more potent B695-40 analogue lower blood pressure by stimulating serotonin_{1A} receptors at the ventral surface of the medulla. An action of these drugs at serotonin_{1A} receptors was confirmed by receptor binding studies. Urapidil, B695-40 and WB-4101 were found to be potent competitors for serotonin_{1A} sites labeled by [^{3}H]8-OH-DPAT in rat brain. The affinities of WB-4101 and B695-40 for these sites were 2-10 nM, and the affinity of urapidil was approximately 250 nM.

(Supported by funds from Marion Laboratories, Inc.)

224.5 IN VIVO RATE OF SEROTONIN SYNTHESIS IN THE DOG BRAIN MEASURED BY POSITRON EMISSION TOMOGRAPHY. M. Diksic*, T.L. Sourkes, H. Nakai*, T. Chaly*, K. Missala* and Y.L. Yamamoto*. Brain Imaging Centre, Montreal Neurological Institute, and Dept. of Psychiatry, McGill Univ., Montreal, Que. H3A 1A1.

Sourkes and associates [Fed Proc 30 (1971) 897] have shown that α-methyl-L-tryptophan (AMTP) follows L-tryptophan (L-Trp) metabolism at least in the 5-hydroxylation metabolic pathway in the brain. Thus, AMTP is a substrate for tryptophan hydroxylase and the product is acted upon by aromatic amino acid decarboxylase, resulting in the formation of α-methylserotonin. Recently, we labelled AMTP with ^{11}C and ^{14}C in the α-methyl position [J Nucl Med 27 (1986) 1047]. After preliminary evaluation of the tracer in the rat brain, the kinetic behaviour was evaluated in the dog brain by means of PET and dynamic scanning. The biological model representing kinetics of AMTP formation in the brain was approximated by considering three compartments. The rate constants for the transfer of AMTP from plasma to brain and back (k_1 and k_2) and the hydroxylation by the rate-limiting enzyme (tryptophan hydroxylase; k_3) were calculated by fitting integral equations representing the radioactivity in brain tissue as a function of time, with rate constants as parameters. Our preliminary data for these rate constants are k_1 = 0.197 ml/g per min (transfer from blood to brain); k_2 = 0.247 min^{-1} (brain-blood transfer) and k_3 = 0.0076 min^{-1} (hydroxylation by tryptophan hydroxylase) in animals with normal plasma tryptophan. Increasing the plasma L-Trp concentration resulted in the decrease of k_1 and increase in k_2; however, k_3 probably does not change. Loading with L-Trp also resulted in the reduction in the volume of distribution. To relate the rate of α-methylserotonin synthesis to the rate of serotonin synthesis, we need constant accounting mainly for the different enzyme affinities for L-Trp and AMTP, because the distribution volume for AMTP and L-Trp are approximately the same. The latter assumption is supported by comparision of our data on AMTP and literature data for L-Trp. The lumped constant (mainly a correction for difference in the hydroxylase activity for two substrates) was estimated to be 0.46 ± 0.12 with values for V_{max} and apparent K_m for AMTP and L-Trp obtained from the literature. From these data the estimate of K_m (apparent) and V_{max} for tryptophan transport through the BBB was 303 ± 54 μM, and 63 ± 10 nmol/g per min, respectively. The average rate of brain serotonin synthesis in the anesthesized dog calculated from these data was 93 pmol/g per min at normal plasma tryptophan concentration (13.2 μM), 1.51 nmol/g per min at total plasma L-Trp of 85.1 μM and 3.1 nmol/g per min at 120.5 μM total plasma L-Trp. This rate of serotonin synthesis measured in dog brain in vivo compares favourably to the rates reported earlier in rat brain.

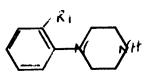
224.6 CLOZAPINE AS A 5-HT_2 ANTAGONIST IN MAN. Meltzer, H.Y., Bastani, B., Kwon, K., Ramirez, L. and Nash, J.F., Case Western Reserve University School of Medicine

Various preclinical behavioral, endocrine, and temperature studies suggest that clozapine and other atypical neuroleptic drugs are serotonin$_2$ (5-HT) antagonists. We compared the ability of clozapine to block the effect of MK-212, a 5-HT_2 agonist, to increase serum cortisol and prolactin levels with that of typical neuroleptic drugs in schizophrenic patients. MK-212 produced significant increases in cortisol and prolactin levels compared to placebo in 10 unmedicated schizophrenic patients. Clozapine treatment in 8 patients at doses of 400-900 mg/day for 3-6 weeks significantly blocked the MK-212-induced increase in serum cortisol and prolactin whereas administration of typical neuroleptics (e.g. chlorpromazine, trifluperazine, haloperidol) in therapeutic dosages to 10 patients significantly increased the cortisol response to MK-212. The prolactin response to MK-212 was not effected by treatment with the typical neuroleptic drugs. Clozapine treatment inhibited the prolactin response to MK-212. These results are consistent with the hypothesis that clozapine is a 5-HT_2 antagonist. Because of the intricate interaction between dopamine (DA) and 5-HT in the nigrostriatal and limbic systems, this 5-HT antagonism may be important to the unique therapeutic properties of clozapine. Chronic administration of clozapine also decreased basal prolactin levels in patients. This may reflect increased turnover of DA in the tuberoinfundibular DA neurons as previously demonstrated in rats. It might also reflect inhibition of a tonic serotonergic influence on prolactin release which might be mediated through DA or a prolactin releasing factor.

224.7 BLOCKADE OF CONDITIONED AVOIDANCE RESPONDING BY SEROTONERGIC PHENYL PIPERAZINES WITH NO APPARENT AFFINITY FOR DOPAMINE RECEPTORS. G.E. Martin, M.K. Scott*, D.L. DiStefano*, C.L. Fedde*, J.M. Kesslick*, C.B. Davis*, R.P. Shank and W.J. Baldy, Jr*. McNeil Pharmaceutical, Spring House, PA 19477.

The ability to block conditioned avoidance responding (CAR) in animals is a property shared by all clinically effective antipsychotic agents. Potency in blocking CAR has also been shown to be highly correlated with potency in blocking the D-2 dopamine receptor. Hence, block of CAR and D-2 dopamine receptor binding interactions have been used as in vivo and in vitro screening techniques, respectively, for novel antipsychotic agents. We report herein that a series of ortho-substituted phenyl piperazines, with no apparent affinity for the dopamine D-2 binding site, are active in blocking CAR in the rat. In receptor binding studies, these compounds did interact with the serotonin S-1 receptor binding site, but did not markedly inhibit binding of ligands for the α-1, D-1, D-2, or S-2 binding sites.

Male Fisher 344D rats were trained with a discrete trial-lever press CAR paradigm to avoid a 0.7mA footshock. A test session consisted of 60 trials spaced at 1 min. intervals in which the shock was signalled by a tone and a light in the operant chamber. All test agents were given i.p. 30 min. prior to the test session and CAR was compared to that from a control session (no drug) from the previous day. The results were as follows:



	ED50 (mg/kg)	Binding Studies K_i Values (nM)				
R_1	i.p.	D_1	D_2	S_1	S_2	α1
OH	26.2	>1000	>1000	-	>1000	>1000
OCH_3	4.4	>1000	>1000	28	>1000	510
$O(CH_2)_3CH_3$	11.2	NT	>1000	19	>1000	-
$OCH(CH_3)_2$	4.8	NT	64	15	235	84
F	5.0	>1000	>1000	58	>1000	373
CN	5.7	>1000	>1000	26	>1000	214
MCPP	2.4	>1000	>1000	25	40	>100

The CAR block produced by MCPP, which interacts with both the S_1 and S_2 receptor is blocked by the serotonin receptor antagonist metergoline. The block of CAR produced by the S_1 selective agent ortho-methoxyphenylpiperazine, however, was not reduced by metergoline. These are the first S-1 selective agents reported to block CAR in the rat.

224.8 SEROTONERGIC ANTAGONIST PROPERTIES OF ATYPICAL ANTIPSYCHOTICS. Gary Gudelsky, J. Frank Nash* and Herbert Y. Meltzer, Departments of Psychiatry and Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

The use of atypical antipsychotics (e.g., clozapine, melperone, setoperone) appears to be associated with a low incidence of extrapyramidal side effects. Recently, atypical antipsychotics, in contrast to typical antipsychotics, have been demonstrated to possess a relatively higher affinity for 5-HT_2 receptors than for D_2-dopamine receptors. It has been suggested that the 5-HT_2 antagonist property of atypical antipsychotics may contribute to the unique clinical profile of these agents. We have proposed that 5-HT_2 receptors mediate the increase in serum corticosterone concentrations and body temperature produced by the 5-HT agonist MK-212 (6-chloro-2[1-piperazinyl]piperazine), since these effects are antagonized by the 5-HT_2 antagonist ketanserin. In the present study we have assessed the ability of typical and atypical antipsychotics to antagonize the 5-HT_2 receptor-mediated corticosterone secretion and hyperthermia produced by MK-212. MK-212 (2.5 mg/kg, i.p.) produced a marked increase in serum corticosterone concentrations in male, Sprague-Dawley rats. Clozapine (CLZ), melperone (MEL) and setoperone (SET) produced a dose-dependent suppression of MK-212-induced corticosterone secretion. In contrast, neither haloperidol (HAL) nor chlorpromazine (CPZ) affected this response to MK-212. The specific 5-HT_{1A} agonist 8-OH-DPAT (0.1 mg/kg, s.c.) also elevated serum corticosterone concentrations. CLZ, MEL and SET failed to antagonize the 8-OH-DPAT-induced secretion of corticosterone. Similarly, the typical antipsychotics HAL and CPZ also had no effect on corticosterone secretion induced by 8-OH-DPAT. The body temperature of the heat-adapted rats was increased approximately 1°C by MK-212 (1 mg/kg, i.p.). This hyperthermic response was antagonized in a dose-dependent manner by the atypical antipsychotics CLZ, MEL and SET but not by the typical neuroleptics CPZ or HAL. The atypical antipsychotics were essentially equipotent in this regard, and a significant inhibition of MK-212-induced hyperthermia was achieved by 1-3 mg/kg of CLZ, MEL or SET. The administration of 8-OH-DPAT (0.05 mg/kg, s.c.) produced a hypothermic response which was unaffected by CLZ or MEL. These data are supportive of the view that atypical antipsychotic agents, in contrast to typical antipsychotics, possess 5-HT_2 antagonist properties in vivo. Furthermore, these agents appear to act selectively at 5-HT_2 sites, inasmuch as they did not alter 5-HT_{1A} receptor-mediated changes in corticosterone secretion or body temperature.

224.9 EFFECTS OF SEROTONIN RECEPTOR AGONISTS AND ANTAGONISTS ON NEUROENDOCRINE FUNCTION IN RHESUS MONKEYS. G.R. Heninger, D.S. Charney and A. Smith*, Dept. of Psychiatry, Yale Univ. Sch. of Medicine, New Haven, CT 06508.

The 5-HT system plays an important role in modulating neuroendocrine function (i.e, prolactin (PRL) growth hormone (GH) and cortisol (CORT) release), but the subtypes of 5-HT receptors mediating these effects are unknown. Since species differences exist in 5-HT subtypes, the current study was conducted in Rhesus monkeys so the data would be more applicable to humans.

METHODS: Eight male Rhesus monkeys (7-10 kgs) slept in their home cages and were chaired daily. Once weekly at 8 a.m., they had a catheter inserted in the superficial lower leg vein where, following a 90 min. adaptation period, all drugs were administered IV and blood was sampled for PRO, GH and CORT measured using standard RIA methods. The 7 agonists studied (with dose range) included: one nonspecific 5-HT precursor, tryptophan (TRP) (200 mg/kg); three specific 5-HT1a agonists, 8-OH-dipropylaminotetralin (8-OH-DPAT), (0.01 to .3 mg/kg) gepirone (GEP) (.1 to 2 mg/kg) and ipsapirone (IPS) (.2 to 1 mg/kg); one 5-HT1b agonist m-chlorophenylpiperizine (MCPP) (.2 to 2 mg/kg); one mixed 5-HT1a agonist and dopamine antagonist buspirone (BUS) (.1 to 2 mg/kg); and one a 5-HT2 agonist, mescaline (MES) (.2 to 5 mg/kg). The specific 5-HT2 antagonist, ritanserin (RIT) (.1 mg/kg) and the nonspecific 5-HT antagonist metergoline (MET) (.25 mg/kg) were administered prior to agonist infusions.

RESULTS: A dose of .25 mg/kg of GEP (.25 mg/kg = .63 uMole/kg) stimulated an average of 20 ng/ml peak release of prolactin (peak minus base). The average relative rank order of the 7 agonists (in uM/kg - with GEP = to 1) in stimulating an approximate 20 ng/ml peak release of prolactin was: BUS (<.2), 8-OH-DPAT (1), GEP (1), IPS (2), MCPP (4), MES (13), and TRP (>1000). The average per cent inhibition of agonist stimulated PRL response was:

	GEP	MCPP	MES	TRP
RIT	0	50	90	18
MET	90	100	-	97

In comparison to PRL, agonist effects on GH and CORT release were much more variable and they did not follow the same rank order as for PRL. On a uM/kg basis 8-OHDPAT was 3 times more sedative than GEP. Variable amounts of behavioral sedation was observed with the other agonists.

DISCUSSION: The exceptional potency of BUS on PRL release would appear to be due to blockade of dopamine receptors. The relative potency of the other agonists, and the lack of blockade of 5-HT1a agonists by RIT, but the full block of MES by RIT indicates that 5-HT1a receptors are more sensitive in stimulating PRO release than 5-HT2 receptors, although the data also indicates that 5-HT2 receptors are also involved in stimulating PRO release. Supported by USPHS MH36229, MH25642, MH39029.

224.10 PHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERIZATION OF THE RESPONSES OF GUINEA PIG NEOCORTICAL NEURONS TO SEROTONIN. M.F. Davies, R.A. Deisz*, D.A. Prince and S.J. Peroutka. Stanford University of Medicine, Dept of Neurology, Stanford, CA, 94305.

The characteristics of the intracellularly recorded responses of cortical pyramidal neurons and interneurons to exogenously applied 5-hydroxytryptamine (5-HT) were investigated in slices of guinea pig somatosensory cortex maintained in vitro by conventional techniques. In 68% of pyramidal neurons, pressure application of 5-HT (10^{-5}-10^{-3} M) caused a slow long-lasting depolarization (0.5-5 mV) which was associated with decreased conductance (20.6 ±1.8%). Current voltage plots suggested that a resting K^+ conductance was reduced by 5-HT and the resistance-voltage plots revealed no voltage dependency. This depolarization was insensitive to tetrodotoxin (TTX) and therefore appeared to be a postsynaptic response. Desensitization was prominent during repeated 5-HT applications. The 5-HT_2 antagonists, ritanserin (10^{-6}M) and cinanserin (10^{-5}M) blocked the depolarization indicating that the 5-HT_2 receptor mediated this response. In 26% of the cells, 5-HT application evoked a slightly faster hyperpolarization (0.5-2.0 mV), secondary to an increased conductance state. This response was also resistant to TTX. The hyperpolarizing response was not secondary to activation of interneurons as it was still observed in the presence of bicuculline or TTX. The 5-HT_{1A} agonist 8-OH-DPAT mimicked and 5-HT_2 antagonists did not block this hyperpolarization. When applied to interneurons, 5-HT always caused a slow depolarization (2-9 mV) which was also associated with a decreased resting conductance.

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224.11 CORRELATION OF PSYCHOTROPIC DRUG EFFECTS AT NEUROTRANSMITTER RECEPTORS WITH PHOSPHATIDYLINOSITOL HYDROLYSIS IN RAT CEREBRAL CORTEX P.A. Pierce* and S.J. Peroutka (SPON: S. Hameroff). Departments of Neurology and Pharmacology, Stanford University, Stanford, CA 94305.

The ability of six psychotropic agents to interact with neurotransmitter receptor sites was compared with their ability to stimulate and/or modulate phosphatidylinositol (PI) hydrolysis in rat cerebral cortex. The drugs studied were (±) 3,4 methylenedioxymethamphetamine (MDMA), (±) 3,4 methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-N-ethylamphetamine (MDE), 2,5-dimethoxy-4-iodoamphetamine (DOI), 2,5-dimothoxy-4-bromoamphetamine (DOB), d-lysergic acid diethylamide d-LSD). The neurotransmitter binding sites analyzed included: 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) sites labeled with ^{3}H-8-OH-DPAT; non-5-HT_{1A} sites labeled with ^{3}H-5-HT; 5-HT_2 sites labeled with ^{3}H-spiperone; putative high affinity 5-HT_2 sites (labeled with ^{3}H-DOB); 5-HT uptake recognition sites labeled with ^{3}H-paroxetine; alpha$_1$-adrenergic sites labeled with ^{3}H-WB 4101; alpha$_2$-adrenergic sites labeled with ^{3}H-rauwolscine; dopamine$_1$ sites labeled with ^{3}H-SCH 23390; dopamine$_2$ sites labeled with ^{3}H-spiperone.

The ring-substituted amphetamines were found to display micromolar affinity for all 5-HT binding site subtypes. By contrast, d-LSD, DOI and DOB display nanomolar potency for 5-HT_2 sites (both those labeled by ^{3}H-spiperone and those labeled by ^{3}H-DOB). In addition, d-LSD displays nanomolar affinity for 5-HT_{1A}, non-5-HT_{1A}, alpha$_2$-adrenergic and dopamine$_2$ sites. The other psychotropic drugs displayed micromolar affinity for all other neurotransmitter binding sites.

The ability of 5-HT to stimulate PI hydrolysis in rat cerebral cortex has been attributed to activation of 5-HT_2 receptors. The rank-order potencies of the above psychotropic agents in modulating PI hydrolysis in rat cerebral cortex was also determined. An attempt will be made to determine if a correlation exists between psychotropic drug effects on PI hydrolysis and a single neurotransmitter receptor.

224.12 ICI 169369 IS AN ANTAGONIST OF 5-HT IN TISSUES CLAIMED TO CONTAIN 5-HT_{1A} AND 5-HT_{1C} RECEPTORS Blackburn, T.P., Cox, B., Grant, T.L. and Growcott, J.W. (SPON: R Pastel). ICI Pharmaceuticals Division, Research Department 2, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, U.K.

ICI 169369 (2-(2-Dimethylaminoethylthio)-3-phenylquinoline hydrochloride) is a new 5-HT antagonist that is currently undergoing clinical trials in migraine, schizophrenia and anxiety/depression. It is an orally active antagonist in tests designed to measure activity at 5-HT_2 receptors but is devoid of effects at other neurotransmitter receptors (Blackburn et al.,Br.J. Pharmac., 90, 256 and 277P 1987). Some authorities divide 5-HT receptors into 3 separate sub-types; 5-HT_1, 5-HT_2 and 5-HT_3 (Bradley et al., Neuropharmac., 25, 563, 1986) and then divide the 5-HT_1 receptor even further into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D}. This report compares the effects of ICI 169369 with another 5-HT_2 antagonist ketanserin on tissues claimed to contain either the 5-HT_{1A} (the isolated rabbit basilar artery preparation) or the 5-HT_{1C} (the isolated rat stomach fundus preparation) subtype.

5-HT caused dose-related contractions of both the basilar artery and the fundus preparations with EC_{50}'s of 5.9×10^{-8} and 6×10^{-9}M respectively. ICI 169369 caused a dose related inhibition of 5-HT (EC_{50}) on the basilar artery over the concentration range 10^{-8} to 10^{-6}M, with an IC_{50} of 7.4×10^{-8}M. Ketanserin on the other hand was inactive even at concentrations as high as 10^{-6}M. ICI 169369 (10^{-7} to 10^{-5}M) produced a non-competitive inhibition of 5-HT on the rat fundus preparation, but ketanserin was inactive. 8-OHDPAT an agonist claimed to be selective for the 5-HT_{1A} receptor subtype caused dose-related contractions of both tissues, but much higher concentrations were required when compared with 5-HT (EC_{50} values of 10^{-6} to 10^{-5}M). ICI 169369 (10^{-7} to 10^{-6}M) caused a dose related inhibition of 8-OHDPAT on the rat fundus preparation and at 10^{-6}M caused an 86% inhibition of a submaximal response to 8-OHDPAT on the basilar artery. Ketanserin was inactive on both preparations.

These results indicate that the attempts to divide the so called 5-HT_1 receptor into different subtypes based only on the use of agonists is premature and that ICI 169369 can act on receptors that are not currently defined as 5-HT_2 (as classified by the use of ketanserin). These additional properties of ICI 169369 might afford it a wider clinical opportunity since the therapeutic applications of drug interaction with the various 5-HT receptor subtypes remains to be determined.

225.1 THE ROLE OF INFRALIMBIC AND INSULAR CORTEX IN OLFACTORY HEART RATE CONDITIONING IN THE RAT. C. B. Sananes and B. A. Campbell, Dept. Psychology, Princeton Univ., Princeton, NJ 08544

We have recently found that classical conditioning of heart rate to an olfactory stimulus depends on the integrity of the central nucleus of the amygdala (ACE) in the infant rat (Sananes & Campbell, 1986). The aim of the present study was to determine the olfactory pathways to ACE which must be intact for the maturation of this conditioned response.

We examined the role of infralimbic (IL) and insular (INS) cortex on olfactory heart rate conditioning. Both cortical regions receive olfactory input (Krettek & Price, 1977) and project to the central nucleus of the amygdala (Otterson, 1982). Testing was carried out in groups of rat pups with bilateral electrolytic lesions confined to one area (INS; n=6) or the other (IL; n=6). Sham and unoperated control groups were also tested. Three days following surgery, 21-day-old rat pups were tested, using a 10-sec presentation of grape juice odor as the CS, and a 0.5 sec .35-mA subcutaneous shock as the US. A sensitization control with the odor explicitly unpaired with shock was also run. Following testing, the rats were sacrificed and histology performed. In unoperated and sham control groups, the CS elicited a marked conditioned tachycardia, significantly different from their sensitization controls [$F(1,11)=28.14$, $p<.01$) and $F(1,11)=87.61$, $p<.01$)], respectively. Conditioned tachycardia was also seen in the INS group, and differed significantly from the sensitization controls [$F(1,11)=7.75$, $p<.05$)]. In contrast, rat pups that received bilateral lesions of IL showed a severe attenuation of the heart rate CR, which did not differ significantly from the sensitization controls [$F(1,11)=2.89$, p.12)]. None of the lesions interfered with basal heart rate, or unconditioned heart rate changes.

These data demonstrate that infralimbic cortex, and not insular cortex contributes to olfactory heart rate conditioning, and may do so by relaying olfactory information to the central nucleus of the amygdala. We offer a speculative explanation for the slow ontogenetic maturation of olfactory heart rate conditioning in the infant rat.

225.2 APPROPRIATE LESIONS OF THE INTERPOSITUS NUCLEUS COMPLETELY AND PERMANENTLY ABOLISH THE CONDITIONED EYELID/NM RESPONSE IN THE RABBIT. Richard F. Thompson, Joseph Steinmetz and Paul F. Chapman. Department of Psychology, Stanford University, Stanford, CA 94305.

Welsh et al, (Neurosci Abst, 12, 270.12, 1986) reported that following interpositus lesions that abolished the eyelid/NM CR in the CS period, a small amplitude (1.32 mm), infrequent (9-22%), late (within 250 msec of CS offset) "CR" occurred on test trials over the 12 days of post-operative training. In approximately a dozen separate studies, neither we nor other laboratories have observed such responses, other than occasional spontaneous responses of random latency. But we have now repeated the experiment yet again. To date we have run 5 animals. Animals are trained using standard conditions (250 msec tone CS, 100 msec corneal airpuff US, coterminating, CS alone every 9th trial 108 trials per day), to a criterion of 8 CRs in 9 consecutive trials, given 2-4 days of over training and given interpositus lesions. Following 7 days of recovery, animals were trained for up to 24 days and behavioral responses recorded for up to 3 sec following tone CS offset. Two of these animals were given 11-12 days of training on the standard paradigm and 11-12 days of training on the Welsh, et al, idiosyncratic paradigm. None of the animals showed any CRs at all, either early or late. Inspection of the actual data Welsh et al, obtained indicates that their lesions were not completely effective, in that they did not completely abolish the short latency CR in the CS-US onset interval, in contradistinction to their abstract, but in full agreement with previous work on effects of incomplete lesions from our laboratory (Clark et al, Br Res, 291, 125-136, 1984).

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225.3 ISCHEMIC INDUCED HIPPOCAMPAL INJURY IN RATS CAUSES DISSOCIATED WORKING MEMORY IMPAIRMENT ON MODIFIED T-MAZE. B.T. Volpe, B. Waczek*, P. Colombo*, H.P. Davis. Cornell University Medical Center, NY, NY, 10021. Burke Center, White Plains, NY, University of Colorado, Colorado Springs, Col.

In order to develop an animal model for the amnesic syndrome that may follow cardiac arrest, and that is characterized by relatively circumscribed CA1 hippocampal injury, we have been examining the behavior of animals that have been exposed to transient forebrain ischemia. Post ischemic (PI) animals demonstrate severe neuron loss in the CA1 hippocampus and dl-striatum. On radial 8 and 12 arm mazes, PI animals demonstrate working memory performance deficits compared to control animals, yet demonstrate normal reference memory performance . Because this dissociation may be a function of task, we employed a split stem T-maze to measure memory for invariant, trial independent information (REFERENCE), and memory for variable trial dependent information (WORKING) using a second, different task. Reference performance required the animals to chose either the rough (r-positive) or smooth (s-positive) surfaced alleyway on the stem. Animals were randomly assigned r-positive or s-positive and remained so for all trials. Surfaces were randomly placed on the right or left alleyway. Working performance required the animals to alternate choice of goal arm for a food reward. A paired run procedure was used, so that an animal received an information run which included a reference judgement in the stem and a forced choice to a goal arm. For the memory run, an animal repeated the reference judgement but was permitted to chose either goal arm. There were ten pairs of runs per trial. Correct working memory performance was scored when the animal alternated goal arm choice. Animals were trained for 20 preoperative trials, exposed to ischemia and returned for 25 postoperative trials. Results showed no difference in preoperative performance ($p > .5$) between PI and sham controls. On post-operative trials there was no difference between groups on REFERENCE memory performance ($p > .1$). However, PI animals performed the WORKING memory task worse than sham controls ($p < .01$). This preliminary data confirms, using another task, the previous observed dissociation of working and reference memory performance, and extends the development of reliable behavioral measures for animal models of human disease. In addition it sets the stage to test whether focal CA1 injury alone or in combination with subiculum or amygdala lesions is sufficient to cause the working memory impairment.

225.4 INCREASED RATE OF FORGETTING OF SPATIAL INFORMATION IN RATS WITH IBOTENATE LESIONS OF HIPPOCAMPUS. L.E. Jarrard, J.M. Ringland*, and L.S. Johnson*. Dept. of Psychology, Washington and Lee Univ., Lexington, VA. 24450.

Hippocampal lesions made with conventional lesion techniques (aspiration, electrolytic) result in rats being impaired in performance of most spatial tasks. However, when the cells in the hippocampus are removed with multiple injections of ibotenic acid (IBO), a procedure that spares fibers of passage and damage to the vasculature and adjacent structures, performance on spatial tasks is less affected. It was found in preliminary research that rats with IBO hippocampal lesions could learn a spatial task that required the animals to return to the same place (match-to-sample (MTS)) on subsequent trials. Thus, the MTS task was used in the present experiment to study forgetting of spatial information in lesioned and control rats.

Animals were divided into 2 control (operated, unoperated) and 2 lesion groups. Multiple injections of small amounts of IBO were used to remove cells in the hippocampus in one lesion group, and in the subiculum in the other group. After recovering from the operations, the animals underwent training on the MTS task using an 8-arm radial maze. Following an information trial, which consisted of raising the door to the arm that was correct on that day and allowing the rat to obtain the food reinforcement, the subjects received 5 trials where only the correct arm was repeatedly baited and the rats allowed to choose from among all 8 arms. On any one day the same arm was correct, while different arms were correct on subsequent days. Following acquisition, forgetting was determined by inserting delays of varying lengths (0, 5, 20, 60 min) between Trials 4 and 5.

Even though rats in the hippocampus group made more errors in acquisition, after 24 days of training their performance did not differ from that of controls. When delays between Trials 4 and 5 were introduced, hippocampals made increasingly more errors as the delays were increased while performance of subiculum and control rats remained the same. Performance of subiculum and control rats did not differ in either acquisition or forgetting.

These findings indicate that removal of the hippocampus results in an increased rate of forgetting of spatial information, thus an impairment similar in many ways to that reported for humans with temporal lobe dysfunction.

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225.5 SYNAPTIC PLASTICITY: THE ROLE OF SYNAPTIC CURVATURE. E.J. Markus and T.L. Petit. Div. of Life Sciences, Univ. of Toronto, Scarborough, Ont. M1C 1A4, Canada

One of the plastic aspects of synaptic structure is synaptic shape. Synapes can be divided into four types: smile, flat, frown, and irregular shapes. Smile shaped synapses refer to those contacts in which the presynaptic (axon) element protrudes into the postsynaptic (dendritic) process. These synapses, when oriented such that the presynaptic terminal is above the postsynaptic terminal, resemble smiles. Frown shaped synapses refer to those contacts where the postsynaptic process pushes into the presynaptic terminal. Flat shaped synapses are those which show no curvature, and irregular synapses show no consistent curvature. Recent research has indicated that synaptic curvature is an important plastic feature of the synapse.

There is an increase in the proportion of frown shaped synapses and a decrease in smile shaped synapses with development in the rat neocortex:

Developmental Period	% Smile	% Frown	N
P1-10	37	30	76
P15-30	26	41	438
P60-90	11	49	307

Following repetitive activation with kainic acid in the adult hippocampus (Petit & Markus, in Neuroplasticity, Learning and Memory, Milgram, MacLeod, Petit, eds, A.R. Liss, 1987) or LTP (e.g. Desmond & Levy, J Comp Neurol 253:466-482, 1986) there is an increase in the proportion of smile shaped synapses.

The mechanisms underlying changes in synaptic curvature can be categorized into two types. 1) Passive membrane conformation to the surrounding terminal: changes in curvature could be related to changes in terminal shape or the location of the synapse on the dendritic process. 2) Active local mechanisms: changes in curvature could be related to changes in junction proteins, vesicle release sites, receptors, or internal cytoskeleton.

These mechansims and implications of plasticity in synaptic curvature will be presented.

225.6 MEMORY IMPAIRMENT AND LOSS OF CA1 NEURONS IN THE HIPPOCAMPUS PRODUCED IN THE FOUR VESSEL OCCLUSION (4-VO) ISCHEMIC RAT MODEL OF STROKE. J.M. Ordy, B. Volpe, G. Thomas, and P. Colombo*. Pennwalt Corp., Rochester, NY 14623 & Cornell Medical Center, New York, NY 10021.

Stroke and cardiac arrest represent 2 leading causes of death and in survivors a circumscribed amnesia or memory loss has been reported for events that occur after global ischemia. Neuropathological examinations of patients with cerebral ischemia due to stroke or cardiac arrest have indicated a selective loss of neurons from the CA1 zone of the hippocampus, an area critical for short-term, working, or representational memory. Although clinical evidence for amnesia and CA1 cell loss in cerebral ischemia has been provocative, until recently, animal models of stroke have provided only tenuous evidence for a causal link between hypoxic or ischemic memory impairment and selective vulnerability of CA1 cell loss. More recently, in a four vessel occlusion (4-VO) rat model of stroke, impairments in working but not reference memory in a radial 8-arm maze, with selective cell loss of CA1 neurons, have been demonstrated. Multiple unit maze tests of memory confound working and reference memory as well as motivation and motor performance. The specific aims of this study were to examine the ischemic effects of 4-VO on trial-dependent representational memory of the rat in a T-maze in which ischemic effects on memory can be segregated from effects on motivation and motor performance by separate measurements of start, choice, and goal latencies. Naive and trained male Wistar rats (250-300 g) were subjected to 4-VO by cauterization of vertebral arteries and 30 min. of transient carotid occlusion to reduce cerebral blood flow to less than 3% of control levels in the hippocampus, striatum and cortex. In naive rats tested only after 4-VO, there was a significant decrease in representational memory in the T-maze in eight 4-VO compared to 7 control rats across 4 replications ($F(1,13) = 9.78$, $p = 0.008$), without ischemic effects on start, choice or goal speeds. In rats tested both before and after 4-VO, there was also a significant decrease in memory in nine 4-VO compared to 8 controls at 2 & 4 months post 4-VO ($F(1,15) = 11.12$, $p = 0.004$), without effects on start or choice speeds, but with a significant reduction in goals speeds ($F(1,15) = 4.14$, $p = 0.05$). After memory tests, all 32 rats were perfusion-fixed and paraffin sections (7μM) of anterior dorsal CA1 hippocampus were stained with hematoxylin and eosin, and graded for neuronal damage with light microscopy. In 4-VO rats, there was severe bilateral loss of CA1 neurons in the anterior region with severe to moderate loss in the mid-dorsal and posterior hippocampus. The selective memory deficits and CA1 cell loss in the 4-VO rats of this study provide support for the continued use of this 4-VO rat model for biochemical and pharmacological studies of global cerebral ischemic damage in stroke and cardiac arrest.

225.7 DOUBLE DISSOCIATION OF ITEM AND ORDER (SPATIAL) MEMORY FOLLOWING PARIETAL VS. MEDIAL PREFRONTAL CORTEX LESIONS. R.P. Kesner (Spons: R. Tuckett), Department of Psychology, University of Utah, Salt Lake City, Utah 84112.

Based on Kesner's attribute model of memory, it is assumed that the neocortex represents a critical neural system that subserves knowledge-based memory. More specifically, it is proposed that the medial prefrontal cortex (MPC) mediates temporal cognitive maps, while the parietal cortex (PC) mediates spatial cognitive maps. Furthermore, it is likely that these two neocortical regions can operate independently. In order to provide empirical support for the above assumptions, rats were trained on an eight arm radial maze for Froot Loop reinforcement. After extensive training each animal was allowed on each trial (one per day) to visit four arms in an order that was randomly selected for that trial (study phase). The sequencing of the four arms was accomplished by sequentially opening of Plexiglas doors (one at a time) located at the entrance of each arm. Immediately after the animal had received reinforcement from the last of the four arms, the test phase began. The test for order memory consisted of opening of either the first and second, second and third, or third and fourth door that occurred in the sequence. The rule to be learned leading to an additional reinforcement was to choose the arm that occurred earlier in the sequence. The test for item memory consisted of opening of a door that was previously visited for that trial and a door that was not. The rule to be learned resulting in an additional reinforcement was to choose the arm previously visited during the study phase of the trial (win-stay rule). The order of presentation of the two tests was varied randomly.

Following extensive training, animals performed better than chance for each item position for both tests. The animals then received medial prefrontal cortex or parietal cortex aspiration lesions.

Results indicated that animals with MPC lesions displayed a total deficit for the order memory task, while remembering well the first spatial location within the item memory task. In contrast, animals with PC lesions displayed a total deficit for the item memory task, while remembering well the first choice order of spatial locations within the order memory task. These data suggest that for the first item(s) within a list there is a double dissociation of function between MPC and PC for item and order spatial memory. Thus, the MPC and PC neural regions can probably operate independently and furthermore, item and order (spatial) memory can be coded separately.

225.8 **TACTILE LEARNING IN OCTOPUS IS AFFECTED BY CYTOCHALASIN B.** J. David Robertson., J.Z. Young*, P.H. Lee* and C.B. Bock*, Department of Anatomy, Duke University, Durham, N.C.; Duke University Marine Laboratory, Beaufort, N.C.; Dept. of Experimental Psychology, Oxford, England.

Preliminary electron microscopic study of serial sections of the tactile learning neuropil in the posterior buccal lobe of *Octopus vulgaris* has revealed the presence of numerous complexly related small neuronal processes that resemble filopodia of growth cones. It is postulated that rearrangements of such fine neurites might lie at the basis of learning and memory. Filopodia depend on actin polymerization for activity and this can be blocked reversibly with Cytochalasin B (CB) in vitro. Tactile learning can be nearly abolished by surgical extirpation of the posterior buccal and subfrontal lobes. The present work shows that tactile learning can be significantly decreased by comparision with control animals without affecting visual learning when CB is applied directly to the tactile learning neuropil. In the experiments, animals were positively trained to smooth plastic balls using food rewards and negatively to rough balls using 10VAC shocks.The animals could not distinguish the smooth and rough balls visually but did so readily by touch. 28 animals previously trained positively with smooth balls were anesthetized with $MgCl_2$. Their brains were exposed and the posterior buccal lobes of each were injected bilaterally through a 200 μm beveled Hamilton syringe needle with 0.6-1.0 μl of a solution of artificial sea water containing ~30% horseradish peroxidase as a histological marker, and 2% dimethylsulfoxide with (test) or without (control) 0.6-1.2 % CB. Negative training was begun after the animals were feeding normally (2-3 days). Tactile learning curves from 2 daily sessions of 8 ball presentations each were plotted for 20 sessions and a discrimination index 'I' was calculated according to the formula $I=(x-y)/(x+y)$; where x=takes of positive ball, y=takes of negative ball. The mean index attained by 16 control animals was 0.48 (SE=0.05) and that of 12 CB injected animals was 0.19 (SE=0.05) ($P=0.1$; Wilcoxon). Injection sites were checked histologically in serial sections. Visual learning remained functional in all the injected animals. While they could not discriminate the rough and smooth balls visually, they learned independently from the negative training that the presence of any ball might well lead to shocks, and thus refused to approach the balls as they initially did to obtain food rewards in the positive training. The results support the hypothesis that filopodial activity in the neuropil is necessary for learning and memory. Supported by a gift from RJR/Nabisco Inc.

225.9 **THE HIPPOCAMPAL SYSTEM AND MNEMONIC REPRESENTATION.** H. Eichenbaum, A. Fagan*, P. Mathews*, and N.J. Cohen.
Department of Biological Sciences, Wellesley College, Wellesley, MA 02181

To investigate the role of the hippocampal system in the processing of mnemonic information, the performance of SHAM operated and that of rats with FORNIX lesions were compared on acquisition of different versions of the same two-odor discrimination task that placed different encoding and representational demands on memory. One group of rats was trained using simultaneous presentation of two odor stimuli, and a go-left/go-right response choice, thus encouraging a memory representation based on comparisons both between odor cues and between response choices. FORNIX rats were severly and persistently impaired on this task. A second group of rats was trained with the same response requirement, but the two odors were presented separately and successively across trials, thus hindering odor cue comparison but retaining the same explicit spatial-response choice. FORNIX rats showed a transient learning deficit on this task. A third group was trained with successive odor presentations and a go/no-go response involving only completing or interrupting a single behavioral act. Thus this task both hindered odor comparison and eliminated explicit cues for response choice, and favored a memory representation based on learning the significance of each odor individually. FORNIX rats outperformed SHAM rats on this task. The combined results support an hypothesis that the hippocampal system is critical to a memory representation based on encoding relationships among multiple percepts, and other brain systems support performance adaptations based on encodings of stimuli individually.

To the extent that only the SHAM rats depend on a representation of the relationship between paired S+ and S- odors, one would expect that their performance, and not that of the FORNIX rats, would be disrupted by mismatching the presentation of previously learned odor-pairs. In a separate experiment, we compared response-choice reaction times on "regular" simultaneous-cue trials, composed of previously learned odor-pairs, versus "probe" trials, composed of mismatched S+ and S- stimuli from the same problems. Pairs of FORNIX and SHAM rats were trained on a series of discrimination problems, until the FORNIX rat of each pair reached accurate performance on two different problems. FORNIX rats were severely impaired in learning rate, but each rat eventually acquired two discrimination problems. Then all rats were tested in sessions composed of a mixture of regular trials on both successful problems and on occasional probe trials. Rats of both groups responded correctly on all types of trials. As predicted, SHAM rats had longer response latencies on probes than on regular trials. FORNIX rats had much shorter response latencies on all trials and, in accordance with our prediction, did not evidence a consistent increase in response latency on probe trials. These results support our contention that FORNIX rats fail to process relational information during memory judgements.

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225.10 **HIPPOCAMPAL MOSSY FIBERS AND AVOIDANCE LEARNING IN MICE TREATED POSTNATALLY WITH CORTICOSTERONE: CORRELATIONS WITH EXTENT AND LEFT/RIGHT ASYMMETRY.** D.P. Wolfer*, H.-P. Lipp, H. Schwegler*, and M. Brust*. Inst. of Anatomy, Univ. of Zürich, Switzerland, and Inst. of Human Genetics, Univ. of Heidelberg, FRG.

Two-way avoidance learning of mice was found to correlate negatively with the extent of the intra/infrapyramidal mossy fiber (IIP-MF) projection (Schwegler & Lipp, Behav. Brain Res. 7, 1983). Postnatal corticosterone injections impair avoidance learning of adult rats (Olton et al., Dev. Psychobiol. 8, 1977). To test a possible relation between hypercorticism, IIP-MF and two-way avoidance, forty pups of the mouse strain DBA/2 were injected postnatally for 12 days either with total 4.8 micrograms of corticosterone (CORT) or solvent (OIL). The adult mice were tested in an open-field and in a shuttlebox. In 33 mice, the extent of the mossy fiber projections was measured on Timm-stained horizontal sections from the midseptotemporal level. Unexpectedly, CORT- and OIL-mice did not differ in open field and two-way avoidance, but both groups showed fairly poor two-way avoidance, atypical for DBA/2 mice. Hippocampal morphometry revealed no group differences, but considerable variability, anomalies, and asymmetries of the mossy fiber projection in both groups. In the pooled sample of mice, the magnitude of the averaged (left and right) IIP-MF projection was positively correlated with avoidance performance in the early stages of conditioning (day 2, $r=0.48$, $p<0.01$), as well as with various scores of pre- and intertrial locomotor activity, while the averaged extent of the suprapyramidal mossy fiber projection correlated positively with avoidance performance in the later stages (day 6, $r=0.50$, $p<0.01$). Curiously, a (dimension-free) asymmetry-index of the IIP-MF was correlated with the overall avoidance performance, mice having relatively more IIP-MF at left showing superior avoidance ($r=0.58$, $p<0.05$, $n=15$). This was observed in CORT-mice only, in which asymmetry indices of the IIP-MF were also correlated with measures from the open-field, more IIP-MF at left being associated with lower locomotor activity. These results and data from studies in progress indicate that variations of the IIP-MF are not correlated with a specific behavior itself, but appear to modulate the likelihood of behavioral change - the behavior being context-dependent - through stabilizing (more IIP-MF) or de-stabilizing (less IIP-MF) complex patterns of neuronal activity in each hemisphere. Thus, if the initial behavior in the shuttle-box is favorable for learning (e.g., high activity levels), more IIP-MF may lead to positive correlations with avoidance. If the initial behavior is inappropriate (e.g., immobility), more IIP-MF may yield negative correlations. Supported by SNF 3.041 and DFG Schw/252.

225.11 INTRACEREBELLAR LIDOCAINE PRODUCES A REVERSIBLE DECREMENT IN CONDITIONED AND UNCONDITIONED NICTITATING MEMBRANE RESPONSES IN THE RABBIT. J. P. Welsh*, N. Bormann*, P. Iannuzzelli*, and J. A. Harvey. Departments of Psychology and Pharmacology, Univ. of Iowa, Iowa City, IA, 52242.

Destruction of the nucleus interpositus (IP) of the cerebellum has been reported to decrease the frequency and amplitude of a previously acquired conditioned nictitating membrane response (NMR) and to prevent its reacquisition (Clark et al., Brain Res. 291, 1984). In addition, such lesions have been reported to increase the latency of conditioned responses (CRs) (Welsh, Gormezano & Harvey, Neurosci. Abst. 12, 978, 1986). Our recent finding that IP lesions also affect the unconditioned NMR has led us to further investigate the role of the cerebellum in the performance of both the conditioned and unconditioned NMR. Reversible inactivation of the cerebellum has proven to be a valuable method for elucidating the neural bases of motor control (Brooks, Rev. Physiol. Biochem. Pharmacol. 95, 1983). Therefore, we employed intracerebellar infusions of the local anesthetic lidocaine to examine changes in both CRs and unconditioned responses (UCRs). Naive rabbits were implanted with cannulae aimed at the anterior portions of the IP nucleus. Pavlovian conditioning of the nictitating membrane reflex was accomplished by pairing a 300-msec tone or light conditioned stimulus (CS) with a 100-msec unconditioned stimulus (UCS) consisting of a puff of air directed at the right cornea. The offset of the CS coincided with onset of the UCS. Conditioning occurred over 3 or more training sessions until a criterion of 80% CRs was achieved. Each session consisted of 120 pairings of CS and UCS with an intertrial interval of 30-sec (range 25-35 sec). An extension of the NMR of 0.5 mm or greater was scored as a conditioned responses (CRs) if its onset occurred during the 300 msec presentation of the CS and as a UCR if it occurred after UCS onset. Histological examination revealed that five animals had cannula tips located immediately dorsal to the more anterior and lateral portions of IP (n=5). These animals demonstrated a complete loss of CRs within one minute of lidocaine infusion. This effect persisted for 5 to 25 min (mean 15 min) and recovery to preinfusion levels of responding (95% CRs) occurred within 35 minutes of infusion. The elimination of CRs produced by lidocaine was accompanied by a reduction in UCR amplitude with no change in UCR latency. These effects of lidocaine on CR frequency and UCR amplitude could be obtained with repeated injections. Infusion of lidocaine into animals with cannula tips located in regions surrounding the lateral portions of IP had a smaller effect on responding while those even further removed were without effect. The results of this study indicate that the IP is necessary for the normal motoric expression of both conditioned and unconditioned NMRs.

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226.1 CHARACTERIZATION OF RAT DOPAMINE β-HYDROXYLASE DEDUCED FROM A cDNA CLONE. Anne McMahon* and Esther L. Sabban, Department of Biochemistry, New York Medical College, Valhalla, N.Y. 10595.

In order to characterize dopamine β-hydroxylase (DBH), the enzyme which catalyzes the formation of norepinephrine, we utilized a cDNA clone for rat DBH which we isolated. This clone of 1131 nucleotides was sequenced and the 211 C-terminal amino acids were deduced followed by 497 nucleotides at the 3'-non-coding region. The amino acid composition deduced was consistent with the overall amino acid composition and acidic properties of DBH. A striking feature is a hexanucleotide (GAGCCG) which is tandemly repeated 10 times and corresponds to part of a 13-fold tandem repeat of the dipeptide glu-pro. The glu-pro dipeptide also occurs 5 additional times in surrounding sequences.

We did not detect any significant homology to the other catecholamine biosynthetic enzymes which have been cloned - rat tyrosine hydroxylase and bovine phenylethanolamine N-methyltransferase.

The mRNA contains a long (at least 500 nucleotide) 3'-non-coding region. There appears to be a single mRNA for DBH in pheochromocytoma cells, of 2100-2200 nucleotides as observed on Northern blot analysis, which leaves at most 1600 nucleotides for the coding region. Therefore, it is likely that the actual molecular weight of DBH is less than the apparent Mr of 67,000 observed by gel electrophoresis of the immunoprecipitated product from translation of the mRNA. Such a discrepancy between actual and apparent molecular weight is not too uncommon for acidic and proline-rich proteins.

DBH in the adrenal and in noradrenergic neurons is present as membrane-bound and soluble forms. It appears that the membrane-bound form contains subunits which are post-translationally processed, possibly by proteolysis, to the soluble form in pheochromocytoma cells and in the locus coeruleus in the brain (Sabban et al, J. Biol Chem., 258:7812, 1983, J. Neurosci, 7:192, 1987). A plot of the average hydrophilicity of the amino acid sequence deduced from the cDNA clone showed that the C-terminal region is quite hydrophilic. Therefore, if there is a polypeptide "anchor" which is proteolytically cleaved during the conversion of the membrane-bound to soluble form of DBH, it is unlikely to reside in the C-terminus of the protein. (Supported by NIH Grant NS 20440).

226.2 EXPRESSION CLONING OF DOPAMINE β-HYDROXYLASE USING LAMBDA gt11. K. P. Decker*, and D. L. Wong. (SPON: R. D. Ciaranello). Department of Psychiatry, Stanford Univ. Sch. of Med. Stanford, CA 94305

We are interested in studying the regulation of dopamine β-hydroxylase [3,4-dihydroxyphenylethylamine, ascorbate:oxygen oxidoreductase (β-hydroxylating)], (DBH), at the transcriptional and genomic levels. For these studies, a cDNA clone for DBH is required. We have isolated such a probe using a lambda gt11 expression cloning system. A rat brain cDNA library (the generous gift of J. Eberwine) was screened with rabbit anti-bovine DBH polyclonal antiserum as primary antibody. To reduce artifactual positive signals, the primary antiserum was pre-absorbed with an E. coli lysate. Iodinated, affinity-purified goat anti-rabbit serum was used as secondary antibody and recombinant clones were identified by autoradiography.

Using the above method for screening the phage library, one plaque out of 180,000 yielded a positive signal. This candidate was plaque-purified until subsequent screens demonstrated positive signals from most plaques. Preliminary experiments showed that the signal produced by the candidate clone could be blocked by preincubation of polyclonal antiserum with purified DBH. DNA was isolated from the recombinant phage, and a 250 bp insert in the Eco R1 site of lambda gt11 was found. This R1 site lies within the structural gene for β-galactosidase, and DNA inserted into this site can therefore be expressed by the phage as a fusion protein composed of β-galactosidase and the protein of interest.

The β-galactosidase portion of the fusion protein thus generated comprises 114.2 kD of molecular weight, as the R1 restriction site interrupts the structural gene 52 bp prior to the transcription termination site. Accounting for the weight of the truncated β- galactosidase, a new polypeptide of apparent molecular weight 10.6 kD is encoded by the insert. Lysogens of the candidate phage were made in another bacterial strain, and the putative fusion protein was also demonstrated in lysates made from this strain. The protein reacted with both anti-β-galactosidase and anti-DBH antiserum in Western blots.

Work is in progress to verify by other methods that the 250 bp insert does, in fact, code for DBH. Once verified, this probe will allow examination of regulatory changes in DBH at the transcriptional and genomic levels, and to isolate a full-length cDNA for the gene.

226.3 BOVINE ADRENAL MEDULLARY DOPAMINE β-HYDROXYLASE SUBUNIT PRIMARY STRUCTURE. D. L. Wong. Dept. of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305.

Soluble bovine adrenal medullary dopamine β-hydroxylase (DBH) consists of three non-identical subunits, α, β and γ, with relative molecular weights of 71, 75 and 78 kD. These are present at a ratio of 1:2:1 and have been purified to homogeneity by sucrose density sedimentation, gel filtration chromatography, affinity chromatography, and preparative SDS-polyacrylamide gel electrophoresis. When the purified subunits are treated with endoglycosidase F, which removes high and complex mannans, a single molecular weight species of 66 kD is generated for each. Intermediate deglycosylation products include species with molecular weights identical to the lower molecular weight subunits and a 68 kD species as well. Lectin blotting shows that concanavalin A (Con A) reacts with the subunits and their partial deglycosylation products confirming that carbohydrate substitution consists primarily of high and complex mannans. Con A reactivity decreases with decreasing molecular weight until the 66 kD product shows no lectin binding. Immunoblotting with a rabbit polyclonal antisera indicates that the subunits and their deglycosylation products are immunoreactive. The antisera must therefore recognize epitopes specific to the primary structure of the enzyme, and the polypeptide backbones of the subunits must be very similar. Amino terminal sequencing of the purified enzyme shows, as suggested by others, that the native protein consists of two non-identical polypeptide chains which are very similar if not identical with the exception that one chain has three additional amino acids at its N-terminus. We have generated three families of polypeptides for the subunits by cyanogen bromide cleavage, formic acid cleavage and proteolytic cleavage with an arginine specific protease. The cyanogen bromide fragments range in molecular weight from 8.8 to 24.5 kD; the formic acid peptides range in size from 16.5 to 68.0 kD; and the arginine cleavage products range in size from 12.0 to 52.5 kD. Immunoreactivity of the cleavage products varies. For the CNBr fragments, only those peptides greater than 11.9 kD are immunoreactive. All of the formic acid peptides are highly immunoreactive although they do not stain well with silver, while none of the arginine cleavage products are immunoreactive. Each set of cleavage products thus appears to generate distinct peptides. Some of these may contain epitopes unique to DBH based on their immunostaining. Furthermore, the various cleavage products may provide us with overlapping peptide fragments. Cyanogen bromide peptides have already been purified preparatively and are currently being sequenced. Sequencing of various overlapping fragments should permit us to piece together the polypeptide backbones for the two nonidentical but very similar polypeptides of DBH.

226.4 **MECHANISM OF ACTIVATION OF TYROSINE HYDROXYLASE.** *C. Abate and T. H. Joh.* Laboratory of Molecular Neurobiology, Cornell Univ. Med. Coll., New York, N.Y. 10021.

Tyrosine hydroxylase (TH), the first and rate limiting enzyme in catecholamine biosynthesis, can be activated by mild proteolysis with trypsin. Trypsin-digested TH (tTH) is a 34 KD fragment that retains catalytic activity and thus can be defined as the catalytic domain of the enzyme. We have previously reported (Abate et al., *Soc. Neurosci. Abst. 12*:601, 1986) that the catalytic domain as represented by tTH extends from amino acid 158 to approximately 454 in the native sequence. This region does not include regulatory phosphorylation sites identified by Campbell et al. (*J. Biol. Chem., 261*:10489, 1985). We sought to characterize the mechanism of activation of TH by comparison of activation by proteolytic removal of the regulatory region with that produced by modification of this region by phosphorylation.

The native form of TH (nTH) was partially purified from rat caudate nuclei as previously described (Joh et al. *PNAS 75*:4744, 1978). tTH was generated by incubation of nTH with 0.2mg/ml of trypsin for 7 min at 30°C; proteolysis was assesed by Western blot analysis. Alternatively, nTH was subjected to phosphorylating conditions in the presence of cAMP-dependent protein kinase. Initial velocity patterns of nTH, tTH and phosphorylated native TH (pTH) were obtained by incubation of enzyme with varying concentrations of cofactor $6MPH_4$ or varying concentrations of substrate tyrosine. Enzyme activity was determined by the method of Coyle (*Biochemical Pharm. 21*:1935, 1972).

As an index of activation, the kinetic parameters of tTH and pTH were assesed. Lineweaver-Burke plots of activity vs. $6MPH_4$ indicate that tTH and pTH share the same K_m for cofactor (1.0×10^{-4} M) and this value is 20 fold lower than that of the native enzyme (2.0×10^{-3} M). In contrast, the K_m of tTH for substrate tyrosine (2.6×10^{-5} M) is 4 fold lower than either the non-phosphorylated or phosphorylated enzyme (9.0×10^{-5} M). In order to determine if digestion to 34 KD is required for activation, nTH was subjected to limited proteolysis in the presence of 10 fold lower conc. of trypsin (0.02mg/ml) for 0 - 30 min. The pattern of proteolysis, identified by Western blot analysis, indicates that under these conditions the enzyme is sequentially digested from 60 KD to 58, 57, and 56 KD; the 56KD band is predominant at 30 minutes. Enzyme activity of TH subsequent to 30 min digestion is 175% of control, non-digested enzyme when assayed in the presence of 1×10^{-3} M $6MPH_4$ and 1×10^{-4} M tyrosine. However, in the presence of 2.5×10^{-5} M tyrosine (1×10^{-3} M $6MPH_4$) activity is 300% of control and in the presence of 1×10^{-4} M $6MPH_4$ (1×10^{-4} M tyrosine) activity is 600% of control. This suggests that proteolysis of to 56 KD results in an increase in affinity for both substrate and cofactor, similiar to the effect produced by more extensive proteolysis to 34 KD.

Thus, proteolysis of TH results in an increase in the affinity of the enzyme for both substrate tyrosine and cofactor $6MPH_4$. This effect corresponds to proteolytic removal of 4000 Daltons which presumably includes N-terminal phosphorylation sites. These data suggest that the mechanism of activation of TH entails modification of a regulatory domain that partially impedes access of substrate and cofactor to the catalytic site. Reversible modification by phosphorylation selectively increases access of cofactor without altering substrate binding.

226.5 **PHOSPHORYLATION SITES ON TYROSINE HYDROXYLASE IN PC12 CELLS.** J.P. Mitchell* and P.R. Vulliet. Department of Physiology, Colorado State University, Fort Collins, CO 80523.

Rat PC12 cells were used to study phosphate incorporation into specific sites on tyrosine hydroxylase (TH) in response to KCl depolarization or nerve growth factor (NGF). PC12 cells were preincubated at 37°C in Krebs-Ringer-HEPES (KRH) buffer containing 0.25 mCi/ml ^{32}P-orthophosphate (carrier-free) to prelabel intracellular ATP stores. After one hour, the ^{32}P-containing KRH buffer was removed and replaced with either KRH buffer alone (control), KRH buffer containing 56 mM KCl (NaCl content reduced to maintain constant tonicity), or KRH buffer containing 50 ng/ml NGF. After 10 minutes, buffers were removed and cells were lysed by adding 200 ul of ice-cold lysis buffer (50 mM tris-acetate, 0.27 M sucrose, 100 mM NaF, 1 mM EDTA, 4 ug/ml leupeptin, 0.2% triton X-100, pH 8.0). Lysates were centrifuged for 5 minutes at 13,000 x g. The TH activity of the supernatants was measured by a coupled decarboxylase procedure. Supernatants were mixed with SDS-sample buffer, heated at 95°C for 5 minutes, and proteins separated on 10% SDS-polyacrylamide gels. Proteins were electrophoretically transferred to nitrocellulose and autoradiographs were made from the nitrocellulose sheets. The major phosphorylated band at 60 kDa, shown previously to be predominantly TH, was cut from the nitrocellulose and digested with trypsin. Over 90% of the radioactivity was released from the nitrocellulose by trypsin. These tryptic phosphopeptides were analysed by isoelectric focusing (pH 2.5-10) and compared to tryptic phosphopeptide standards obtained from purified TH labeled by purified protein kinases (Ca^{++}/calmodulin-dependent and cAMP-dependent kinases).

Both KCl depolarization and NGF treatment increased TH activity in the supernatant; however, these increases in enzyme activity were characterized by different kinetic parameters for each treatment. Total phosphate incorporation into TH increased with both treatments. Both treatments increased phosphorylation of peptide C, previously identified as the serine 19-containing tryptic phosphopeptide. Two additional peptides had increased phosphate incorporation when compared to peptides isolated from control cells. The relative degree of phosphorylation of these three peptides was different for KCl depolarization and NGF treatment, possibly explaining the differences seen in the enzyme kinetics.

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226.6 THE PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN PC12 CELLS AFTER DOWN REGULATION OF PROTEIN KINASE C. A.L. Cahill and R.L. Perlman, Kennedy Mental Retardation Research Center and Departments of Pediatrics and of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637

Treatment of the superior cervical ganglion (SCG) or PC12 pheochromocytoma cells with a variety of agonists, including phorbol esters, cAMP analogues, cholinergic agonists, and depolarizing agents, increases the phosphorylation of tyrosine hydroxylase (TH) in these tissues. TH can be phosphorylated in vitro by a number of different protein kinases, including the cAMP- and cGMP-dependent protein kinases, a type II Ca^{2+}/calmodulin-dependent protein kinase, and protein kinase C. We have been interested in determining the roles of these various protein kinases in regulating the phosphorylation of TH in situ. One approach to this problem is to analyze the site-specific phosphorylation of TH in response to various agonists. Six different tryptic phosphopeptides can be isolated from TH from the SCG and from PC12 cells. One of these peptides, known as peptide T3, has been reported to contain a specific protein kinase C phosphorylation site (McTigue et al., J. Biol. Chem. 260, 9047-9056, 1985). In the SCG, phosphorylation of this peptide was increased only by treatment with phorbol dibutyrate (PDBu). In PC12 cells, however, phosphorylation of this peptide was increased not only by phorbol esters, but also by treatment with cAMP analogues, depolarizing agents, and NGF. These results imply that all of these agents may activate protein kinase C in PC12 cells. To investigate the role of protein kinase C in the phosphorylation of TH in PC12 cells, we have depleted cells of protein kinase C and then compared the basal and stimulated phosphorylation of TH in these cells to that in control cells. PC12 cells were depleted of protein kinase C by incubation for 24 h with 1 μM PDBu (Matthies et al., J. Neurosci. 7, 1198-1206, 1987). After 24 h the cells were washed to remove PDBu, labeled with $^{32}P_i$, and then treated with the desired agents. ^{32}P-labeled TH was isolated by SDS-PAGE and subjected to complete tryptic hydrolysis. ^{32}P-labeled phosphopeptides were separated by two-dimensional thin-layer electrophoresis and chromatography and visualized by autoradiography. In protein kinase C-depleted cells, basal phosphorylation of peptide T3 was reduced, and treatment with PDBu did not increase the phosphorylation of peptide T3. Down regulation of protein kinase C appears to provide a useful technique for studying the role of this kinase in the phosphorylation of TH.

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226.7 TYROSINE HYDROXYLASE IS AN INTEGRAL COMPONENT OF SECRETORY GRANULES FROM ADRENAL MEDULLA. D.M. Kuhn. Lafayette Clinic, 951 E. Lafayette, Detroit, MI 48207

Intact secretory granules isolated from bovine adrenal medulla express tyrosine hydroxylase (TH) activity. Membrane ghosts prepared from granules retain TH activity while the granule contents are free of hydroxylase activity. When granule membrane proteins are resolved on SDS-polyacrylamide gels and transferred to nitrocellulose, antibodies directed against TH recognize a protein which is slightly lower in molecular weight than TH from PC12 cells but which can be positively identified as TH by various criteria. TH catalytic activity and immunoreactivity can be removed from granule membranes by detergent treatment and by increasing the pH of a ghost preparation but not by increasing ionic strength. TH also remains associated with ghost membranes after treatment with a phosphatidylinositol-specific phospholipase. Trypsin treatment of intact granules or granule membranes releases only small amounts of TH immunoreactivity but causes the total loss of catalytic activity. Biotinylation of intact granules or exposure to the catalytic subunit of cAMP-dependent protein kinase in the presence of (γ-^{32}P)ATP in order to label proteins on the cytoplasmic surface, followed by immunoprecipitation of TH, reveals that TH is apparently labelled by both procedures. These results indicate that TH is an integral component of the granule membrane. Furthermore, TH appears to be imbedded in the cytoplasmic side of the membrane where it is exposed to its substrates and other cytoplasmic and membrane constituents. Plasma membranes were also purified from adrenal medulla and were found to contain both TH immunoreactivity and enzyme activity. Taken together, these results suggest that TH becomes a part of the plasma membrane during exocytosis-induced granule fusion. Furthermore, membrane bound TH may serve as a marker of exocytosis in the adrenal medulla.

226.8 **TUMOR FORMATION AND DOPAMINE PRODUCTION BY PC12 CELLS ON EHS-DERIVED EXTRACELLULAR MATRIX.** C. L. Bethea and T. K. Borg*. Division of Reproductive Biology and Behavior, Oregon Regional Primate Research Center, Beaverton, OR 97006 and Department of Anatomy, Univ. of South Carolina School of Medicine, Columbia, SC 29208.

We previously reported that PC12 cells flatten extensively, increase dopamine secretion, and decrease dopamine storage when cultured on extracellular matrix (ECM) produced by bovine corneal endothelial cells (*Mol. Cell. Endocrinol.* 37:319, 1984). Here, the effect of EH mouse sarcoma-derived (EHS) ECM (Kleinman et al., *Biochem.* 25:312, 1986) on PC12 cell morphology, growth, and dopamine production was examined. PC12 cells (10^5) were plated onto plastic and 4 thicknesses of EHS-ECM. Variable thickness of the ECM was achieved by aliquoting 100, 150, 200 or 250 μl of EHS gel extract into 35 mm dishes. Phase contrast micrographs and scanning electron micrographs obtained on days (D) 2, 4, 6 and 8 after plating showed that cells on plastic grew into a single-layered lawn of cells, whereas cells on 200-250 μl of EHS-ECM grew into large tumors. PC12 cells on 150 μl of ECM first grew into tumors, but by day 8 they appeared to dissolve the ECM and then migrate out from the tumors. One hundred μl of EHS gel did not distribute evenly and intermediate regions of cell piling were observed.

^{3}H-thymidine (THY) incorporation for 30 min, dopamine secretion (ng/ml) and cell content (ng) were then compared between cells cultured on plastic and 250 μl EHS-ECM (thickest). Dopamine (DA) levels were determined with a catechol-o-methyl transferase radioenzymatic assay (2X blank = 40 pg). The results of one experiment are shown below. (n = 3/group)

		D2	D4	D6	D8
^{3}H-THY x 10^3	EHS	4.5	11.0	52.5	164.8
	Plastic	34.6*	46.4*	143.3*	267.6*
DA(ng/ml)	EHS	nd	nd	1.1	3.9
	Plastic	nd	nd	3.1*	7.9*
DA(ng)	EHS	1.5	4.8	20.6	58.1
	Plastic	4.7*	14.1*	55.8*	119.6*

*p<0.05; nd=nondetectable.

Dopamine secretion and content are reduced in EHS-ECM cultures, but this can be attributed to a decrease in cell number. DA/ ^{3}H-THY yields similar ratios for EHS-ECM and plastic on D 4, 6 and 8. Visual examination and ^{3}H-THY incorporation suggest that fewer cells survive plating on EHS-ECM than plastic, but the subsequent doubling times appear comparable. Norepinephrine was undectable in either group with the radioenzymatic assay (2X blank = 83 pg). Therefore, these data suggest that, although EHS-ECM alters plating viability, cell-cell and cell-substratum association, this may not change dopamine production on a per cell basis.

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226.9 POSSIBLE ORIGIN OF SULFOCONJUGATED CATECHOLAMINES IN THE PLASMA: THE PERIPHERAL SYMPATHETIC NEURONS. N.T. Buu, W. Debinski*, C. Lussier*, and O. Kuchel*. Lab. of the Autonomic Nervous System, Clinical Research Institute of Montreal, Montreal, Quebec H2W 1R7, Canada.

The origin of plasma sulfoconjugated catecholamines (CA), the predominant (90 percent) fraction of CA in human blood remains unknown. Although sulfoconjugating enzymes which participate in maintaining CA homeostasis, are present in most endocrine tissues (e.g. kidney, heart, adrenal glands) and in blood platelets, recent studies have discounted these tissues as the sources of conjugated CA in the plasma. On the other hand, except for epinephrine, a major portion of plasma CA is derived from the peripheral sympathetic neurons but whether these sympathetic neurons can also be the origins of plasma sulfoconjugated CA remains undetermined.

This study investigates the sulfoconjugation activity in the peripheral sympathetic neurons by measuring the formation of dopamine (DA) sulfate following L-dopa administration in richly innervated tissues of the superior cervical ganglia (SCG), vas deferens and spleen of adult Sprague Dawley control rats and rats pretreated with pargyline, an inhibitor of monoamine oxidase (MAO). Free and sulfoconjugated CA were measured by HPLC with electrochemical detection.

In control rats L-dopa administration led to a significant elevation of DA sulfate in the plasma and tissues. In the SCG the concentrations of DA sulfate became equal to that of free DA (9.9 ± 1.5 ng/pair of ganglia) while in the spleen and vas deferens they accounted for 10 percent of the total DA. In MAO-inhibited rats the same L-dopa treatment caused a two-fold increase in DA sulfate in SCG and a five-fold increase in the other tissues but the concentrations of free DA also doubled.

The results indicated that DA can also undergo sulfoconjugation in the peripheral sympathetic neurons, and this sulfoconjugation, like that in the brain (J. Neurochem. 45:470, 1985), appeared to be regulated by MAO. Thus, the peripheral sympathetic neurons which release the major fraction of free CA into the blood, may also be the major sources of plasma sulfoconjugated CA.

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226.10 EXTRANEURONAL SYNTHESIS OF EPINEPHRINE IN BRAIN. I.N. Mefford*, G.M. Foster*and N.K. Garrick (SPON: J.M. Tolliver) Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20892.

Pharmacological evidence supports the view that epinephrine in hypothalamus and perhaps in other brain regions is not synthesized in catecholamine neurons but is formed in some extraneuronal element. The bulk of epinephrine found in rat brain appears to be stored in noradrenergic nerve terminals. Immunohistochemical data suggest that neurons or other cells which contain phenylethanolamine N-methyltransferase (PNMT) in the forebrain do not contain other biosynthetic enzymes, tyrosine hydroxylase and/or dopamine--hydroxylase. Destruction of ascending projections from the A1, A2 and C1, C2 cell body groups or destruction of hypothalamic norepinephrine terminals using 6-hydroxydopamine cause depletion of both norepinephrine and epinephrine, but no decrease in PNMT activity. Stimulation of the C1 area results in marked efflux of epinephrine into extracellular space but no change in extracellular norepinephrine concentration in posterior hypothalamus. This suggests that an N-methyltransferase provides a metabolic barrier to the accumulation of norepinephrine in extracellular space in this brain region.

Two approaches have been taken to address the source and mechanism of this apparent rapid conversion of norepinephrine to epinephrine. Cervical spinal fluid was continuously collected from chair adapted Rhesus monkeys prior to and during infusion of anesthetics and other drugs. Samples were analyzed by microbore HPLC with amperometric detection for epinephrine, norepinephrine and dopamine. Rapid and marked enhancement of epinephrine in cerebrospinal fluid was observed following only a modestly sedating dose of pentobarbital (6 mg/kg i.v.).

The possibility that the extraneuronal synthesis of epinephrine could occur in non-neuronal elements such as glia was examined in astrocyte cultures. The capacity to N-methylate norepinephrine was observed in astrocyte cultures prepared from several brain regions including hypothalamus and cerebral cortex. Using norepinephrine as substrate and S-adenosyl methionine as cofactor, enzyme activity and specificity were compared to adrenal PNMT. Immunocytochemical experiments are presently under way to further characterize this enzyme. The presence of a norepinephrine N-methylating enzyme in astrocytes provides an efficient mechanism for rapid extraneuronal conversion of norepinephrine to epinephrine, perhaps providing a metabolic and diffusional barrier between released norepinephrine and extrajunctional alpha-2 adrenoreceptors at which epinephrine may be the primary agonist.

226.11 ADRENERGIC PROPERTIES OF 2- AND 6-FLUOROEPINEPHRINES. C. Creveling, F. Gusovsky, A. Adejare*, J. Daly, and K. Kirk*. Laboratories of Chemistry and Bioorganic Chemistry, NIDDK, NIH, Bethesda, Maryland 20892.

We have extended our studies on the effects of aromatic fluorine substitution on the chemical and biological properties of catecholamines to epinephrine. 2- and 6-Fluoroepinephrine (2-FEpi, 6-FEpi) were synthesized by a sequence of N-formylation, hydride reduction and hydrogenolysis of previously synthesized fluorinated dibenzyloxyphenethanolamines. Similar to the dramatic change in adrenergic selectivity seen with norepinephrine (Science, 204, 1217, 1979; J. Med. Chem. 22, 1493, 1979), fluorine substitution on the 2- or 6-carbon of the aromatic ring alters the selectivity of epinephrine towards alpha- and beta-adrenergic receptors. Thus, 2-FEpi is a relatively specific beta-adrenergic ligand while 6-FEpi is a relatively specific alpha-adrenergic ligand. However, unlike the effect on norepinephrine but similar to the effect on phenylephrine (J. Med. Chem. 29, 1982, 1986) fluorine substitution can markedly increase the potency of epinephrine as well as induce selectivity. Thus 2-FEpi shows a 3-fold increase in affinity relative to epinephrine for beta-receptors as well as a greatly reduced affinity towards alpha receptors. 6-FEpi, on the other hand, not only has a greatly reduced affinity for beta-receptors, but shows a 3-fold increase in affinity towards alpha-1 receptors. 6-FEpi is equipotent with epinephrine toward alpha-2 receptors. Thus the increase in potency observed with 6-FEpi is specific for alpha-1 receptors. We previously reported that the alpha selective agonist 6-fluorophenylephrine showed a 2-fold increase in potency relative to phenylephrine for both alpha-1 and alpha-2 receptors.

DISPLACEMENT OF ALPHA- AND BETA-SPECIFIC LIGANDS FROM RECEPTORS IN MEMBRANES FROM RAT CEREBRAL CORTEX.

	Alpha-1	Alpha-2	Beta
		-(IC_{50})-	
Agonist	[^{3}H]WB4101	[^{3}H]Clonidine	[^{3}H]Dihydroalprenolol
(-)Epi	4.8uM	9.1nM	6.0uM
(±)2-FEpi	76.	110.	3.5
(±)6-FEpi	3.2	14.	130.

226.12 NEUROTRANSMITTER INDUCED MODIFICATION OF ARACHIDONATE TURNOVER IN CEREBRAL PHOSPHOLIPIDS. L.R. Murthy* and A.I. Barkai*, (spon. W.C. Clark), N.Y.S. Psych. Inst. and Columbia Univ. Coll. P & S., New York, N.Y. 10032.

Arachidonic Acid (AA), the major precursor of eicosanoids, plays an active role in the deacylation-reacylation cycle of membrane phospholipids. This cycle regulates the levels of lysophospholipids, free fatty acids and eicosanoids, which may alter membrane functions and affect neuronal signal transduction. The present study was undertaken to investigate the effects of norepinephrine (NE) and serotonin (5-HT) on AA turnover in cerebral phospholipids. Cross chopped slices of cerebral cortex were incubated for 30 min, with or without varying concentrations of NE, or 5-HT. ^{3}H-AA, ATP, CoA and Mg^{++} were then added and the incubation was continued for additional 5 min. The reaction was stopped by addition of chloroform: methanol (1:2) and lipids were extracted and seperated by HPTLC for radioactive analysis. Incorporation data revealed a dose related increase of ^{3}H-AA in phosphatidylinositol (PI), but a decrease in other phospholipids. The NE effect on PI was attenuated by mepacrine, a phospholipase A_2 inhibitor and also by acyltransferase inhibitors such as indomethacin and tetrahydrocannabinol. The results suggest a phospholipase A_2 activiation by NE with, a concomitant stimulation of a specific lysophosphatidylinositol acyltransferase.

Supported by NIMH grant 33690 to A.I.B

227.1 **CHOLINERGIC VASOPRESSOR MECHANISM IN ROSTRAL VENTROLATERAL MEDULLA IS MEDIATED BY THE M_2 MUSCARINIC RECEPTOR SUBTYPE.** *R. Giuliano, P. Ernsberger, and D.J. Reis.* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

In vitro binding, autoradiographic and functional studies support the existence of subclasses of muscarinic acetylcholine receptors (mAChR) in peripheral tissues and regions of CNS. Recent studies have demonstrated a cholinergic pressor mechanism in the rostral ventrolateral medulla (RVL), a brainstem region containing adrenaline (C1) neurons, which is critically involved in cardiovascular control (*JPET* 231:457, 1984; *Soc. Neurosci. Abstr. 12*:964, 1986). We sought to (1) characterize the binding properties of mAChR in the RVL and (2) define the mAChR subtype which mediates pressor responsiveness to cholinergic activation of the RVL.

Radioligand binding assays were carried out using membranes prepared from ventrolateral medulla of bovine brain and incubated with ^{3}H-quinuclidinyl benzylate (^{3}H-QNB) in Tris buffer (60 min at 25°C). Nonspecific binding was defined by 1μM atropine. ^{3}H-QNB specifically labeled numerous sites (B_{max} = 183 ± 22 fmol/mg protein) with high affinity (K_D = 88 ± 11 pM). In competition experiments against ^{3}H-QNB (70 pM) the following agents were tested: oxotremorine (OXO), the M_1- selective agonist pilocarpine (PILO), scopolamine (SCOP), the novel M_2- selective antagonist AF-DX 116 and the M_1-selective antagonist pirenzepine (PZ). The order of potency for inhibition of ^{3}H-QNB binding was, for agonists: OXO >> PILO, and for antagonists: SCOP > AF-DX 116 = PZ.

In anesthetized, paralyzed and ventilated rats i.v. physostigmine (PS) produced a marked, reproducible rise in AP (44 ± 2 mmHg; n=14). Antagonism of the pressor response to this fixed dose of PS was determined in rats microinjected bilaterally into the C1 area (50 nl) with SCOP (3 pmol to 3 nmol), AF-DX 116 (3 pmol to 3 nmol) or PZ (15 pmol to 3 nmol). Initial resting AP fell after administration of 0.03 nmol AF-DX 116 (23 ± 7 mmHg, n=4). At a dose 100-fold greater (3.0 nmol) SCOP produced a comparable decrease (24 ± 2 mmHg, n=8), but PZ had no effect (1 ± 4 mmHg, n=10). AF-DX 116 inhibited the PS-induced rise in AP in the same dose range as SCOP (% inhibition at 0.3 nmol: 47 ± 12, n=6 vs. 50 ± 10, n=5, respectively, and at 3.0 nmol: 85 ± 10, n=5 vs. 81 ± 3, n=6, respectively) ($p < 0.001$). PZ was ineffective even at 3 nmol (% inhibition: 1 ± 13, n=6). Bilateral microinjection of PILO into the C1 area (10 nmol) had no effect on AP (increase of 7 ± 2 mmHg, n=4), whereas local administration of OXO evoked a dose-related increase (threshold dose, 2 pmol; maximal effect at 25 nmol, 50 ± 3mmHg, n=5). SCOP and AF-DX 116 antagonized the rise in AP elicited by OXO, inhibiting the pressor response to 1 nmol (45 ± 6 mmHg, n=11) approximately 50% at 0.015 nmol SCOP (58 ± 9%, n=5) and 0.15 nmol AF-DX 116 (55 ± 16%, n=4) ($p < 0.001$). PZ exhibited lower potency, antagonizing the OXO pressor effect (59 ± 4%, n=4) at 0.75 nmol, a dose 50 or 5 times higher than that of SCOP or AF-DX 116, respectively.

We conclude that (1) mAChR, predominantly the M_2 subtype, are present in RVL; (2) the muscarinic agonist OXO, but not PILO, binds with high affinity to the M_2 mAChR in RVL; (3) the pressor effect of systemically administered PS or locally-injected OXO is mediated by the M_2 mAChR in RVL; and (4) M_2 mAChR in RVL have an important role in central cardiovascular control.

227.2 **CHOLINERGIC-AUTONOMIC REGULATION: ANATOMICAL SUBSTRATES.** *D.A. Ruggiero, R. Giuliano, R. Stornetta, M. Anwar* and D.J. Reis.* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

While there is ample physiological and pharmacological evidence for cholinergic (Ch) control of arterial pressure (AP) and vasopressin release (e.g., by rostral ventrolateral medulla (RVL) or paraventricular nucleus of hypothalamus (PVN)), the anatomical substrates are poorly understood. The presence of cholinergic or cholinoceptive elements in central autonomic nuclei, or the pathways involved, are either unknown or have not been studied in detail. In this study we sought to define regions in the CNS where cholinergic interactions with central autonomic neurons occur. Rats were anesthetized (Nembutal, 0.4 ml i.p.; 50 mg/ml) and perfused (4% para formaldehyde in 0.1M phosphate buffer, pH 7.4); 25μm sections were processed using antisera against choline acetyltransferase (ChAT), the acetylcholine synthesizing enzyme, by the peroxidase-antiperoxidase technique. The use of a sensitive immunocytochemical procedure combined with darkfield illumination revealed new groups of cholinergic perikarya and terminal fields in several brainstem and spinal autonomic substructures.

In **hypothalamus,** ChAT-positive terminals (but not perikarya) were distributed to discrete autonomic nuclei (zona incerta, dorsal, lateral, posterior and subparafascicular nuclei). No terminals were labeled in PVN. In **pons,** stained cells and terminals occurred in n. Koelliker-Fuse (KF); terminals also occupied dorsal and medial divisions of parabrachial n., periventricular gray (PVG) (excluding locus ceruleus) and raphe. In **medulla,** cells and processes occurred in medial and commissural divisions of n. tractus solitarii. Cells were labeled in raphe obscurus and magnus, PVG and RVL. Cells in RVL lay in proximity to the ventral surface and medial to adrenergic perikarya of the C1 area. In RVL, terminals, of unknown origin, and dendrites of the retrofacial nucleus, were adjacent to or overlapped C1 neurons. In **spinal cord,** ChAT-labeled processes overlapped Ch-sympathetic preganglionic neurons (SPN's) of the intermediomedial and intermediolateral cell columns; processes also occurred in dorsal and ventral horns. Since Ch-perikarya occur in brainstem areas known to project to SPN's, we sought to determine the source(s) of supraspinal Ch-afferents to the cord. WGA-HRP was injected into the thoracic cord and sections reacted with tetramethylbenzidine and processed immunocytochemically for ChAT and phenylethanolamine N-methyltransferase (PNMT, the adrenaline synthesizing enzyme). After thoracic injections, neurons were retrogradely labeled in nuclei containing ChAT- and PNMT-positive cells. No cells were labeled for both ChAT and WGA-HRP; in contrast many cells, concentrated in the RVL, were dually-labeled for PNMT and WGA-HRP.

In conclusion: 1) Ch-cells and terminals or a comingling of both elements occurred differentially in areas known to regulate AP; 2) The absence of Ch-terminals in PVN suggests that Ch-control of vasopressin may be mediated by adjacent cells or by synapses on processes lying external to this nucleus; 3) Newly identified groups of Ch-cells suggest additional substrates for Ch-control of autonomic functions; 4) Ch-afferents on SPN's may be of intrinsic origin. These data, collectively, suggest that Ch-control of cardiovascular functions involves multiple sites throughout the neuraxis.

227.3 **CHOLINE ACETYLTRANSFERASE IN THE RAT ROSTRAL VENTROLATERAL MEDULLA: ULTRASTRUCTURAL LOCALIZATION AND SYNAPTIC INTERACTIONS WITH NEURONS CONTAINING CATECHOLAMINE-SYNTHESIZING ENZYMES.** *T.A. Milner, V.M. Pickel, C. Abate and D.J. Reis.* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Pharmacological and biochemical studies suggest interactions involving cholinergic and catecholaminergic neurons, particularly those of the C1 adrenergic cell group in the rostral ventrolateral medulla (RVL) that have been strongly implicated in cardiovascular control. Ultrastructural localization of choline acetyltransferase (CAT) and its relation to neurons exhibiting immunoreactivity for tyrosine hydroxylase (TH) or phenylethanolamine N-methyltransferase (PNMT) were examined in the RVL using dual immunoautoradiographic and peroxidase anti-peroxidase labeling methods to simultaneously localize antisera from two different species.

By light microscopy, the CAT-immunoreactive cells were located both dorsally (i.e., the nucleus ambiguus) and ventromedially to those labeled with TH or PNMT. A few CAT-labeled processes were dispersed among TH- or PNMT-containing neurons with the majority of overlap immediately ventral to the nucleus ambiguus. By electron microscopy, CAT-immunoreactivity was detected in neuronal perikarya, dendrites and axon terminals and in the vascular endothelial cells of certain blood vessels. The CAT-labeled perikarya were medium sized (15-25μm) and contained abundant cytoplasm and a slightly indented nucleus. Synaptic junctions on CAT-immunoreactive perikarya and dendrites were primarily symmetric with 75% (45 out of 60) of the terminals unlabeled. The remaining terminals were immunoreactive for CAT (18%) or TH/PNMT (7%). Terminals with CAT-immunoreactivity were large (0.8-2.0μm) and contained numerous small clear vesicles and 1-3 dense-core vesicles. Seventy seven percent (112 out of 145) of CAT-labeled terminals formed symmetric synapses with unlabeled perikarya and dendrites; whereas only 8% were with TH- or PNMT-labeled perikarya and dendrites and 15% were with CAT-immunoreactive perikarya and dendrites. Additionally, 60% of the processes immunoreactive for CAT or TH/PNMT, although seen in the same field, were without any apparent synaptic or cellular interrelations. The proportionally small number of observed (symmetric) synapses (in both directions) involving CAT and TH- or PNMT-containing neurons suggests that cholinergic and adrenergic neurons may exert reciprocal inhibition of each other and thus may modulate their response to the more abundant input from unlabeled afferents in the rat RVL. Furthermore, the detection of CAT-immunoreactivity in vascular endothelial cells is analogous to that previously reported in certain cortical and peripheral blood vessels. The results therefore suggest that cholinergic regulation of arterial pressure through the RVL may involve local interneurons. (Supported by NIH grant HL 18974.)

227.4 **GLUTAMATE RECEPTOR ANTAGONIST BLOCKS THE RESPONSES OF SYMPATHETIC PREGANGLIONIC NEURONS (SPN) TO STIMULATION IN THE C1 AREA OF THE ROSTRAL VENTROLATERAL MEDULLA (RVL).** *S. F. Morrison and D. J. Reis,* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, N.Y. 10021.

In the rat, the region of the RVL containing the C1 adrenergic cell group (C1 area) is the site of sympathoexcitatory neurons that project to the spinal sympathetic intermediolateral nucleus (IML). The projection is over myelinated and unmyelinated axons with conduction velocities comparable to those estimated for the PNMT containing axons of the lateral funiculus of the spinal cord (Soc. Neurosci. Abstr. 12:1157, 1986). In the present study, we sought to characterize the responses of SPN's to RVL stimulation and to determine if these responses were mediated by activation of glutamate receptors on SPN's. Electrical stimuli (80μA, .8ms, .4Hz) were applied to the RVL of rats anesthetized with urethane, paralyzed, and artificially ventilated. Sympathoexcitatory responses evoked in the preganglionic splanchnic sympathetic nerve consisted of two peaks of excitation with latencies of 61 ± 2 ms and 142 ± 8 ms (n = 8). Both peaks in the sympathetic response to RVL stimulation were abolished by injection of kynurenic acid (25 nmol) into the central canal at the level of the calamus scriptorius, but were unaffected by similar application of the NMDA receptor antagonist, AP5. The SPN's contributing to the splanchnic nerve were antidromically identified in the IML of T7-T9 using stimuli applied to their axons in the splanchnic nerve. The mean antidromic response latency of 42 splanchnic SPN's was 27 ms (range 5 - 51 ms). Individual splanchnic SPN's responded to RVL stimulation in one of three ways: (a) an early activation with a modal response latency of 28 ± 2 ms (n = 12); (b) a late activation with a modal response latency of 112 ± 4 ms (n = 6); or (c) both an early and late excitation with similar latencies to (a) and (b) (n = 5). Both the early and the late excitations of splanchnic SPN's were antagonized in a current-dependent manner by iontophoretically (40-100nA, .2M) applied kynurenic acid. We conclude that both the rapid and slowly conducting sympathoexcitatory pathways from the RVL to the IML produce their effects through activation of glutamate receptors on SPN's which appear to be of the quisqualate or kainate type. These results raise the possibilities (a) that glutamate may be released by the terminals of C1 adrenergic neurons that project from the RVL to the IML or (b) that this sympathetic pathway involves a glutamatergic spinal interneuron.

227.6 CAUDAL VENTROLATERAL MEDULLARY PROJECTIONS TO THE C1 CELL GROUP. L. Jeske and D.O. Nelson, Dept. of Physiology, Northwestern Medical School, Chicago, IL 60611

Noradrenergic projections, arising from the A1 cell group in the caudal ventrolateral medulla (CVLM), are thought to innervate neurons residing in the C1 region of the rostral ventral lateral medulla (RVLM). This projection has been implicated in baroreflex and tonic control of sympathetic activity. In this study we examined the neuroanatomical connections between the CVLM area and the pressor region of the C1 cell column located in the paragigantocellularis area (PGCL) and the paraventricular nucleus (PVN) using a combination of retrograde fluorescent tracers and immunohistofluorescence. Rhodamine (50 nl/25% vol) or fluorogold (100 nl/4% vol) was stereotaxically injected into the PGCL region or the PVN of ketamine-xylazine anesthetized male rats through a glass micropipette (tip diameter 10-50 μm) connected to a custom micropressure injection system. Following a 4 day to 2 week recovery period, brain tissue was processed with fluorescein isothiocyanate (FITC) immunohistofluorescence for dopamine-ß-hydroxylase (DBH) and examined under epifluorescence for double labelling. Labelling was quantitated by carefully counting and mapping the location of retrograde and double labelled neurons in each brain section from 1.5 mm caudal to obex to the level of obex. Less than 5% of the noradrenergic neurons comprising the A1 cell column contained retrograde label after an injection into the PGCL region. The few double labelled neurons were scattered along the length of the A1 column with no apparent rostral-caudal organization. However, intense retrograde staining was evident caudal to obex in a column of cells lying medial and dorsal to DBH reactive cells. Heavy retrograde labelling was also observed at several other medullary and hypothalamus sites, including the PVN. In contrast to the A1 cell group, double labelling of approximately 75% of the A5 and A6 cell groups was observed following retrograde tracer injection into the RVLM. Injection of tracer into the PVN resulted in double labelling of approximately 90% of the CVLM noradrenergic cells in the entire rostral-caudal extent of the A1 cell column. A number of retrograde labelled non-noradrenergic neurons were located dorsal and medial to the A1 cell column in the area where neurons projecting rostral to the PGCL region were found. This region located dorsal-medial to the A1 cell group in the CVLM was found in our previous studies (Fed. Proc, vol 46:1243, 1987) to contain bulbospinal neurons. Thus cells in this region give rise to projections to the PGCL pressor region and to the intermediolateral cell column (IML). In conclusion the noradrenergic projection from the A1 region to the PGCL pressor region of the C1 cell column is minor and as such probably does not play a major role in controlling these cells. CVLM innervation of the PGCL arises primarily from non-noradrenergic neurons located near the A1 cell group. Most noradrenergic innervation of the PGCL region appears to arise from the A5 and A6 cell groups. In contrast, A1 cells do give rise to a heavy projection to the PVN. These data support the work of Guyenet et. al., in questioning the role of A1 to C1 projections in controlling sympathetic outflow and arterial pressure. (Supported by HL29033 and NS23423).

227.7 PNMT NEURONS OF THE C1 CELL GROUP WITH PROJECTIONS TO BOTH LOCUS COERULEUS AND SPINAL CORD. J.R. Haselton* & P.G. Guyenet. (SPON: L. Heimer) Dept. of Pharmacology, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

The locus coeruleus (LC) receives a dense innervation from neurons in the retrofacial portion of the nucleus paragigantocellularis lateralis (PGCL). This projection originates in part from phenylethanolamine N-methyltransferase - immunoreactive (PNMT) cells of the C1 group (Guyenet & Young, Brain Res., 406, 171-184). Since most of the PNMT neurons of this rostral region project to the spinal cord (Ross et al., Neurosci. Lett., 25, 257-262), an attempt was made to identify PNMT neurons which send collaterals to LC and the spinal cord (SC). Rats, anesthetized with sodium pentobarbital (50mg/kg, i.p.), received unilateral injections of the tracers Fluorogold (FG; 1ul, 4% in water) in SC and rhodamine-tagged microbeads (RB; 100nl) in the ipsilateral LC. The animals were perfused 10-14 days later, the brains removed and sliced (40um) on a Vibratome. Coronal tissue slices, taken from the caudal pole of the facial nucleus to 1.8 mm caudally, were processed for the immunohistochemical detection of PNMT using an FITC-conjugated secondary antibody. PNMT neurons which send collaterals to both LC and SC were found scattered throughout the C1 cell group. Because FG fluorescence shone through the FITC filters, a quantitative analysis of triple labeled cells could not be achieved. However, unambiguous examples of triple labeled neurons could be identified in those cases in which the FG label was of adequately low intensity. Two other populations of afferents to LC were also observed in the retrofacial PGCL: one group was PNMT immunoreactive but did not project to SC, and the second group was PNMT negative and also did not project to SC. This latter group contained relatively few cells rostrally, but many such cells were found ≥400um posterior to the caudal pole of the facial nucleus.

In rats anesthetized with urethane (1.1g/kg, i.p.) and sodium pentobarbital (25mg/kg, i.v.) the C1 area was explored with recording microelectrodes while stimulating in LC. Numerous antidromically activated single-units were found. The vast majority (46 of 50) of these neurons were silent, and were characteristically observed as negative potentials. Their mean antidromic latency was 8.1 ±0.6ms, which indicates a mean conduction velocity of 2.1m/s assuming a straight line distance of 3.9mm between the stimulation and recording electrodes; the mean threshold was 197.6 ±35.5uA. All four units tested for responsivity to iontophoretically applied glutamate were excited, indicating that the recordings were from neuronal cell bodies. In several cases a latency shift was observed upon changing the stimulus strength, suggesting that at least some of the antidromically evoked potentials were driven from the terminal field of this projection. None of the reticulo-coeruleal neurons shared any characteristics with the sympathoexcitatory neurons previously reported by this laboratory, although they were recorded in close proximity to the latter. Nor could any of the 20 reticulospinal sympathoexcitatory neurons tested be antidromically driven from LC even when very high stimulus currents (up to 5mA) were used.

Several conclusions are drawn from these data. 1) The projection to LC from the retrofacial PGCL appears to arise in part (36%) from PNMT neurons. 2) At least some PNMT neurons have collaterals which project to both LC and SC. 3) The neurons which project to the LC have different electrophysiological properties than the PGCL sympathoexcitatory neurons previously identified in this laboratory.

Supported by HL28785.

227.8 ANATOMICAL RELATIONSHIP BETWEEN RESPIRATORY AND CATECHOLAMINE NEURONS IN VENTROLATERAL MEDULLA OF RAT. H.H. Ellenberger, W.-Z. Zhan* and J.L. Feldman, Systems Neurobiology Laboratory, Kinesiology Department, UCLA, Los Angeles, CA 90024-1568.

The ventrolateral medulla is a probable site of central coordination of respiration and circulation, containing neurons implicated in control of each system. The ventral respiratory group (VRG) and the A_1 and C_1 catecholamine groups form longitudinal cell columns extending the length of the ventrolateral medulla. These populations were simultaneously labeled to determine their local organization. VRG neurons were retrogradely labeled by injection of Fluoro-Gold™ (FG) into the phrenic nucleus and/or by injection of Rhodamine-impregnated latex beads (Rh) into the contralateral VRG. The A_1 and C_1 groups were labeled by the immunohisto-chemical staining of cell bodies containing dopamine-β-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT), respectively. FG and/or Rh labeled neurons formed a column extending the entire length of the medulla. These VRG cells congregated in two clusters: a dense ventrolateral cluster of multi-polar, triangular and fusiform cell bodies, and a dorsomedial band of fusiform shaped cell bodies. DBH and PNMT labeled cell bodies also formed a continuous column extending the length of the medulla constituting the A_1 and C_1 groups, respectively. Only DBH cells were labeled in the caudal medulla. DBH and PNMT cells were intermingled at an intermediate level, with PNMT cells concentrated medially and DBH cells concentrated laterally. In the rostral medulla only PNMT labeled cells were present. The VRG and the A_1/C_1 groups formed parallel cell columns in the ventrolateral medulla; the VRG being immediately dorsomedial to the A_1/C_1 groups. Some A_1 and C_1 cells were intermingled with labeled VRG cells, but VRG cells were not double-labeled for either DBH or PNMT. The caudal portion of the A_1 group coincided with the level of the caudal VRG. The level of A_1/C_1 overlap coincided with the level of the rostral VRG, and the level of C_1 coincided with the level of the Bötzinger Complex. The proximal relationship between VRG neurons and the A_1/C_1 neurons, with the overlapping of their dendritic fields suggests that local signal processing and interactions among these cells in the ventrolateral medulla is important for the central coordination of respiratory and cardiovascular control. Supported by NIH Grants HL07363, NS24742 and HL37941.

227.9 TOPICAL APPLICATION OF GLYCINE TO THE ROSTRAL VENTROLATERAL MEDULLA CAUSES UNEQUAL DECREASES IN ACTIVITY OF RENAL AND MESENTERIC SYMPATHETIC NERVES. R.D. STEIN, C.P. YARDLEY*, C.L. FITZSIMONS* AND L.C. WEAVER. John P. Robarts Research Institute and Dept. of Physiol., Univ. Western Ontario, London, Canada, and Dept. of Physiol., Michigan St. Univ., E. Lansing, MI.

Transection of the spinal cord causes a significant decrease in activity of renal sympathetic nerves, but no change in discharge of mesenteric sympathetic nerves. These results suggest that ongoing activity of renal nerves is more dependent upon supraspinal excitatory drive than is activity of mesenteric nerves. Experiments were designed to test this hypothesis further and to determine if decreases in nerve activity seen after blocking of excitatory drive from the rostral ventrolateral medulla (RVLM) can be attributed to the "unmasking" of tonically active sympathoinhibitory systems. Experiments were performed on chloralose-anesthetized cats that were artificially respired. Baroreceptor sensitive multifiber activity of 6 mesenteric and 6 renal nerves was recorded using standard electrophysiological techniques. Neurons in the RVLM were inhibited by bilateral topical application of small cotton pellets soaked with glycine (200 mg/ml, pH 7.37 - 7.43). Application of glycine significantly decreased activity of renal nerves from 42 to 10 μV (pooled SE = 6.8) and activity of mesenteric nerves changed from 35 to 27 μV (SE = 2.6). Activity of renal nerves decreased significantly more than that of mesenteric nerves. The neural responses were accompanied by significant decreases in mean arterial pressure (from 123 to 70 mmHg, SE = 6.8) and heart rate (from 248 to 214 beats/min, SE = 5.2). Application of cotton pellets soaked in physiological saline did not affect nerve activity, blood pressure or heart rate. Responses to glycine began within 20 seconds; mesenteric nerve activity was reduced for 5 minutes, renal nerve discharge was decreased for 20 - 30 minutes and blood pressure returned to control after 15 - 20 minutes. Having established the neural and cardiovascular responses to glycine, this procedure was repeated and the spinal cord was transected at the first cervical segment to eliminate potential descending sympathoinhibitory influences. The second application of glycine decreased renal nerve activity from 53 to 13 μV (SE = 9.5) and changed mesenteric nerve activity from 38 to 32 μV (SE = 4.3). Transection of the spinal cord during the glycine response did not alter renal nerve activity, whereas discharge of mesenteric nerves was significantly increased (32 to 45 μV, SE = 4.3). These findings indicate that tonic excitatory drive from the RVLM affects renal but not mesenteric sympathetic outflow. The small decrease in mesenteric nerve activity seen after blockade of excitatory drive may be attributed to tonically active sympathoinhibitory systems. Supported by NIH, HL21436.

227.10 EVIDENCE THAT TONIC EXCITATORY DRIVE FROM THE ROSTRAL VENTROLATERAL MEDULLA IS NOT UNIFORMLY DISTRIBUTED TO RENAL AND MESENTERIC NERVES. C.P. Yardley,* R.D. Stein, C.L. Fitzsimons* and L.C. Weaver. John P. Robarts Research Inst. and Dept. of Physiol., Univ. West. Ont., London, Canada, and Dept. of Physiol., Mich. St. Univ., E. Lansing, MI.

The rostral ventrolateral medulla (RVLM) is a region crucial for the maintenance of basal levels of arterial blood pressure. Whether the spontaneous activity of neurons in this region is uniformly directed to all sympathetic nerves maintaining vasomotor tone, or preferentially distributed to nerves innervating certain vascular beds, has yet to be elucidated. These alternatives were investigated by microinjecting 0.5-1.0 μl of the inhibitory amino acid glycine, bilaterally into the RVLM of chloralose-anesthetised cats via a cannula electrode while recording blood presure, heart-rate, renal (RNA) and mesenteric nerve activity (MNA). These two sympathetic nerves may not be dependent to the same degree upon supraspinal drive for basal discharge (Stein, Hayner and Weaver, Fed. Proc. 44:1726, 1985). Electrical stimulation of the sites in the RVLM prior to microinjection always elicited increases in blood pressure and activity of both nerves. Five min after the microinjection of glycine blood pressure decreased significantly from 113 to 90 mmHg (pooled SE=2.9) in all 6 cats. Heart-rate was not significantly reduced. Simultaneously, RNA decreased significantly from 42 to 28 μV (SE=2.8). In contrast, the small variation in MNA, 22 to 18 μV (SE=1.3), was not significant. In 4 of 6 cats the depressor response was preceded by an increase in blood pressure from 112 to 174 mmHg, an increase in MNA and variable changes in RNA. Such variable changes in RNA may have been due in part to secondary effects of baroreceptor activation. The early responses lasted 1-4 min and were always followed by a depressor response. The responses to glycine were not due to the volume of injection, as microinjection of 1.0 μl of saline into the same site failed to evoke significant changes in blood pressure or nerve activity. Neither was the glycine acting on neurons in a depressor region near the RVLM as kainic acid microinjected (1.0 μl) into the same region elicited large increases in blood pressure accompanied by increases in MNA and variable changes in RNA. Subsequent histology confirmed that the electrode tips were positioned in the well-defined pressor region of the RVLM. These results demonstrate that vasomotor neurons in the RVLM may be driving sympathetic nerves in a non-uniform manner. Such findings also suggest that the maintenance of blood pressure may depend on the activity of some sympathetic nerves more than others, and that in the past, the role of the mesenteric nerves in maintaining BP may have been overstated. Supported by NIH grant, HL21436.

227.11 STUDIES ON THE CAUDAL VASODEPRESSOR AREA OF THE CAT VENTROLATERAL MEDULLA, P.J. Gatti*, I.J. Namath* and R.A. Gillis* (Spon: V.J. Massari). Depts. of Pharmacology, Howard Univ. College of Medicine and Georgetown Univ. Schls. Med. & Dent., Washington, D.C.

We have recently discovered an area in the caudal ventrolateral medulla of the cat from which profound decreases in arterial blood pressure (BP) and heart rate (HR) can be elicited by microinjecting the gamma aminobutyric acid (GABA) receptor antagonist drug bicuculline (Gatti and Gillis, Soc. Neurosci. Abstr. 12:536, 1986). This area is located from obex to 4 mm rostral to obex, 4 mm lateral to the midline and 1-2 mm beneath the ventral surface of the medulla. From these data, it appears that at this site there is a tonically active GABAergic tone responsible for keeping BP and HR elevated since removal of it (with bicuculline) decreases these indices of cardiovascular function. Since GABA at this site tonically keeps BP and HR elevated, it is presumed that this area is sympathoinhibitory. To test this hypothesis, we microinjected the neuroexcitant amino acid, L-glutamic acid, into this same area while monitoring BP and HR in chloralose-anesthetized artificially respired cats. Unilateral microinjection of L-glutamic acid (10-20 nl of a 100 mM solution) using a picospritzer resulted in a transient fall in BP and HR (n = 4, observations = 7). BP fell by 49 ± 7 mmHg ($p < .05$) and HR by 35 ± 11 bpm ($p < .05$). Injection of an equal volume of the saline vehicle did not affect cardiovascular function. L-glutamic acid lowered BP and HR in an area approximately 2-3 mm rostral to obex 4 mm lateral to the midline and 1-2 mm below the ventral surface of the medulla. This area surrounds the rostral portion of the lateral reticular nucleus and overlaps the bicuculline sensitive site. In an attempt to block neuronal conduction in this area and thus reversibly eliminate this sympathoinhibitory area, the local anesthetic lidocaine was microinjected into this site. Unilateral microinjection of lidocaine (40 nl of a 4% soln) raised BP by 38 mmHg and HR by 10 bpm. These data are therefore consistent with the hypothesis that this area which is under tonic GABAergic inhibition is sympathoinhibitory.

227.12 MONOAMINE RELEASE FROM ROSTRAL VENTROLATERAL MEDULLA IS SYNCHRONOUS WITH CHANGING BLOOD PRESSURE. D. Bhaskaran and C.R. Freed, Depts. of Medicine and Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

Many neurons in the rostral ventrolateral medulla (C1) contain phenylethanolamine-N-methyltransferase (PNMT), the enzyme which converts norepinephrine to epinephrine. The C1 region is known to be involved in the maintenance of arterial pressure and may constitute a vasomotor center (Brain Res. 273: 356, 1983). In our laboratory, we have shown how changes in blood pressure can alter neurotransmitter metabolism in nucleus tractus solitarius, dorsal raphe nucleus (Life Sci. 37: 1783, 1985) and locus coeruleus (Neurosci Abst. 61.19, 1985). We have now studied the neurochemical responses in the C1 nucleus which follow drug induced hypertension and hypotension. Male Sprague-Dawley rats weighing 250-350 g were anesthetized with urethane and had carbon paste in vivo electrochemical electrodes placed in C1. Linear sweep voltammetry at a rate of 5 mV/sec every 5 min was performed using a DCV-5 cyclic voltammetry apparatus (Bioanalytical Systems) with semiderivative signal processing. Phenylephrine was infused i.v. to raise blood pressure by 50 mm Hg and nitroprusside was used to lower blood pressure by 20 mm Hg. Phenylephrine-induced hypertension reduced the catechol peak by -25 + 6% while the indole peak increased by 40 + 8%. Nitroprusside-induced hypotension produced reciprocal results. During hypotension there was an increase in the catechol peak (18 + 5%) and a reduction in the indole peak (-20 + 7%). Interestingly, this is the only nucleus we have examined in which the neurochemical responses to hypotension were the reciprocal of those seen for hypertension. Pharmacological studies with inhibitors of catechol synthesis and catabolism including a-methylparatyrosine (tyrosine hydroxylase), fusaric acid (dopamine-B-hydroxylase), pargyline (monoamine oxidase), and LY134046 (PNMT) showed that the signal measured in that nucleus was likely to be epinephrine with a possible contribution by norepinephrine. The same experimental protocol was used to study the C1 area in Wistar spontaneously hypertensive (SHR) and Wistar-Kyoto normotensive (WKY) rats with findings similar to those observed in Sprague-Dawley animals. These results contrast with our observations in nucleus tractus solitarius (Neurosci. Abst. 114.9, 1986) in which each strain (SHR, WKY, and Sprague-Dawley) showed different changes in neurochemical profiles during changes in blood pressure. The present results show that catechol and indole metabolism in the C1 nucleus is closely related to the direction and magnitude of blood pressure change. Because similar changes are observed across different genetic strains, it is likely that the neurochemical responses reflect basic relationships between blood pressure and neurotransmitter utilization.

227.13 **THE VASODEPRESSOR RESPONSE TO CLONIDINE IS MEDIATED BY IMIDAZOLE AND NOT α_2-ADRENERGIC RECEPTORS IN THE ROSTRAL VENTROLATERAL MEDULLA.** *D.J. Reis, P. Ernsberger, R. Giuliano, R. Willette, and A.R. Granata.* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Clonidine, an imidazol(in)e, acts within the ventrolateral medulla (VLM) to lower arterial pressure (AP). It is widely assumed that clonidine acts as an α_2-adrenergic agonist. However, in the VLM clonidine binds not only to α_2-receptors but also to imidazole sites which are distinct from both adrenergic and histaminergic receptors (Ernsberger et al., *Eur. J. Pharmacol.* 134:1, 1987). Furthermore, selective blockade of α_2-receptors in the C1 area of the VLM fails to reverse the hypotensive effect of clonidine applied in the same region (Willette et al, *Fed. Proc.* 46:1460, 1987). We sought to determine whether vasodepressor responses correlate with actions on imidazole or on α_2-receptors in the VLM. A series of compounds structurally-related to clondine were tested: (a) *in vitro* to measure binding affinity at imidazole and α_2 sites, and (b) *in vivo* to establish vasodepressor potency upon microinjection into the C1 area of the rostral VLM. Affinity at imidazole sites was defined as the IC_{50} at binding sites in bovine VLM membranes labeled by the high-affinity clonidine analog ^{3}H-p-aminoclonidine (^{3}H-PAC; 1 nM) in the presence of 10 µM norepinephrine to mask α_2-receptors. Affinity at α_2-receptors was defined as the IC_{50} at ^{3}H-PAC binding sites in bovine frontal cortex membranes, which lack imidazole sites. Vasodepressor potency was defined as the maximum fall in AP elicited by microinjection of the test compound (1 nmol in 50 nl) into the C1 area of the rostral VLM in a male rat anesthetized with urethane (1 g/kg, i.p.), paralyzed, and artificially ventillated.

Clonidine exhibited high affinity at both imidazole and α_2-receptors (IC_{50} = 6 nM for both) and elicited a potent depressor response (40 mmHg). Guanabenz failed to bind to imidazole sites and lowered AP only slightly (10 mmHg), although it had a higher affinity at α_2-receptors than did clonidine (IC_{50} = 3 nM). Norepinephrine and phenylephrine also failed to bind to imidazole sites and elicited little or no change in AP, despite high α_2-affinity (IC_{50} = 122 and 400 nM, respectively). Naphazoline was equipotent with clonidine at α_2-receptors (IC_{50} = 8 nM) but over 40-fold less potent at imidazole sites, and elicited a much smaller fall in AP (6 mmHg). Cimetidine failed to bind to α_2-receptors but exhibited high affinity at imidazole sites (IC_{50} = 30 nM), and elicited a potent depressor response (29 mmHg). Imidazole-4-acetic acid also lacked α_2 binding activity but did bind to imidazole sites with moderate affinity (IC_{50} = 560 nM) and elicited a moderate depressor response (13 mmHg). Vasodepressor potency was strongly correlated with affinity at imidazole sites ($r = 0.937$, $n = 4$) but not with α_2-affinity ($r = 0.002$, $n = 3$). We conclude that stimulation of imidazole and not α_2-receptors mediates depressor activity among clonidine-like compounds. An imidazole receptor in the C1 area of the VLM may participate in the vasodepressor response to clonidine. An endogenous clonidine-displacing substance (CDS), which is the putative natural ligand of these receptors (Ernsberger et al., *J Hypertension* 4(Suppl. 5):S109, 1986), may participate in cardiovascular control.

BRAIN METABOLISM II

228.1 CEREBRAL PERFUSION STUDIES WITH CONTRAST ENHANCED MRI AT 4.7 TESLA. B. Kaplan*, T.A. Kent*, M. Quast*, E. Amparo*, A. Suttle*, H. Eisenberg. Depts. of Neurology, Psychiatry, Pharmacology, Division of Neurosurgery, and Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX.

We have been interested in developing techniques to noninvasively measure cerebral perfusion using paramagnetic contrast agents and magnetic resonance imaging (MRI). Gd-DTPA shortens T1 relaxation times. When conjugated to albumin, Gd-DTPA remains in the intravascular space (Schmiedel, et al, Radiology, 1987) and has promise as an indicator of cerebral blood volume (CBV). Unconjugated, it may be useful as an indicator of blood:brain barrier permeability to moderate sized particles (MW 590) (Kent, et al, JCBF Met, Suppl, in press). In this study, we determined the feasability of measuring regional cerebral ischemia using Gd-DTPA-albumin in anesthetized rats at 4.7 Tesla (GE CSI-II). Cerebral ischemia was produced by bipolar coagulation of both vertebral arteries and then cannulation of a carotid artery. Cerebral blood flow (CBF) was reduced by 30% as determined by radioactive microsphere technique, and CBV was reduced by a similar amount as determined by the ^{51}Cr-EDTA space. There was no histological evidence of infarction. In some cases, both CBF and CBV were increased on the side with carotid occlusion, possibly due to anomalous collaterization. After a baseline MRI to determine T1 (by progressive saturation using a series of five TR's and a 14 msec TE), the Gd-DTPA albumin (.1 ml/kg i.v., 15 Gd-DTPA molecules per ablumin) was injected. In 8 of 11 cases, there was less T1 shortening (approximately 50%) on the occluded side, suggesting reduced CBV, since T1 shortening should be proportional to CBV. In the remainder, there was greater enhancement on the occluded side. In general, these corresponded to the ^{51}Cr-EDTA values. We are attempting to quantitate the CBV by establishing calibration curves of the relationship between T1 shortening and known concentrations of Gd-DTPA-albumin in blood. Preliminary application of these curves to the in-vivo data resulted in calculated CBV of 2-5%. It may also be possible to determine CBF by rapid sequence scanning and determining the rate of enhancement. We are applying these methods to newer superparamagnetic agents and these results will be presented.

228.2 NON-INVASIVE DETERMINATION OF CEREBRAL VENOUS HEMOGLOBIN SATURATION IN THE DOG BY NEAR INFRARED SPECTROSCOPY. M. Ferrari*, J.F. Hartmann*, D.A. Wilson*, D.F. Hanley, and R.J. Traystman. (Spon: K. Kubos). Dept. of Anesthesiology/Critical Care Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Pulse oximetry (PO) provides a non-invasive measure of arterial systemic oxyhemoglobin saturation (SaO_2). However, O_2 dependent organs such as the brain and heart regulate their O_2 delivery independently of changes in arterial O_2 content. Thus, the effects of spontaneous changes in systemic O_2 availability on these organs may not be characterizable by PO. Moreover, PO is restricted to changes occurring in the 80-100% SaO_2 range. Near infrared spectroscopy (NIRS) was developed to monitor changes in tissue hemodynamics and O_2 utilization. However, it is limited to monitoring changes from an uncertain initial state. Derivative NIRS (DNIRS) (Hruschka, W.R. and K.H. Norris, Appl. Spectr. 36:261, 1982) has been extensively applied in agriculture. The technique utilizes the changes in absorption spectra and, potentially, may overcome uncertainties regarding absolute optical density (OD). In this study we evaluated the potential of DNIRS for cerebral venous hemoglobin saturation (SvO_2) measurement using a canine model.

Methods. In 6 barbiturate anesthetized, paralyzed, mechanically ventilated dogs, catheters were placed to collect arterial and superior sagittal sinus (SSS) blood. Two fiber optic bundles from a fast scanning spectrophotometer were affixed firmly to the skull such that the SSS was transilluminated. The breathing mixture was made hypoxic (HH) decrementally and samples of SaO_2 and SvO_2 were determined. A steady state spectrum was recorded at each HH level. The derivatized spectrum was then regressed against the measured SvO_2.

Results. In 4 dogs 40 spectra, with SvO_2 ranging from 1.5-70%, the regression equation $SvO_2 = b_1 + b_2(A_1/A_2) + b_3A_3$ yielded a substantial correlation between the spectral changes and SvO_2 (residuals SD = 3.5; multiple R = .98; $b_1 - b_3$ = regression coefficients; A_1-A_3 = wavelength). In 2 dogs this equation was used to predict SvO_2 in 26 separate spectra of varying HH intensity. The prediction SE was 2.82 and when regressed against sampled SvO_2 yielded an r value of 0.97.

Discussion. The cerebral SvO_2 is directly influenced by the balance between cerebral O_2 delivery and cerebral O_2 uptake. The results of this study indicate that it is possible to build a predictive algorithm for non-invasive SvO_2 measurement in the dog using DNIRS. The small variation encountered in the coefficients describing the relationship suggests that SSS transillumination may also be possible in humans whereby SvO_2 and its changes could be quantified.

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228.3 **STRATEGIES FOR THREE-DIMENSIONAL ANALYSIS AND DISPLAY OF HUMAN REGIONAL CEREBRAL BLOOD FLOW.**

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Ongoing studies have been performed to evaluate regional cerebral blood flow (**rCBF**) using the radiopharmaceutical I^{123}-Iodoamphetamine, and Emission Computed Tomography (**ECT**). **ECT** generates images of slices through the brain, with the value of each picture element (*pixel*) corresponding to the relative **rCBF** measured for each volume element (*voxel*). The set of serial contiguous slices are stacked to form a volume consisting of a three-dimensional array of voxels.

The **CARP** system (**C**omputer **A**ided **R**econstruction **P**ackage), developed in the Neuroscience Imaging Laboratory at UTHSCD, includes a volume processor which can manipulate and form images from the three-dimensional array. Objects within the volume can be isolated and compared to other portions of the volume, or to objects extracted from other patient studies. These objects are stored in a hierarchical database, which allows selective transformations to be performed on any defined structure within the volume.

The three-dimensional approach is critical for comparing across studies because of differences in patient positioning, brain size and shape, and amount of total scintigraphic activity. These problems are addressed through the use of three-dimensional translation, rotation, scaling, and non-linear warping of selected objects. The volume processor can generate images which demonstrate local hemispheric activity from any viewing position. Computer generated 3D images of **rCBF** have been found to improve communication to referring physicians in terms of location and extent of abnormal regions, and provide quantitative comparisons which are more precise than subjective evaluation of slice images.

(Support from the Biological Humanics Foundation, NIH-DA2938, and NIH-AA3901)

228.4 CEREBRAL BLOOD FLOW AND OXIDATIVE GLYCOLYSIS ARE UNCOUPLED DURING SOMATOSENSORY STIMULATION IN HUMANS. M.E. Raichle, P.T. Fox, and M.A. Mintun*. Departments of Neurology and Radiology and the McDonnell Center for Studies of Higher Brain Function. Washington University School of Medicine, St. Louis, Missouri, USA

Dynamic regional regulation of cerebral blood flow (CBF) by metabolic demand is widely accepted. A recent study (1) reported that physiological increases in neuronal activity (somatosensory stimulation) produced local augmentation of CBF (29%) far in excess of the increase (6%) in cerebral oxygen consumption ($CMRO_2$). As glucose metabolism (CMRGlu) is known to increase with neuronal activity, that study (1) suggested nonoxidative glycolosis as an acute accommodation to increased neuronal work. To test this, we measured CBF and CMRGlu responses to neuronal activation (finger vibration) (1).

Eight paired studies (alternating stimulus side) were performed in four normal volunteers. Each study included 2 CBF scans (resting and stimulated) and 1 CMRGlu scan (stimulated). CBF was measured using an intravenous bolus injection of $H_2{}^{15}O$. CMRGlu was measured using standard ^{18}F-DG methods, with arterial blood samples.

For each study, the zone of maximal activation (parietal cortex) and a control region (frontal cortex) having the same resting CBF were identified from the paried CBF studies. Response magnitudes for CBF and CMRGlu were determined identically, by comparing the parietal and frontal regions in the stimulated state. CBF in the parietal region of interest increased 24% (9 ml/100 gm·min, ± 1 sd; $p < 0.0001$) in agreement with our previous study (1). CMRGlu, however, increased by only 11% (0.8 mg/(100 gm·min), ± 0.2 sd; $p < 0.0025$). This difference in response magnitude was significant ($p < 0.0025$). Comparison of the same regions during stimulation of the ipsilateral hand (the complementary study of each pair) showed no responses for CBF or CMRGlu.

We conclude that neuronal activity increases local blood flow 1 to 3 fold the task-induced local increases in glucose consumption during intense somatosensory stimulation (finger vibration) in humans. Oxygen consumption and glucose consumption, however, are probably coupled both at rest and during acute activation. Whether task-induced increases in local blood flow will exceed local increases in metabolism under other stimulus conditions (e.g. visual) remains to be determined.

1. Proc Nat Acad Sci USA (1986) 83:1140-1144.
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228.5 MECHANISTIC PET STUDIES: DEMONSTRATION OF A DEUTERIUM ISOTOPE EFFECT IN THE MAO CATALYZED BINDING OF [^{11}C]L-DEPRENYL IN LIVING BABOON BRAIN. J. S. Fowler*, R. R. MacGregor*, S. L. Dewey, D. J. Schlyer*, J. Logan*, A. P. Wolf* and B. Langstrom* (SPON: P. Brink). Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973 and Institute of Chemistry, University of Uppsala, Box 531, S-751, Uppsala, Sweden

PET is uniquely capable of probing biochemical transformations in the living human and animal body providing that the rate limiting reactions responsible for the concentration of radioactive products in a region of interest can be identifed and characterized.

L-Deprenyl is a suicide inactivator of monoamine oxidase B (MAO B) and is used in the treatment of Parkinson's disease. We have reported the feasibility of using [^{11}C]L-deprenyl to map monoamine oxidase (MAO) in the living human brain (Science 235: 481, 1987). These studies included the demonstration of stereoselectivity and are consistent with the irreversible covalent attachment of the catalytically activated labeled tracer to MAO.

We report here the observation of a significant kinetic isotope effect in the binding of [^{11}C]L-deprenyl to living baboon brain using PET. In these studies, a direct comparison of the brain uptake of [^{11}C]L-deprenyl with [^{11}C]L-deprenyl-α,α-d_2 (N-[^{11}C]methyl-α-methyl-N-2-propynyl-1,1-d_2-benzeneethanamine) was made over the time period 0-90 minutes in three different baboons. For each study a complete arterial plasma curve as well as the amount of unchanged [^{11}C] tracer was measured. Using this information, blood-to-brain influx constants (k_1) were determined for each study (Patlak et al. J. Cereb. Blood Flow Metab. 3:1, 1983). The ratio of k_1(hydrogen):k_1(deuterium) ranged from 1.8 to 2.5 demonstrating that the MAO catalyzed removal of a hydrogen atom from the alpha methylene carbon atom of the propargyl group is rate determining in the mechanism responsible for the retention of L-deprenyl in brain.

Although the deuterium isotope effect has been observed for many substrates and inhibitors of MAO, this is the first example of the in vivo visualization of the deuterium isotope effect with MAO in its native environment demonstrating the power of PET for discriminating kinetic isotope effects in the living body and identifying the specific bond-breaking process which give rise to the PET image.

Research supported by USDOE, OHER, NIH NS-15380 and NS-15638.

228.6 LOCAL CEREBRAL GLUCOSE UPTAKE ABNORMALITIES IN PATIENTS WITH MILD CLOSED HEAD INJURY WITH PERSISTING COGNITIVE DEFICITS, DESPITE NORMAL CT AND MRI. M.S. Humayun*, S.K. Presty, N.D. LaFrance*, B. Gordon*, R.F. Dannals*, D. Clough*, J. Links*, H.N. Wagner*, and D.M. Long. Depts. of Neurology, Neurosurgery, and Nuclear Medicine. Johns Hopkins Univ. School of Med., Baltimore, MD 21205

Mild closed head injury (CHI) is often followed by neuropsychologic impairments in recent memory and attention, despite the absence of structural abnormalities shown by CT or MRI. Biomechanical models of CHI (Ommaya et al., Head Injuries: Proc. of the 2nd Chicago Symp. on Neural Trauma 49-75, 1976) predict that the cortical regions in proximity to rough bony protrusions (i.e. temporal and frontal cortices) will suffer greater injury compared to regions covered by smooth surfaces (i.e. occipital cortex). To explore the possibility that CHI may result in glucose metabolism abnormalities, we used the fluorodeoxyglucose (FDG) technique with PET imaging to measure local cerebral glucose uptake (Huang et al., Am. J. Physiol. 238:69-82, 1980) in patients 3-12 months following mild CHI (n=3) and matched controls (n=3). All CHI patients had normal CT, MRI, and EEG and negative drug screens. CHI patients all showed deficits in attention and recent memory on neuropsychologic testing. In order to increase the sensitivity of the PET scan, all subjects were engaged in a computer-based continuous performance task throughout the initial 30 minutes following FDG administration. Local cerebral glucose uptake (LCGU) rates were calculated as ratios of regional activity to mean whole brain activity for each individual. Group comparisons were made using t-tests with $p < .05$ level of significance after Bonferonni correction. Significant differences in LCGU between groups were found in temporal and frontal cortices and the caudate nucleus. The CHI group exhibited decreased LCGU in medial temporal, posterior temporal, and posterior frontal cortices, as well as in the caudate nucleus. LCGU was increased, relative to controls, in the anterior temporal and anterior frontal cortices. There were no significant group differences found in whole brain LCGU. These preliminary results suggest that CHI patients with no structural brain injury show compromised functional activity in neural regions, some of which are consistent with the biomechanical brain injury models. Changes in regional glucose metabolism have been shown to be associated with closed head injury and may contribute to the post-traumatic neuropsychologic deficits.

228.7 **TREATMENT OF INTRACRANIAL ARTERIOVENOUS MALFORMATIONS EVALUATED BY POSITRON EMISSION TOMOGRAPHY**

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Positron emission tomography (PET) was performed on 7 patients before and after excision and/or percutaneous embolization of an intracranial arteriovenous malformation (AVM) and the results were compared to data from normal controls. The patient population included 5 males and 2 females with a mean age of 37 years; two AVM's were > 11 cm, three AVM's were 7-11 cm, and two were less than 6 cm in diameter on angiography. Two additional patients with AVM's 6-11 cm in size were each studied on two occasions, one two and one three years apart, without treatment. Parameters measured included cerebral blood flow (CBF), blood volume (CBV), oxygen extraction and utilization (OEF and CMRO2) and glucose utilization (LCMRGl). Five patients underwent attempts at embolization of their AVM, one had surgical resection, and one had both procedures. Initial PET scans showed significant effects of the AVM's on remote cortex when patient data were compared to data from normal controls, with decreased LCMRGl (mean=27µmol/100g/min, S.E.M.=0.3, p<.001), increased CBV (mean=12% brain weight, S.E.M.=0.4, p<.001), and decreased CBF/CBV ratio (mean=5.3, S.E.M.=0.6, p<.005) in remote ipsilateral cortex. Values in the contralateral cortex were at the lower limit of the normal range. These findings imply that the shunting of blood through an AVM has widespread deleterious effects on brain, with oxygen metabolic function being maintained through compensatory mechanisms. Embolization resulting in incomplete long-term obliteration of the AVM did not result in improvement in hemodynamic or metabolic parameters as measured by PET. Surgical treatment resulting in a significant long-term decrease in AVM size was associated with moderate increases in CMRO2 in cortex remote from the AVM. Follow-up of two patients without treatment showed no significant changes in the parameters measured. These data suggest that partial treatment of AVM's may not result in long-term clinical benefit compared to the natural history of the untreated condition.

228.8 FDG/PET SCAN METABOLIC ASYMMETRIES IN ASYMPTOMATIC HIV SEROPOSITIVE SUBJECTS.

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We studied 7 young male homosexual subjects who were completely asymptomatic but HIV seropositive and 10 age-matched male normal volunteers who were HIV seronegative, with resting PET/FDG studies and MRI scans. Mean age and S.D. of the controls was 31.1 ± 9.9 years and of the HIV seropositive subjects was 36.1 ± 6.7 years (p = N.S.).

Mean cerebral metabolic rates for glucose (CMRglc) in the controls was 6.93 ± 2.0 mg/100g/min and in the HIV seropositive subjects was 7.21 ± 1.3 mg/100g/min (p = N.S.). When the regional CMRglc (rCMRglc) values for 24 separate brain lobules were compared no differences between the groups were found. Similarly, the ratio of lobular to mean CMRglc ($r/\bar{x}$) for any lobule was compared and no difference was found between the two groups. However, an index of regional or hemispheric metabolic asymmetry (the unsigned value for [(R-L)/(R+L)/2] showed significant differences between the two groups. For example, the asymmetry index for the whole hemisphere was 0.061 ± 0.035 in HIV seropositives and 0.019 ± 0.018 in the controls ($p < 0.02$); in the prefrontal lobule it was 0.078 ± 0.048 in HIV seropositives and 0.027 ± 0.024 in HIV seronegatives ($p < 0.03$). These results suggested that certain or many regions of the brain in asymptomatic HIV seropositive subjects were significantly asymmetric in either direction (R > L or L > R) when compared to controls. Furthermore, when the asymmetry index in 7 seropositive subjects was individually compared to the mean values for asymmetry in the controls, by the use of Z-scores, it became apparent that the asymmetry was being produced by four of the seven subjects only. The regions showing the major asymmetries in these four subjects were the prefrontal, mid temporal (lateral) and medial temporal regions and the cerebellum (Z scores ranged between 2.24 and 6.5). In conclusion, in HIV seropositive subjects we have studied, no abnormalities in the MRI scans were noted and in the PET scans one half of the subjects had abnormal asymmetry in frontal and temporal regions, indicating probable focal reductions in rCMRglc in these regions on the right or the left sides.

228.9 HUMAN OPIOID ABUSERS SHOW REGIONAL DECREASES IN CEREBRAL GLUCOSE UTILIZATION DURING MORPHINE EUPHORIA.

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The 2-deoxy-D-[1-^{14}C]glucose method allows measurement of the regional cerebral metabolic rate for glucose (rCMRglu), an index of brain function (Sokoloff, L. et al., J. Neurochem., 28: 897, 1977). It has been used to identify brain areas affected by opioids and other abused drugs in rats (London, E. D. et al., Clin. Neuropharmacol., 9, Suppl. 4:208, 1986), and can be applied in human studies, with [^{18}F]fluorodeoxyglucose (FDG) and positron emission tomography (PET) (Phelps, M. E. et al., Ann. Neurol., 6:371, 1979). Due to interest in identifying brain circuits involved in opioid euphoria, we used the PET FDG method in a double-blind, placebo-controlled, crossover study comparing morphine (M) and placebo (P) effects on rCMRglu in men. Subjects were volunteers (21-45 yr) with a history of opioid abuse, but no current drug dependencies except nicotine. In 3 tests before PET, spontaneous EEG was recorded in a 5-min baseline period and for 45 min after M (15 or 30 mg, i.m.) or P. Also recorded were self-reports on a 4-item questionnaire relating to feeling and liking the drug, and a 6-item analogue scale, measuring the strength and quality of the drug effect and the subject's liking for it. Only subjects with positive M responses continued in the study. Positive response criteria included the following: decreased EEG alpha power or frequency or increased theta or delta power, feeling opiate-specific sensations, recognizing M as "dope", and liking it at least slightly. Of the first 13 subjects entering the study, 7 fulfilled the criteria, and underwent 2 PET scans, usually a week apart, with 30 mg M or P given 15 min before FDG (~5 mCi, i.v.), while they were blindfolded and listening to a white noise tape presenting a beep every min. At the sound of each beep, the subject rated his euphoria on a scale of 0-4.

Levels of rCMRglu were measured in 22 areas. M generally decreased rCMRglu, with significant decrements (10-15% P) in 8 brain areas (anterior cingulate cortex, superior & middle frontal gyri, insula, amygdalohippocampal complex, putamen, midbrain, thalamus; Hotelling's $T^2 = 43.234$, $p < .0001$). M did not alter arterial blood gases or pH when FDG was injected, indicating that rCMRglu decreases did not result from hypercapnia. A significant correlation was obtained between temporal pole rCMRglu after M and integrated euphoria scores during the FDG incorporation period ($r = -.80$, $p = .05$). The results indicate that M decreases cerebral oxidative metabolism, and implicate specific neuroanatomical sites as mediators of opioid euphoria.

228.10 PET Study of Frontal Cortical Glucose Metabolic Rate in Schizophrenia

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Introduction: This study is a preliminary analysis of an ongoing study to replicate the finding of decreased frontal/occipital metabolic activity in schizophrenia. This finding of hypofrontality was first noted in cerebral blood flow studies (Ingvar, 1974) and was subsequently found on PET scan studies by some (Buchsbaum, 1982, 1984) investigators but not by all (Sheppard, 1983). Frontal lobe dysfunction has been hypothesized to play a role in schizophrenic symptomatology.

Methodology: Thirteen patients (11M, 2F age 28 ± 6.7 yrs) who met DSM-III criteria for schizophrenia and eighteen normals (14M, 4F, age 26.2 ± 8.1 yrs) participated. Subjects were given 5 mCi of 18F-2dg and then performed the Continous Performance Test (CPT) for 35 minutes. Subjects were then scanned on CTI Neuroecat IV (FWHM = 7.6 mm). Nine scans parallel to the CM line were obtained. Glucose use was calculated according to the Sokoloff model.

Three slice levels (61%, 41% and 28% of the distance between the canthomeatal line and the vertex of the brain) were chosen for analysis of cortical metabolism. Cortical metabolism was measured by peeling off a 2.2 cm cortical rim from the slice. This rim was divided into four equal quadrants in each hemisphere.

Results: A significant four-way anova [diagnostic group (normals vs schizophrenics) by anteroposterior (4 cortical sectors front to back) by hemisphere (right,left) by level (61%,41%,28%)] was found ($F=2.82$, $p= .04$, d.f. 3,81) which reconfirmed our earlier finding. Exploratory post hoc t-tests showed that schizophrenics had lower relative right frontal lobe, and higher relative occipital lobe and lower right parietal and temporal lobe.

228.11 TEST-RETEST rCMRgl ACTIVITY PATTERNS IN SCHIZOPHRENIC PATIENTS: AN ASSESSMENT OF MEASUREMENT STABILITY. GK THAKER, HH HOLCOMB, CA TAMMINGA, E MATTHEW, H LOATS, B GORDON, J LINKS, R DANNALS and HN WAGNER*. Univ. Md. Psych. Res. Ctr. and Johns Hopkins Hospital, Baltimore, Md. 21205; and Westminster, Md. 21157.

Behavioral dependent changes in regional cerebral metabolic rate of glucose (rCMRgl) can be quantitatively assessed with positron emission tomography (PET). Large inter-individual variations in metabolic activity profiles constitute the principal statistical impediment to PET image analysis. It is, therefore, useful to determine an individual's 'control' rCMRgl profile and the physiological stability of the measurements that determine that profile. Here we describe measurements on four medicated patients with schizophrenia (two women and two men) who were studied with PET twice in similar behavioral conditions. The interscan interval was two days to two weeks. During the initial 30 minute 18F-2DG uptake period, the subjects performed a computer driven visual vigilance task. ROI were measured from the twelve scan planes obtained from each study and plotted. Corresponding images from the two studies were digitally aligned and subtracted from one another. This subtraction method provides a visual representation of pixel-by-pixel differences in rCMRgl. Digital registration with corresponding XCT scans also provided verification of spatial fit between scans. ROI and digital image subtraction analytic methods indicate that medicated, clinically stable patients with schizophrenia appear to have stable, but individually unique, metabolic activity patterns.

228.12 CORTICAL GLUCOSE METABOLIC ASYMMETRIES IN ALZHEIMER'S DISEASE. E.J. Metter, W.H. Riege*, D.E. Kuhl *, D.G. Fujikawa *, G.W. Small (SPON: M.K. Menon). Laboratory of Nuclear Medicine, UCLA School of Medicine, Los Angeles, CA 90024, and Veterans Administration Medical Center, Sepulveda, CA 91343.

Positron emission tomography using (F18)-fluorodeoxyglucose has demonstrated left-right hemispheral and frontal-parietal asymmetries in Alzheimer's disease (AD) patients studied in a resting state. In a previous study of 14 AD patients, we noted that the changes in these two ratios appeared to be independent, and were not found in normal subjects. In this report an additional 30 patients with AD were evaluated (giving a total sample of 44 subjects), to determine whether an association exists between the ratios. A frontal-parietal (F/P) and left right (L/R) ratio were computed for each subject by averaging multiple measures from each region. Normal ranges were determined from 21 age-matched control individuals. For the 44 AD patients, the F/P ratio was larger than normal in 30, while 14 had ratios within the normal range. For the left-right ratio, 14 subjects had higher left than right metabolism, 16 were symmetric and 14 had higher right than left. In this manner six subgroups could be identified. The groups did not differ in the severity of dementia, age, verbal, nonverbal or frontal performance scores. The presence of these prominent asymmetries in AD suggest that major functional changes occur throughout the brain. Previous studies have demonstrated that the left-right differences are associated with differences in visuospatial and language function, but it is unclear what the role of the frontal-parietal asymmetries is in the behavioral changes associated with AD.

INVERTEBRATE LEARNING AND BEHAVIOR III

229.1 DEMONSTRATION OF CLASSICAL CONDITIONING OF EYE WITHDRAWAL IN THE GREEN CRAB. R. D. Feinman and C. I. Abramson*. Department of Biochemistry. SUNY Health Science Center at Brooklyn. Brooklyn, NY 11203.

In the eye withdrawal reflex of the green crab Carcinus maenas aversive stimulation of the anterior portion of the animal causes a rapid reflex retraction of the eye into the carapace. We conditioned eye withdrawal using a 5 s low amplitude vibration to one side of the carapace as a conditioned stimulus (CS). Repeated pairings of this CS with an unconditioned stimulus (US), a 0.5 s puff of air (0.25 psi) to the eye on the same side, resulted in the reliable appearance of the retraction response during CS presentation. We found that there was significantly less responding in control groups subjected to backward conditioning or specifically unpaired presentation of stimuli. We also observed little effect on control animals with presentation of CS alone, US alone or simply time in the apparatus. We subjected 8 experimental animals and 5 control groups of 8 animals each to 100 trials. The number of CS responses of exerimentals reached a plateau after about 60 trials. A summary of total responses:

subject	mean response	SD
paired	48.1	13.2
unpaired	12.3	12.1
backward	11.3	5.4
CS alone	1.1	1.3
US alone	0.4	0.7
blank	0.0	0.0

The paired group was most resistant to extinction as measured by responses to 20 CS alone trials directly following acquisition. We also found some response to the CS in the contralateral eye even though this eye did not respond during training. After 24 hrs rest, the response of paired animals to CS alone presentation was not substantially different from controls, but re-acquisition was dramatically enhanced indicating that there was savings over this period. These results demonstrate that the eye withdrawal reflex can be conditioned in a manner formally identical to classical (Pavlovian) conditioning. The relative simplicity of the underlying neuronal circuit suggests that this may be a useful learning model.

229.2 ASSOCIATIVE AND NON-ASSOCIATIVE LEARNING IN SPINY LOBSTERS: BEHAVIORAL DISCRIMINATION OF ODORANT MIXTURES. J.B. Fine-Levy*, P.C. Daniel*, and C.D. Derby (SPON: D. Edwards). Dept. of Biology, Georgia State University, Atlanta, GA 30303.

We are using the Florida spiny lobster, Panulirus argus, as a behavioral model for the study of quality coding in olfaction, and have developed both a discriminative aversive conditioning paradigm and an habituation paradigm to assay for behavioral discrimination of odorant mixtures. Four artificial mixtures (crab, mullet, oyster, and shrimp) were presented to the animals each at 0.05 mM and 0.5 mM concentrations. These mixtures elicited several types of feeding responses from unconditioned animals. For the aversive conditioning paradigm, twelve lobsters were distributed into four groups of three, each group being trained to avoid both concentrations of one of the mixtures. A total of ten pairings of the aversive stimulus with the conditioned stimulus over five test days was sufficient for associative learning to occur. Following conditioning, changes in responses to the four mixtures were categorized as either active or passive avoidance, and merged to form an index of aversion for each conditioned mixture. Comparisons of these indices indicate that crab mixture and shrimp mixture are perceived as being more similar to each other than to mullet mixture and oyster mixture, while mullet mixture and oyster mixture, though more similar to each other than they are to crab mixture and shrimp mixture, are still perceived to be relatively distinct from each other. Similar results were obtained with the habituation paradigm. A 66-100% decrease in response to the crab mixture was accomplished through 2-min presentations of 5 ml of alternating concentrations (0.05 mM and 0.5 mM) of crab mixture, repeated every five minutes for a total duration of 3 to 3.5 hr. Habituation, measured as relative decrease in response to a stimulus, was greatest to crab mixture (90%), followed by shrimp mixture (65%), oyster mixture (49%), and mullet mixture (47%). The degree of similarities among the mixtures as perceived by the animals is in close parallel with similarities in the overall compositions of the mixtures as indicated by multivariate analyses. Behavioral discriminations of chemical mixtures by these animals, as elucidated by use of these associative and non-associative learning paradigms, may be compared with neurophysiological data (Girardot and Derby, abstract at this meeting) to provide an indication of the type of neural code that allows the animals to perform these discriminations.

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229.3 HABITUATION OF ESCAPE SWIMMING IN TRITONIA APPEARS TO INVOLVE MULTIPLE SITES OF CIRCUIT MODIFICATION. W.N. Frost and P.A. Getting, Dept. of Physiology & Biophysics, Univ. of Iowa, Iowa City, IA 52242.

The neural mechanisms underlying learning and memory are currently being investigated in a number of preparations. Much of this work has concentrated on behaviors such as reflexes. Little is known, however, about the mechanisms underlying modification of certain other types of behaviors, such as rhythmic movements produced by central pattern generators.

In the mollusc Tritonia, noxious epithelial stimuli trigger escape swimming consisting of a series of dorsal/ventral flexions. Abraham and Willows (Com. Behav. Biol., 6:271, 1971) reported that the escape swim undergoes habituation: with repeated stimulation the number of cycles per swim progressively decrements. We are beginning a study of the cellular mechanisms underlying habituation of this behavior.

Our first goal was to characterize the site(s) of plasticity. Habituation was produced by repeated application of a salt solution (150 ul, 4M NaCl, ISI=2 min) to the animal's tail. The number of cycles per swim decremented from an average of 7.3 for the first stimulus to 1.7 by the tenth (n=6). Since this change was a decrement in number of cycles, the habituation was not likely to be due to plasticity in the effector system.

In isolated brain preparations, electrical stimulation of pedal nerves evokes the neural correlate of swimming (a series of coordinated bursts in identified flexion neurons). With repeated electrical stimulation (10 trials, ISI=2 min) the number of burst cycles/swim decreased from an initial level of 5.4 to a final level of 1.9 (n=7). The habituation, therefore, is not likely to be due to receptor adaptation. It appears that a major component of the plasticity underlying habituation was located within the central ganglia.

The synapses made by sensory neurons onto their follower cells show a striking homosynaptic depression (Getting, J. comp. Physiol., 110:271, 1976) and would thus appear to represent one locus of plasticity. Our current studies, however, suggested that at least one additional circuit modification was involved. Behavioral experiments employing stimulation of two body sites with separate receptor populations (head and tail) revealed that habituation showed stimulus site generalization. Animals were made to swim once every 30 min using head stimulation. This interval produced very little habituation. Between two head stimuli a series of 10 stimuli were applied to the tail (ISI=2 min). We found that following habituation to tail stimulation, the response to the next head stimulus was significantly habituated (n=6, $p<0.05$). Experiments with isolated brain preparations, using electrical stimulation of L and R Pedal Nerve 3 in place of different body site stimulation, also led to generalization. A simple explanation for the generalization of habituation was that a second circuit modification had occurred at an interneuronal locus after the convergence of the two sensory pathways.

We conclude that the memory for habituation of the Tritonia swim appears to involve multiple circuit modifications: one involving homosynaptic depression at the first central synapse of the sensory neurons, and a second, interneuronal locus perhaps located within the pattern generator itself. Supported by NS17325 to PAG.

229.4 **CELLULAR ANALYSIS OF THE REINFORCEMENT PATHWAY IN OPERANT CONDITIONING OF HEAD-WAVING IN *APLYSIA*** D.G. Cook & T.J. Carew, Yale University, Department of Psychology, New Haven, CT 06520.

Aplysia can be operantly conditioned to avoid head-waving to one side of their body, if that response is punished by bright light (Cook & Carew, 1986). An important step in the cellular analysis of this operant learning is to identify the neural pathways which mediate reinforcement. In the present study we have examined the role of input from the optic and rhinophore nerves, which are known to be visual pathways in *Aplysia* (Jacklet, 1980).

Using a split-foot preparation, intracellular recordings were made from pedal motor neurons controlling the neck and body wall (Hening et al, 1979). Animals (N=10) received two types of stimuli: Light Trials (30 sec. bright light); and Blank (Control) Trials. Action potentials were recorded 30 sec prior and then during the stimulus period. First, animals received 3 light and 3 blank trials in random order. Then the optic nerve (O.N.) and rhinophore nerve (R.N.) were bilaterally cut and the same procedure was repeated. Prior to nerve cut, light produced a modest increase in firing ($\bar{x}$= 9%). However, following nerve cut, light induced a highly significant increase in firing ($\bar{x}$=40%, $p<.005$). Thus, surprisingly, there was a significant increase in the amount of light-induced excitation of the pedal motor neurons following O.N. and R.N. transection ($p<.01$). This effect was not due to the direct effect of light on the neurons, since subsequent denervation of the CNS abolished the enhancement. These results show: (1) input from the O.N. and R.N. inhibits light-induced excitation of pedal motor neurons; and (2) the excitation unmasked by nerve transection is mediated via extraocular photoreceptors.

In our behavioral experiments light serves as an aversive reinforcer. Our cellular results suggest that input from the eyes and rhinophores actually reduce the effect of light on the head-waving system. Thus, eliminating these pathways should not retard learning; in fact, it may even enhance learning. To test this hypothesis, 3 groups were examined: A Contingent/Cut-Nerve group (N=25); a Contingent/Sham-Operated group (N=25); and a Yoked Control/Sham-Operated group (N=25). Two days after surgery, animals were trained and tested. Contingent/Shams showed significant conditioning ($\bar{x}$[test - baseline]= 26 sec, $p<.05$). Interestingly, the Contingent/Cut-Nerve group also showed significant learning ($p<.01$), with a score ($\bar{x}$=44sec) almost double that of the Contingent/Shams. Yoked/Shams showed no learning.

In summary, our behavioral results confirm cellular observations and suggest that visual input from the eyes and rhinophores is not essential for the operant conditioning, and may in fact retard it. It will now be of interest to assess the functional significance of this suprising result, and to identify the extraocular pathways which mediate reinforcement.

229.5 PSEUDOCONDITIONING, SENSITIZATION AND HABITUATION OF DIRECTIONAL SIPHON RESPONSES IN APLYSIA. M.T. Erickson* and E.T. Walters, Dept.Physiol. & Cell Biol., U.Texas Med.Sch, Houston, TX 77225.

Recently we described qualitatively different defensive siphon responses in Aplysia that aid in directing ink at a site of attack (J.Comp.Physiol.A, 159:339, 1986). Siphon responses to tactile stimulation of the midbody region displayed pseudoconditioning - following strong unconditioned stimuli (US) to the head or tail the responses to midbody test stimulation came to resemble the respective US response. Evidence for some associative specificity with pairing suggested the involvement of pseudoconditioning mechanisms in a form of stimulus-response (S-R) learning (Soc. Neurosci. Abstr. 12:398, 1986).

As a first step in analyzing these forms of learning we have begun a comparative examination of nonassociative plasticity of the directional siphon responses, using an in vitro preparation with a photocell to measure head-type (constricting) and tail-type (flaring) responses, and suction electrodes to stimulate peripheral nerves. In each experiment 20 test stimuli were applied to a peripheral nerve at 60 sec intervals. In some cases a strong US (3 brief trains in 10 sec) was applied to nerves innervating either the head or the tail following the 10th test stimulus. Ten test stimuli were then applied again 1 hr later. Repeated weak test stimuli to head, tail, or midbody nerves caused similar habituation, with the responses falling to about 10% of their initial amplitude by the 20th stimulus, regardless of whether the test response was flaring or constricting. Mean recovery of the habituated responses at 1 hr was only 25% of baseline for tail nerve elicited flaring responses (n=7), 10% for midbody nerve elicited flaring responses (n=5), 70% for midbody nerve constricting responses (n=5), and 25% for head nerve elicited constricting responses (n=6). All test pathways showed short-term sensitization when the US caused the same type of unconditioned response as the test stimulus (n=6,5,6,5, respectively). Under these conditions sensitization at 1 hr was stronger for head or tail test nerves than for midbody test nerves. Pseudoconditioning (defined as the conversion of a constricting response to a flaring response or vice-versa following the tail or head US) was rarely observed using head nerve or tail nerve test pathways. However, midbody nerve elicited constricting responses showed dramatic short-term pseudoconditioning after a tail nerve US, although no pseudoconditioning at 1 hr (n=6). Midbody nerve elicited flaring responses showed weaker short-term pseudoconditioning to a head nerve US, but this pseudoconditioning was still present at 1 hr (n=5). Preliminary data indicate that similar pseudoconditioning can be produced using cutaneous stimuli.

229.6 CENTRAL SUPPRESSION OF DEFENSIVE REFLEXES IN APLYSIA BY NOXIOUS STIMULATION AND BY FACTORS RELEASED FROM BODY WALL. J.K. Krontiris-Litowitz, M.T. Erickson*and E.T. Walters. Dept. of Physiol. & Cell Biol., Univ. Texas Med. Sch., Houston, TX 77225.

Indirect evidence has supported the possibility that noxious stimulation causes suppressive as well as facilitatory effects on defensive reflexes in Aplysia (e.g. Carew, Walters, and Kandel, J. Neurosci. 1:1426, 1981; Goldberg and Lukowiak, J. Neurobiol. 6:395, 1984). To test this possibility further we have examined the effects of conditioning stimulation applied to a contralateral nerve on stable siphon responses elicited by ipsilateral head or tail nerve test stimuli. Whereas moderate intensity conditioning stimuli cause sensitization (Erickson and Walters, this volume), stronger conditioning stimuli (considered noxious because these levels easily exceed the threshold for copious inking) caused clear suppression of the siphon test responses in 9 of 10 preparations. These effects, which in this preparation are probably neurally mediated, display some interesting similarities to reflex suppression described in mammalian models of "stress-induced analgesia".

In the intact animal endocrine signals might also contribute to stress-induced reflex suppression. One set of candidates includes the "trauma factors" which are released into the wash (SBW) collected from noxiously stimulated isolated body wall and into hemolymph from intact stimulated animals. These factors produce body wall contraction and cardioacceleration (Cooper et al. and Krontiris-Litowitz et al., Soc. Neurosci. Abstr., 12:861, 1986). We tested the effects of 10 min bath application of SBW into an isolated well containing the abdominal ganglion on stable gill withdrawal responses elicited by siphon nerve stimulation at 15 min intervals: 23 preparations showed reflex suppression (0-90% of baseline) that recovered at least partially after SBW washout; 6 showed no recovery 30 min after washout; 2 showed no change; and 5 showed weak facilitation (110-140% of baseline). Preliminary evidence suggests that at least some of the suppression is produced by factors with molecular weights between 1000 and 10,000 daltons. Central suppression of the tail withdrawal reflex was also seen in 3 of 6 preparations, but tail withdrawal appeared to be more affected by complicating sensitization (perhaps from the tail pulling against the strain gauge) than gill withdrawal (measured with a photocell). SBW suppression of the gill withdrawal reflex was accompanied by suppression of evoked spike activity in LDG gill motor neurons (6 of 6 cases), in gill motor neuron L7 (5 of 7 cases), and in 6 of 9 unidentified neurons displaying simple excitatory responses to siphon nerve stimulation. We are presently examining effects on LE and VC sensory neurons.

229.7 **ANALYSIS OF THE DEVELOPMENTAL EMERGENCE OF SENSITIZATION IN *APLYSIA* REVEALS AN INHIBITORY EFFECT OF A FACILITATORY STIMULUS.** C.H. Rankin and T.J. Carew, Department of Psychology, Yale Uniersity., New Haven, CT 06520

Previous studies have shown that dishabituation and sensitization, produced by tail shock in the siphon withdrawal reflex in *Aplysia*, emerge as separate processes during development. Moreover, tail shock has been shown to exert an inhibitory effect on the reflex in animals in which sensitization had not yet emerged (Rankin and Carew, 1986, 1987). In these studies a single strong intensity (100 V) tail shock was used. In the present study a range of intensities was used in order to: (1) confirm the developmental separation of dishabituation and sensitization, and (2) to analyze the inhibitory process by examining its interaction with dishabituation.

Animals in early stage 12 were studied. In the first experiment, sensitization was examined in two groups: a Weak Shock group (N=10) received a 3 V tail shock which produced a small contraction and no inking; and a Strong Shock group (N=10) received a 150 V shock, which produced a massive contraction of the entire body and copious inking. To assess sensitization, siphon responses (measured as percent reduction of siphon area) to water-jet stimuli prior to shock were compared to responses following shock. Confirming previous experiments, sensitization was absent in this stage: Weak Shock, $\bar{x}$ pre=40.65, $\bar{x}$ post=37.9; Strong Shock, $\bar{x}$ pre=37.8, $\bar{x}$ post=27.3. In addition, tail shock produced inhibition of the reflex. Interestingly, the inhibition appeared to be greater for strong shock (28% reduction from pre-shock level) compared to weak shock (7% reduction).

We next asked whether inhibition could be revealed by competing it with a facilitatory process (dishabituation) known to exist at this stage. Furthermore, if strong shock produces greater inhibition than weak shock, then dishabituation produced by strong shock should be less than that produced by weak shock. Animals were first habituated (60 stimuli, 3 sec ISI) followed by either the weak (N=10) or the strong (N=10) tail shock. Three more stimuli were then delivered to assess dishabituation. Each group showed significant habituation ($p<.005$). In addition the Weak Shock group exhibited significant dishabituation ($p<.01$). In contrast, the Strong Shock group exhibited no significant dishabituation, consistent with the hypothesis that the strong shock produces sufficient inhibition to block dishabituation.

In conclusion, we have confirmed and extended our previous findings that early in development (1) dishabituation is present, while sensitization is absent; and (2) in the absence of sensitization, an inhibitory process can be revealed. This inhibitory process can be detected both by reflex reduction and by retardation of dishabituation.

229.8 **ANALYSIS OF NON-DECREMENTED EPSPs PRIOR TO THE EMERGENCE OF SENSITIZATION REVEALS AN INHIBITORY PROCESS IN *APLYSIA*.** T.G. Nolen and T.J. Carew. Departments of Psychology and Biology, Yale University, New Haven, CT 06520.

Behavioral studies of the siphon withdrawal reflex of juvenile *Aplysia* have shown that habituation, dishabituation and sensitization emerge according to different developmental timetables, and that prior to the emergence of sensitization, a facilitatory stimulus (tail shock) produces significant reflex inhibition (Rankin and Carew, 1987; and this volume). To explore the cellular basis of this inhibitory process, we have examined the effect of a facilitatory stimulus on afferent input from the siphon, monitored in the identified neuron R2, at different times during development.

Stage 12 of juvenile development was divided into 3 substages: Early, Mid and Late. As an analog of sensitization, we examined heterosynaptic facilitation of non-decremented EPSPs evoked in R2 by brief shocks to the siphon nerve. A brief train of shocks to the right connective (one of the neural paths for the tail input) served as the analog of tail shock. In Late Stage 12, connective stimulation produced significant facilitation of non-decremented EPSPs (N=8, $p<.004$). Likewise, in Mid Stage 12, significant facilitation was observed (N=6, $p<0.031$). However, in Early Stage 12 there was no significant facilitation (N=10, $P>0.4$). Thus there was a significant developmental trend in the emergence of the cellular analog of sensitization ($p<.01$), which paralleled the emergence of behavioral sensitization (Rankin and Carew, 1987).

Since the cellular analog of sensitization emerged between Early and Mid Stage 12, we compared the effects of connective stimulation in these two substages. Whereas the EPSPs in Mid Stage 12 were facilitated above their initial baselines (median= 125%), those in Early Stage 12 were depressed below baseline (median=83%). Thus, there was a significant and opposite effect of connective stimulation in the two substages ($p<.007$), reflecting the transition from inhibition to facilitation.

In conclusion, in Early Stage 12 *Aplysia*, the cellular analog of sensitization was not only absent but, as in the behavior (Rankin and Carew, 1987), significant inhibition was evident. Thus, we have found that tail shock and its analog (connective stimulation) can activate either facilitatory or inhibitory processes in the juvenile CNS, depending upon the stage of development. Because the inhibitory process emerges before the facilitatory process, it can be studied in relative developmental isolation, providing a useful preparation in which to explore the mechanisms underlying these opposite processes, which are known to be triggered in parallel in the adult (Marcus et al, this volume).

229.9 **A QUANTITATIVE ANALYSIS OF THE DEVELOPMENT OF THE CNS IN JUVENILE *APLYSIA*.** D. Cash* and T. J. Carew. Dept. of Psychology, Yale University, New Haven CT, 06520.

Several simple forms of learning emerge during juvenile development in *Aplysia* (Rankin and Carew, 1986, 1987). To analyze the cellular mechanisms underlying this development of learning, it is important to understand basic features of neuronal ontogeny during the juvenile phase. In this study we carried out a systematic analysis of the growth of the CNS by counting all the neurons in all the major central ganglia (buccals, cerebrals, pedals, pleurals, and abdominal) at different stages of juvenile development: Stage 9, 10, 11, Early 12 (12E), Late 12 (12L), and Adult. All ganglia were fixed, sectioned at 10μ, stained with cresyl violet, and scored with a blind procedure.

A striking pattern of neuronal proliferation was observed throughout juvenile development: there was a highly non-linear increase in cell number, with an initial stable phase followed by a dramatic increase during Stage 12. The mean number of neurons in the total CNS per stage was: Stage 9=1058 (N=7); Stage 10=1126 (N=10); Stage 11=1418 (N=10); Stage 12E=2860 (N=10); Stage 12L=7874 (N=2); Adult (50g)=9511 (N=2). Thus there was a highly significant increase in neurons across stages [$F(4,29)=361$, $p<.001$]. However, there were no significant differences between the early stages (9-11,about 40 days) but clear and significant differences among all later stages ($p<.01$ in each case), with the greatest increase in cell number occurring between Stage 12E and 12L(also about 40 days). This same pattern across developmental stages was seen in each of the central ganglia ($p<.001$ in each case). In addition, the relative contribution of each ganglion to the total CNS neurons remained constant throughout the 6 stages, indicating that the basic pattern of non-linear proliferation was reiterated in each of the central ganglia.

In conclusion, our results show that the proliferation of neurons during juvenile development in *Aplysia* is highly non-linear, and that Stage 12 is important for the large-scale addition of neurons in the CNS. The simultaneous increase in each of the CNS ganglia implies the presence of a general developmental signal, and in turn raises the question as to the nature of that signal and the factors which cause it to be triggered. In addition, it is interesting that a specific form of learning, sensitization, also emerges between Early and Late Stage 12 in two independent response systems in *Aplysia*: (1) siphon withdrawal (Rankin and Carew, 1987) and its cellular analog (Nolen and Carew, 1987); and (2) escape locomotion (Stopfer and Carew, 1987). These observations suggest that it may be possible in developing *Aplysia* to relate the emergence of particular forms of learning to the emergence of particular neurons and neural circuits.

229.10 **BEHAVIORAL DISSOCIATION OF DISHABITUATION, SENSITIZATION AND INHIBITION IN THE SIPHON WITHDRAWAL REFLEX OF ADULT *APLYSIA*.** E.A. Marcus, T.G. Nolen, C.H. Rankin and T.J. Carew. Departments of Biology and Psychology, Yale University, New Haven, CT 06520.

Several recent lines of evidence suggest that dishabituation and sensitization in *Aplysia* may be mediated by different mechanisms. Hochner et al (1986) have shown that there are two processes involved in presynaptic facilitation, one acting predominantly at decremented synapses, and the other at non-decremented synapses. In addition, behavioral and cellular developmental studies have shown that dishabituation emerges much earlier than sensitization (Rankin and Carew, 1987; Nolen and Carew, 1987). In the present study we asked whether the behavioral dissociation evident in juvenile *Aplysia* could also be observed in adult animals.

In the first experiment, we varied the intensity of a single shock (1 sec) to the tail. Three groups were run: STRONG (100mA; N=34); INTERMEDIATE (50mA; N=18); and WEAK (2.5mA; N=26). Half the animals in each group received dishabituation training (20 habituating water-jet stimuli to the siphon, followed by shock); the other half received sensitization training (no habituation prior to shock). Animals were tested 1, 10 and 20 min after shock. All shock intensities produced significant dishabituation: WEAK ($p<.025$); INTERMEDIATE ($p<.025$); STRONG ($p<.05$). In contrast, none of the shock intensities produced sensitization; in fact, immediately after shock, reflex depression was observed.

Since sensitization could not be produced by a single shock, we next examined whether it could be produced by repeated shocks. One group (N=10) received a single (50mA) shock; a second group (N=10) received four shocks (2 sec ISI). Sensitization (compared to baseline) was assessed 1, 10 and 20 min later. As before, no sensitization was evident in the single shock group; in fact, significant inhibition was evident (1 and 10 min, $p<.005$). In contrast, the four-shock group first exhibited significant inhibition (1 min, $p<.05$), followed by significant sensitization (20 min, $p<.05$). Thus, whereas a single shock is sufficient to produce dishabituation, multiple shocks of the same intensity are necessary to produce sensitization.

In conclusion, dishabituation, sensitization, and inhibition in the siphon withdrawal reflex can be dissociated in two ways: (1) during development by examining their ontogenetic timetables; and (2) in adults by varying the magnitude of a facilitatory stimulus. In light of the mechanisms known to contribute to the dissociation of dishabituation and sensitization in the adult (Hochner et al, 1986), and the recently described inhibitory processes in this reflex (Belardetti et al, 1987), it will be of interest to examine these and perhaps novel processes as they emerge and are assembled during ontogeny.

229.11 CLASSICAL CONDITIONING OF THE GWR OF APLYSIA AFFECTS THE GILL WITHDRAWAL RESPONSE ELICITED BY DEPOLARIZATION OF CENTRAL MOTOR NEURONS. K.D. Lukowiak and E.Colebrook. Neuroscience Research Group. University of Calgary, Calgary, Alberta Canada T2N 4N1.

The gill withdrawal reflex (GWR) of the in vitro siphon, mantle, gill and abdominal ganglion preparation can be classically conditioned by pairing of a weak tactile stimulus (the conditional stimulus, CS) to the siphon with a strong tactile stimulus (the unconditional stimulus, UCS) to the gill. Initially, the CS does not evoke a GWR but following pairing, the CS comes to elicit a GWR. Conditioning only occurs when the CS is paired with the UCS. Accompanying the behavioral change is a change in the synaptic efficacy between the central siphon sensory neurons and the central gill motor neurons. However, the changes in synaptic efficacy observed at this synapse are not necessary and/or sufficient to explain the observed behavioral changes. Changes must occur at other loci. We hypothesize that such a locus might be distal to the motor neurons. If this hypothesis is correct, then the ability of the gill motor neuron to elicit a gill movement should be altered following classical conditioning. We tested this hypothesis by depolarizing a gill motor neuron (L7 or LDG1) before, during and after classical conditioning training. The cells were depolarized to produce a similar number of action potentials on each test. Two control depolarizations were given at an interstimulus interval of 20 minutes. Following this, each preparation received 10 paired CS-UCS trials (intertrial interval 2 min). The gill motor neuron was then depolarized. The preparations were then rested for 1 hr and then the preparations received 10 more conditioning trials. The motor neuron was again depolarized. This procedure was repeated twice more so that each preparation received 40 paired CS-UCS presentations and was tested on 4 different occasions to determine if the efficacy of the motor neuron to cause a gill movement was altered. We found in initial experiments (n=5) that the gill withdrawal movement produced by depolarization of the motor neuron was significantly increased following classical conditioning in 3 of 5 preparations. In one of the other preparations there was no change and in the fifth preparation the ability of the motor neuron to cause a contraction decreased. In 3 control preparations where the CS and UCS were not paired, the ability of the gill motor neuron to cause a gill movement decreased. Thus, it appears that changes occur distal to the motor neuron during the course of classical conditioning and these changes may play a role in the mediation of associative learning in this preparation.
Supported by the MRC.

229.12 OPTICAL MEASUREMENT OF NEURON ACTIVITY DURING THE GILL WITHDRAWAL REFLEX IN APLYSIA. J.-Y. Wu,* D.P. Zecevic, J.A. London, M. Rioult,* and L. B. Cohen. Dept. of Physiology, Yale University School of Medicine, New Haven, CT 06510.

Optical measurements were used to obtain an overview of the number and activity of neurons in the Aplysia abdominal ganglion during the gill-withdrawal reflex. The isolated siphon preparation described by Kupfermann et al (1971) was used. The siphon was stimulated with a motor-driven nylon filament. Action potential activity in cell bodies was monitored via a 124 element photodiode array using absorption measurements on ganglia stained with the voltage sensitive dye, NK3041 (nee RH155). Extracellular electrode recordings were made from nerves and connectives and video-tape recordings were made of the gill movements. In recent experiments we used small adults (2 to 5 g) expecting that the reduced light-scattering of ganglia from smaller animals would improve the signal-to-noise ratios of the recordings.

In one preparation we detected activity in 90 neurons during a gill-withdrawal reflex after the preparation had been habituated with 15 mechanical stimuli given at a rate of 1 per minute. When this preparation was sensitized by shocking a connective, we detected activity in 150 neurons during a withdrawal reflex. stimulus.

We do not know how complete this recording was. In an earlier experiment, measurements during gill withdrawals were followed by a stimulation trial where the nerves and connectives were stimulated electrically to try to activate all of the neurons in the ganglion. By counting the number of neurons whose activity could be detected optically in this trial and comparing this with the number of neurons present in the ganglion, 1,100, (Coggeshall, 1967) we obtained a estimate of the completeness of the those recordings (25-40%). With this value we estimated that between 250 and 420 neurons were active during the withdrawal reflex in a sensitized preparation. Thus the neuronal substrate of the Aplysia gill-withdrawal reflex may be very complex. It may be difficult to determine the contribution of any individual neuron or synaptic connection to the total behavior.
Supported by NIH grant number NS08437.

229.13 THE CONTRIBUTION OF IDENTIFIED GILL MOTOR NEURONS TO GILL BEHAVIORS IN APLYSIA: MODELS AND NEW DIRECTIONS. J.L. Leonard* and K. Lukowiak (SPON: J.I. Goldberg). Dept. of Zoology, Univ. of Oklahoma, Norman, OK 73019 and Dept. of Medical Physiology, Univ. of Calgary, Calgary, AB T2N 4N1 Canada.

Interest in the response of the gill of Aplysia to tactile stimulation of the siphon (GWR) as a model system for analysis of neuronal mechanisms of learning has been based on the hope that it would be possible to identify the contribution of the CNS neurons to the behavior and to correlate changes in their activity with changes in gill behavior. An early model (Kandel 1979) suggested that PNS neurons make a negligible contribution to the behavior. This implies that CNS neurons are necessary for the GWR and changes in CNS neurons are sufficient to alter behavior. Experimental tests have shown that the CNS is not necessary and the PNS is sufficient for the GWR (Peretz et al. 1976, etc.). CNS pathways may be sufficient to mediate a GWR but this has yet to be demonstrated. Learning studies indicate a poor correspondence between changes in CNS neurons and changes in gill behavior (Lukowiak 1986; Colebrook 1986). Changes in CNS neurons may not be sufficient to alter behavior.

We describe here three alternative models: 1) the Parallel, 2) the Motivational, and 3) the Qualitative model. Under the Parallel model, the CNS and PNS are separate and equal. Either is capable of mediating a complete range of gill behaviors. They interact only at the level of gill muscle and "whoever is talking loudest is boss." This model predicts that the contribution of the CNS can only be identified in the absence of activity in the PNS. In the Motivational model, the CNS acts to alter the probability and/or intensity of gill movement but the type of gill movement made is determined by PNS circuits. The PNS is necessary and sufficient for coordinated gill movements, while CNS neurons may modulate but cannot command coordinated gill movements. The contribution of the CNS will be difficult to identify. The Qualitative model, however, predicts that CNS neurons act to determine the type of gill movement made. That is, a change in the activity of CNS neurons produces a qualitative change in behavior. As part of a test of this last model, we have used a blind procedure to characterize the movements evoked by intracellular stimulation of CNS neurons, in terms of our expanded list of gill Actions (Leonard and Lukowiak 1985). The type of elicited movement is consistent for a particular cell and the addition of Actions with increased stimulation is predictable.
Supported by MRC grants to K.L.

229.14 QUANTITATVE MODELING OF HIGHER-ORDER FEATURES OF CLASSICAL CONDITIONING IN APLYSIA. R.D. Hawkins. Ctr. for Neurobiol. & Behav., Columbia Univ., and NYS Psych. Inst., NY, NY 10032.

Recent studies indicate that a cellular mechanism of classical conditioning of the Aplysia gill- and siphon-withdrawal reflex is an extension of a mechanism underlying sensitization of that reflex (Hawkins et al., 1983; Abrams et al., 1985). This finding suggests that the mechanisms of yet higher forms of learning may similarly be based on the mechanisms of these simple forms of learning. Hawkins and Kandel (1984) illustrated this hypothesis by suggesting how several higher-order features of classical conditioning which are thought to have a cognitive flavor, including second-order conditioning, blocking, and the effect of contingency, could be accounted for by combinations of the cellular processes that underlie habituation, sensitization, and classical conditioning in the basic neural circuit for the gill- and siphon-withdrawal reflex. To test the feasibility of these ideas, I have incorporated them in a quantitative model and performed computer simulations of several of these features of conditioning.

The model is based on known cellular processes and circuitry in Aplysia. The basic circuit includes several sensory neurons (for the CSs and US) which synapse on a facilitator neuron and a motor neuron. The facilitator neuron is assumed to act on all of the terminals of the sensory neurons. Like the single-cell model of Gingrich and Byrne (1987), this model includes several subcellular processes in the sensory neurons: 1) synaptic depression, which I assume is due to long-lasting inactivation of Ca^{2+} channels, 2) presynaptic facilitation, which is thought to be due to cAMP-mediated closure of K^+ channels, leading to spike broadening and enhanced transmitter release (Klein and Kandel, 1980), and 3) activity-dependent presynaptic facilitation, which is thought to be due to priming of the adenylcyclase by Ca^{2+} which enters the sensory cells during spike activity (Hawkins et al., 1983; Walters and Byrne, 1983; Abrams et al., 1985). In addition, firing of the facilitator neuron is assumed to accommodate during prolonged stimulation. Free parameters in the model were adjusted by trial and error so that a single set of parameters would produce a wide range of conditioning phenomena. No attempt has been made to fit empirical data, since some of the behavioral phenomena addressed have not yet been tested in Aplysia.

The model successfully simulates acquisition, extinction, differential conditioning, second-order conditioning, blocking, the effect of contingency (degradation of learning by presentation of unpaired USs or CSs), and latent inhibition. These results demonstrate the feasibility of the ideas incorporated in the model. The validity of those ideas may be tested by recording from the neurons during behavioral conditioning. As a first step in that direction, I have shown that a dissected preparation consisting of the mantle organs and abdominal ganglion undergoes classical conditioning of gill withdrawal (mean increase = 278 $\pm$ 114% following paired training and 45 $\pm$ 36% following unpaired training, $p < .05$). It may now be possible to use this preparation to test the assumptions of the model.

230.1 GENETIC ANALYSIS OF THE ALZHEIMER'S-RELATED AMYLOID BETA PROTEIN GENE. R.E. Tanzi,* E.D. Bird, And R.L. Neve. (Spon: W.F. White) Program in Neuroscience Harvard Medical School; Dept. of Genetics, Children's Hospital; Mailman Res. Ctr., McLean Hospital, Boston and Belmont, MA.

We have isolated multiple cDNAs encoding the Alzheimer's related amyloid beta protein (ABP) gene from fetal brain, fetal eye, and HL60 promyelocytic leukemia cell line cDNA libraries. All three libraries yielded an identical (based on restriction mapping) 1.05 kb EcoRI fragment and an identical 1.6 kb EcoRI piece immediately 5' to the 1.05 kb fragment. In addition, a 1.8 kb EcoRI fragment (also immediately 5' to the 1.05 kb fragment) was isolated from the fetal brain library and was found to have a different restriction map from that of the analogous 1.6 kb piece. The 1.6 kb and 1.8 kb fragments detected identical bands on genomic Southern blots with the exception of an additional 9 kb band picked up with the 1.6 kb fragment and not by the 1.8 kb fragment. This result suggests that the differences in the restriction maps of the two clones may reflect rearrangement, insertion or deletion as opposed to simple base pair differences, perhaps involving differential RNA splicing. This hypothesis is supported by the observation of two RNA bands (3.5 kb and 3.7 kb) revealed by Northern blot analysis with all the ABP cDNAs. Further restriction mapping and sequencing are underway to resolve this possibility.

All of the ABP cDNAs map to chromosome 21, suggesting that amyloid plaques in the brains of elderly Down syndrome (DS, trisomy 21) patients may be due to gene dosage. Northern blot analysis shows a 50% increase in expression of the ABP gene in the DS brains compared to normal controls. To study the possibility of ABP gene duplication in patients with Alzheimer's disease (AD), we identified restriction fragment length polymorphisms (RFLPs) for the ABP cDNAs. Both the 1.6 kb and 1.8 kb clones detect RFLPs with EcoRI, BanI, and RsaI, suggesting that the two fragments reside at the same locus. These RFLPs along with an EcoRI RLFP revealed by the 1.05 kb fragment were used to test brain and lymphocyte DNA samples from normal individuals and patients with AD and DS for 2:1 dosage of ABP gene alleles by Southern blot analysis. A third copy of the ABP gene in an individual heterozygous for an RFLP would double the intensity of one of the two allelic bands comprising the RFLP. Visual inspection of 10 sporadic AD samples tested thus far has revealed no obvious duplication, whereas gene dosage is clearly evident in some DS samples. Further investigation using densitometry to document this result is underway.

230.2 ANALYSIS OF BETA AMYLOID mRNA IN ALZHEIMER'S DISEASE BY *IN SITU* HYBRIDIZATION. M.L. Cohen, T.E. Golde, M.F. Usiak, L.H. Younkin, and S.G. Younkin. Depts. of Pathology and Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

We have employed *in situ* hybridization to determine (1) which cells in the CNS express the beta amyloid (bA) gene and (2) if bA genes are overexpressed in Alzheimer's disease (AD). We focussed our analysis on the nucleus basalis of Meynert (nbM) which is composed of large cholinergic neurons known to degenerate in AD. We hybridized with an antisense bA oligonucleotide (bases 1895-1918 in the A4 region of the bA sequence published by Kang et al., Nature 325:733-736, 1987), an antisense human beta tubulin (bT) oligonucleotide (positive control), and a random sequence oligonucleotide (control for non-specific binding). The 3 probes were 24 bases long with G-C contents of 62-67%. In our initial experiments, probes were tailed with ^{35}S-dATP using terminal deoxynucleotidyl transferase. These probes produced erratic high background signals that were particularly severe in white matter. We circumvented this problem by tailing with ^{125}I-dCTP to activities of over 10^8 cpm/ug. Hybridizations were performed on thaw mounted 12 um cryostat sections cut from tissue blocks that had been rapidly frozen by immersion in isopentane cooled with liquid nitrogen. In each experiment, sections from AD and control cases were processed in parallel and the 3 probes were hybridized to 3 serial sections from each block. Sections were fixed 5 min in paraformaldehyde, treated with acetic anhydride, delipidated, and hybridized overnight at 50 C in 1X SSC, 1 mg/ml salmon sperm DNA, 1 mg/ml tRNA, 10% polyethylene glycol, and 1 nM probe. They were then washed 15 min in 0.2X SSC at 37 C X4, 60 min in 0.2X SSC at room T X2, and dipped twice in water. Sections were air dried, dipped in Kodak NTB-2 emulsion, and grains evaluated after a 4-8 day exposure. Tubulin and bA mRNA were present in large neurons of the cerebral cortex and nbM. Tubulin mRNA was readily detected in glial and endothelial cells, but we have so far been unable to detect bA mRNA in these cell types. Hybridization of the bA probe to nbM sections from 5 control and 5 AD cases well matched for age and postmortem interval yielded an average of 385% more specific grains (grains with bA probe - grains with non-specific probe) over cholinergic somata in AD ($p<0.01$). Parallel hybridization of the bT probe to nbM sections from these same cases yielded an average of 23% fewer specific grains over cholinergic somata in AD. Our finding of increased bA mRNA in a neuronal population degenerating in AD is particularly significant because it has recently been reported that a segment of chromosome 21 bearing the bA gene is duplicated in AD. (Supported by AG-06656 and the AFAR).

230.3 A REGIONAL AND LAMINAR ANALYSIS OF THE AMYLOID-β-PROTEIN mRNA IN THE NEOCORTEX OF NORMAL HUMAN, MONKEY AND ALZHEIMER'S DISEASE BRAINS BY IN SITU HYBRIDIZATION. J.H. Morrison*, D.A. Lewis*, G.A. Higgins*, W.G. Young*, D. Goldgaber**, D.C. Gajdusek**, and M.C. Wilson*. *Research Institute of Scripps Clinic, La Jolla CA 92037 and **NINCDS, Bethesda, Maryland 20892.

Using ^{35}S-labeled RNA probes we have determined that significant regional and laminar specificity exists for the distribution of the amyloid-β-protein mRNA and presumably the amyloid-β-protein that has been strongly implicated in the cellular pathology of Alzheimers' Disease (AD). In situ hybridization results demonstrate that in the prefrontal cortex of normal human the majority of large neurons in II, III, and V express an abundant level of amyloid-β-protein mRNA. Fewer neurons exhibit hybridization to amyloid-β-protein mRNA probes in the primary motor and somatosensory areas, and the signal is particularly low in layers IV and VI in these areas. Primary visual cortex is striking for its very high density of labeled cells in III and V and paucity of cells in the sublaminae of IV. The regional and laminar patterns in monkey are similar to normal human with two exceptions: 1) there is a notable heightened density of labeled cells in deep III and superficial V in monkey whereas the labeled cells are more evenly distributed throughout III and V of normal human and 2) primary visual cortex of monkey has a much lower density of labeled cells than human, particularly in layer III. In addition, extensive regional heterogeneity exists in the monkey temporal lobe. Most regions have a relatively high abundance, except for entorhinal cortex, which appears to have the lowest signal intensity of any temporal region. The AD cases studied thus far are highly variable, but in one well-studied case with a high density of plaques and tangles there is a marked decrease in both cell number and amyloid-β-protein mRNA content per labeled cell in prefrontal cortex as compared to the same region of normal human cortex. This decrease is particularly striking in layer III. We interpret the loss of cells and possibly decreased signal per cell in this case to be a reflection of the degeneration or low viability of the large cells prone to degeneration or tangle formation in layer III of prefrontal cortex in AD.

Some of the regional and laminar distribution patterns of amyloid-β-protein mRNA correlate well with the distribution of plaques and tangles in AD. However, exceptions such as the high density of labeled cells in layers III and V of human primary visual cortex, an area of relatively low density of plaques and tangles in AD, suggest that the presence of high intracellular levels of amyloid-β-protein may be necessary but not sufficient to confer vulnerability on a given cell class in AD.

230.4 QUANTITATIVE IN SITU HYBRIDIZATION SHOWS DIFFERENTIAL EXPRESSION OF AMYLOID-β PROTEIN mRNA WITHIN NEURONS OF THE HIPPOCAMPAL FORMATION IN ALZHEIMER'S DISEASE. G.A. Higgins *[1], D.A. Lewis [1], W.G. Young [1], S. Bahmanyar [2], D. Goldgaber [2], D.C. Gajdusek [2], J.H. Morrison [1] and M.C. Wilson *[1] (SPON: W.C. Weiderholt). [1] Research Institute of Scripps Clinic, La Jolla, CA 92037 and [2] NINCDS, Bethesda, MD 20892.

The hippocampal formation is a primary site for the neuropathological deposition of senile plaques (SP) and neurofibrillary tangles (NFT) in Alzheimer's Disease (AD). Recently, cDNA clones have been isolated to β-amyloid mRNA, whose product forms a major component of SP and possibly NFT. Additionally, the locus encoding β-amyloid protein has been genetically linked to inheritance of familial forms of AD and may be duplicated in sporadic AD.

We have mapped the neuroanatomical distribution of amyloid mRNA in the hippocampal formation of monkey, normal human, and in AD to determine which cells express β-amyloid, and whether this distribution correlates with the pathological desposition of amyloid in AD. In situ hybridization was performed on paraformaldehyde-fixed tissue sections treated with proteinase K and hybridized with an 35S-labelled RNA probe homologous to human β-amyloid mRNA.

Our results show that amyloid mRNA is expressed by neurons at high abundance in both normal and AD hippocampal formation and neocortex, but not in the cerebellum. In the hippocampus proper, β-amyloid transcripts are present at high abundance in pyramidal neurons of CA1, CA2, CA3 and the hilus, and in granule cells of the dentate gyrus. In contrast, expression at lower abundance was observed in neurons of the subicular complex and entorhinal cortex of normal human and monkey hippocampus. Quantitative analysis of cellular optical density showed that these entorhinal and subicular neurons exhibit less than half the hybridization signal of CA3 pyramidal neurons (eg. 48+11.75 versus 126+18.62). However, comparison of these same cell populations in AD suggests that entorhinal and subicular neurons have similar abundance of β-amyloid transcripts as CA pyramidal neurons.

Thus, amyloid β-protein mRNA is expressed widely in divergent neuronal populations of the hippocampal formation, including regions such as CA2 and CA3 which are relatively spared from AD neuropathology. However, the elevated abundance of amyloid mRNA in the subiculum and entorhinal cortex of surviving neurons in AD versus normal brain suggests the possibility that overexpression of the β-amyloid gene in these regions may lead to cell death and pathology in AD. Supported by a Hereditary Disease Foundation Fellowship to G.A.H. and NIH grant (NS23038) to M.C.W.

230.5 STRUCTURE AND EXPRESSION OF AMYLOIDOGENIC GLYCOPROTEIN IN RAT BRAIN: IMPLICATION FOR ALZHEIMER'S DISEASE, B. Shivers, C. Hilbich*, K. Beyreuther*, and P.H. Seeburg*. Center for Molecular Biology, University of Heidelberg, D-6900 Heidelberg, F.R.G.

Amyloid is deposited at characteristic locations in human brains from aged individuals and those with Alzheimer's disease or Down's syndrome. These deposits contain amyloid A4 polypeptide (M_r 4,500) which is derived from a larger glycoprotein (1-3) with the structural characteristics of a cell-surface receptor (1). We have cloned and sequenced the cDNA for the rat amyloidogenic glycoprotein and found it to encode a 695 amino acid glycoprotein sharing a 97% identity with its human homologue. Inspection of the 3 amino acid differences between the region encoding rat and human A4 amyloid polypeptide did not reveal why the human protein is more susceptible than the rat protein to proteolytic events which generate the A4 polypeptide. To examine the expression of this glycoprotein in rat brain, we incubated fixed, frozen sections of adult male rat brain with either amyloidogenic glycoprotein cRNA, or with antisera made against 3 peptide sequences found in the glycoprotein. We discovered that rat amyloidogenic glycoprotein is an abundant and ubiquitious brain protein appearing as patches on neuronal cell membranes. Its highest expression in rat brain occurs in regions (e.g. neocortex, hippocampus, olfactory tubercle) which contain amyloid deposits in humans as well as in some other regions which do not contain amyloid deposits in humans (e.g. cerebellar cortex, thalamus). Since aged rat brains rarely contain amyloid deposits, it appears that elevated levels of expression of this highly conserved glycoprotein alone do not invariably lead to amyloid deposition, focussing our attention on local factors which foster aberrant catabolism of this normally-expressed brain protein. The high abundance and wide distribution of amyloidogenic glycoprotein protein in rat brain as well as its patch-like appearance on neuronal membrane surfaces support a role for this protein in cell-cell contact.

1. Kang, J. et al. Nature 325: 733 (1987).
2. Goldgaber, D. et al. Science 235: 877 (1987).
3. Tanzi, R. et al. Science 235: 880 (1987).

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230.6 ANALYSIS OF GENE DOSAGE FOR THE β-AMYLOID PRECURSOR PROTEIN IN NORMAL AGED HUMANS AND PATIENTS WITH ALZHEIMER'S DISEASE. M.B. Podlisny, G. Lee and D.J. Selkoe* (SPON: S. Khoshbin). Center for Neurologic Diseases, Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115.

The deposition of extracellular amyloid filaments in cortical and meningeal microvessels and in the centers of neuritic plaques is an invariable accompaniment of Alzheimer's disease (AD). Protein chemical and immunochemical analyses of partially purified vascular or plaque core amyloid have shown that a major proteinaceous component of these filaments is a 4.5 kDa hydrophobic protein designated the β-protein. The recent isolation of cloned cDNAs for the β-protein precursor in several laboratories has revealed that the gene is localized to the midportion of the long arm of chromosome 21. This finding provides an explanation for the almost constant development of AD-type amyloid deposits in patients with trisomy 21 on the basis of increased gene dosage. Two studies reporting duplication of a small segment of the long arm of chromosome 21 containing the amyloid β-protein in patients with sporadic AD have recently appeared. It is important to confirm these data in a larger number of patients.

We screened a human brain cDNA library with an oligonucleotide encloding the amyloid β-protein residues 20-29 (phe→gly). Of 7 clones detected, the largest clone (designated β-6) contained a 1.6 kb insert containing bases 849 - 2451 of the full-length β-protein precursor cDNA reported by Kang et al.[1] A 0.95 kb Eco R1 fragment of this insert (encoding amino acids 284 - 600 of the precursor) was used to probe Eco R1 digests of human genomic DNA purified from fresh lymphocytes of 11 normal aged individuals (ages 64-89), 4 patients with trisomy 21 (ages 27-54) and 15 patients with clinically typical AD (ages 62-89). On each Southern blot, β-6 plasmid standards representing 0.5, 1, 2, 4 or 8 gene copies/haploid genome were included to establish gene copy number and to verify linearity of the hybridization signals. Each blot was also probed with a cDNA for human MAP2 (chromosome 2; kindly provided by K. Kosik) which served to control for sample uniformity from lane to lane and whose signal was compared to those from the β-6 probe for a relative assessment of gene dosage. The ratio of β-6/MAP2 hybridization signal in Down's samples was ~1.5 times that in normal individuals. Results of the initial 15 AD samples as well as additional cases will be presented. Ascertaining the frequency at which increased β-protein gene dosage occurs in familial and sporadic AD will determine whether alternative mechanisms for the increased deposition of amyloid proteins in AD need to be invoked.

[1] Nature 325:733-736, 1987.

230.7 α-1-ANTICHYMOTRYPSIN, A SERINE PROTEASE INHIBITOR, IS A COMPONENT OF AMYLOID DEPOSITS IN ALZHEIMER'S DISEASE BRAIN. C. R. Abraham*, D. J. Selkoe and H. Potter, Dept. of Neurobiology, Harvard Med. Sch., and Brigham and Women's Hospital, Boston, MA 02115.

Neuropathological analysis of Alzheimer's disease brains reveals three main characteristic lesions: neurofibrillary tangles, senile plaques and fibrous deposits in cerebral and meningeal blood vessels. The senile plaques contain a rim of degenerating or regenerating neurites and an amyloid core. Similar amyloid is also deposited in the blood vessel walls. The amyloid is ultrastructurally and antigenically different from the filaments that comprise the intracellular neurofibrillary tangles, the former having a 6-10 nm width while the latter are two helical filaments with a periodicity of 160 nm and a width of ~ 20 nm. Senile plaques and vascular deposits are present only in normal aged individuals (humans and other mammals), in Alzheimer's disease, and Down's syndrome, whereas neurofibrillary tangles are found in several other conditions. Thus amyloid deposits are a more specific marker of normal aging and Alzheimer's disease.

We used two approaches — molecular cloning and immunochemical analysis — to identify one of the components of Alzheimer's disease amyloid deposits as the serine protease inhibitor α-1-antichymotrypsin. Antibodies produced against isolated amyloid filaments from postmortem human brain which label amyloid cores in senile plaques and amyloid deposits in blood vessels were used to identify normal liver as expressing large amounts of certain amyloid components. The antiserum was then used to screen a liver λgt11 c-DNA expression library, which yielded three related clones. The 1.5 Kb insert was subcloned in pGEM 4 for sequencing and production of labelled probes. Northern blot analysis revealed a 1.5 Kb message which proved that we have a full-length c-DNA. The largest expression was seen in the liver, but some is also present in normal brain, and perhaps in a higher amount in Alzheimer's disease brain. DNA sequence analysis of these clones showed them to contain the c-DNA coding for α-1-antichymotrypsin, a potent protease inhibitor produced in the liver and present at high concentration in the serum. Antibodies to purified α-1-antichymotrypsin stained the amyloid deposits in Alzheimer's brain, confirming the presence of this protein in the lesions. Models in which the protease inhibitor α-1-antichymotrypsin could play an active role in the development of amyloid deposits in Alzheimer's disease, together with the other known component of the amyloid, the β-protein, will be discussed.

230.8 LOSS OF ALKALINE RIBONUCLEASE INHIBITOR PROTEIN ASSOCIATED WITH ALZHEIMER NEURONAL DEGENERATION. R.E. Majocha, E.M. Sajdel-Sulkowska, A. Rodenrys*, M. Ventosa-Michelman* and C.A. Marotta (SPON: A. Pope). Program in Neuroscience and Department of Psychiatry, Harvard Medical School, McLean Hospital and Massachusetts General Hospital, Boston, MA 02114.

In early studies we first demonstrated that postmortem (pm) control and Alzheimer's Disease (AD) brains yield intact RNA in amounts suitable for in vitro translation and for the preparation of recombinant cDNA libraries that contain cDNAs of identified proteins (see Zain et al, these Proceedings, 1987). We and others observed, however, that the yield of AD RNA is often diminished and the decrease is not solely a result of antemortem hypoxia or pm storage since biopsied cortex from living AD patients also contains reduced neuronal RNA (J. Neurol. Neurosurg. Psych. 44:97 and 49:229). Both transcriptional and degradative factors are under investigation. We have observed that the activity of AD lysosomal acid RNase is increased, total alkaline ribonuclease is unchanged and that the activity regulated by the ribonuclease inhibitor protein (rip) is increased in many cases even in the presence of a reducing agent. We prepared a monoclonal antibody (mab) to human rip to examine whether or not the distribution of the protein bears a relation to the neuronal degeneration seen in AD. In frontal cortex of controls, in addition to some glial staining, a dark reaction product in layer III neurons was localized to dendrites and the cell body. In AD cases there were decreased numbers of darkly stained pyramidal cell bodies, and dendrites were often weakly stained. The mab reacted strongly with astrocytes, which is consistent with the high levels of AD GFAP mRNA we routinely obtain (Zain et al, these Proceedings, 1987). Double staining with the mab and with thioflavin S or with antibodies to paired helical filaments demonstrated the progressive loss of neuronal rip that was replaced by neurofibrillary tangles. The immunocytochemical results indicate that the loss of rip activity previously noted in AD is not entirely related to the oxidation state of the protein. Model studies of others support the concept that the normal in vivo metabolism of RNA involves the combined action of acid and alkaline RNases. Cataldo et al (these Proceedings, 1987), using antibodies to lysosomal markers, demonstrated increased immunoreactivity in layer III neurons. The combined action of lysosomal and non-lysosomal enzymes may act to reduce RNA levels in AD neurons. However, the occasional instability observed for pm AD RNA, as assessed by Northern blots, may result from factors unrelated to the normal degradative pathway. Supported by AG04522.

230.9 ELEVATED PHOSPHORYLATION OF THE M_R 60,000 PROTEIN (P60) IN ALZHEIMER'S DISEASE. T. Saitoh, L.A. Hansen*, G. Cole*, R.D. Terry*, and K.R. Dobkins*. Univ. of California, San Diego, Sch. of Med., Neuroscience Dept., M-024, La Jolla, CA 92093.

M_r 60,000 protein (P60) phosphorylation is elevated in cytosol from Alzheimer brain as compared to control brain (T. Saitoh and K. R. Dobkins, Proc. Natl. Acad. Sci. USA, 83:9764-9767, 1986).

Because reactive fibrous gliosis accompanies neuronal degeneration, any increased level of a biochemical marker in Alzheimer's disease might derive from this gliosis. Accordingly, we studied P60 phosphorylation in brain tissue from Pick's disease and multi-infarct dementia where the degree of gliosis is substantial, but P60 phosphorylation in these diseases was not significantly different from the control. Thus, P60 phosphorylation seems to be specifically elevated in Alzheimer tissue.

We tried to correlate P60 phosphorylation with numbers of neurofibrillary tangles and numbers of neuritic plaques, two indices of the severity of Alzheimer's disease. The more tangles observed, the more P60 phosphorylation. The plot of tangle numbers against P60 phosphorylation gave a linear correlation ($r=0.85$; $P<0.001$). Interestingly, the number of plaques, however, did not have a significant correlation with the degree of P60 phosphorylation suggesting that P60 phosphorylation may be involved in the formation of neurofibrillary tangles. Overphosphorylation is a characteristic of neurofibrillary tangle constituents and, as described below, P60 may be a protein kinase.

We asked if P60 is a component of protein kinase. All protein kinases autophosphate. If P60 is a protein kinase or makes a complex with a kinase, increasing the viscosity of reaction medium should not affect the degree of P60 phosphorylation, although this manipulation decreases P60 phosphorylation if P60 kinase is physically apart from P60. Increasing the reaction viscosity by high sucrose decreased the total phosphorylation, but not P60 phosphorylation, supporting autophosphorylation. Another way to test the association of protein kinase and its substrate is to change the protein concentration in the reaction. If protein kinase is associated with substrate (or part of it), reducing the protein concentration does not affect the relative degree of P60 phosphorylation. The result showed that relative P60 phosphorylation does not depend on the protein concentration of the reaction mix, although total protein phosphorylation decreased as proteins get diluted. Both the viscosity experiment and protein concentration experiment are consistent with P60 being either a kinase or associated with a kinase.

We then tested the metal requirement for P60 phosphorylation and found it to be stimulated two-fold by Mn^{2+}. Tyrosine phosphorylation is known to be stimulated Mn^{2+} and, in fact, P60 is phosphorylated at a tyrosine residue.

230.10 THE TAU1 EPITOPE IS NOT IN THE CARBOXY TERMINUS OF A TAU cDNA FUSION PROTEIN K.S. Kosik,* R. Neve, G. Lee* and L.I. Binder.* (Sponsor: M. Yamamoto). Brigham and Women's Hosp. and Harvard Medical School, Boston, MA 02115 and University of Alabama, Birmingham, AL 35294

The microtubule-associated protein, tau, is the most strongly implicated component of the Alzheimer neurofibrillary tangles. Tau-immunoreactivity is also present in the dystrophic neurites observed in wide regions of the Alzheimer cortical neuropil. Recently, we have isolated a cDNA clone for the human tau gene from a human fetal brain expression library (Neve et al., Mol Brain Res, 1986) and confirmed its identity by sequence homology with a mouse tau cDNA. This tau clone obtained by screening the library with a mouse tau cDNA contains the entire coding region of tau as well as some 3' untranslated message. A second human tau clone was obtained from the same library by screening the library with an affinity-purified tau antibody (Drubin et al., J Cell Biol, 1984). The antibody-selected clone hybridized to the original human tau clone. When the restriction map of the expression clone was compared to that of the full-length clone, it was found that only the carboxy one-third of the tau coding region was contained in the expression clone. The resulting fusion protein was tested on Western blots for its reactivity with a number of different tau antibodies. The antibody designated tau1 (Binder et al., J Cell Biol, 1985) is reactive with all the heterogeneous forms of tau in a number of species including human. However, it reacts with Alzheimer neurofibrillary tangles only after treatment with alkaline phosphatase and is thus considered directed at a phosphatase-sensitive site (Grundke-Iqbal, PNAS, 1986). Tau1 did not react with the fusion protein containing the carboxy one-third of tau. It is therefore unlikely that the tau1 epitope is within this carboxy terminal region. It is, however, possible that the epitope is obscured by the presence of the beta-galactosidase fused to the tau fragment. A polyclonal antibody raised against Alzheimer neurofibrillary tangles did react with the fusion protein, as well as anti-beta-galactosidase, both of which demonstrated a fusion protein of approximately 20 kDa. Two other antibodies did not react with the fusion protein. They were 5E2, a monoclonal antibody raised against fetal tau, and a second polyclonal antibody raised against Alzheimer neurofibrillary tangles with apparent specificity for tau on immunoblots. It is likely that Alzheimer paired helical filaments contain more than a single tau epitope. (Supported by NIH grants AG06601 and NS00835)

230.11 THE EVOLUTION, DISTRIBUTION AND MORPHOLOGY OF TAU IMMUNOREACTIVITY SUGGEST A CEREBROVASCULAR ORIGIN OF DEMENTIA OF THE ALZHEIMER TYPE. STRAIGHT OR TWISTED TUBULE IS A MORE APPROPRIATE NAME THAN PAIRED HELICAL FILAMENT. S. Ch. Papasozomenos. Dept. of Path. and Lab. Med., Univ. of Texas Med. Sch., Houston, TX 77225.

In a prospective study, autopsy and biopsy formalin-fixed tissues were taken from throughout the CNS of 4 patients with Alzheimer's disease (AD), 10 patients with senile dementia of the Alzheimer type (SDAT), 3 demented patients with Down's syndrome (DS) and controls. Two monoclonal antibodies against tau, Tau-1 and Tau-2, an antiserum against GFA protein and the peroxidase-antiperoxidase technique at the light and EM levels were used. Both Tau-1 and Tau-2 stained the neurofibrillary tangles (NFTs), the neurites but not the amyloid in senile plaques (SPs), and swollen neurites scattered in the neuropil. Tau-1 required dephosphorylation of sections prior to immunostaining. Tau-2 stained also astrocytes. The distribution and morphology of tau immunoreactivity was similar in all 3 forms of dementia of the Alzheimer type (DAT), but the rapidity of evolution and the amount were most severe in DS and least severe in SDAT. Tau immunoreactivity was first noted in astrocytes in the amygdala, subiculum, Sommer's sector, the neocortical layer V, basal ganglia, brainstem, pontine nuclei, medulla and cerebellum. Tau-2-positive astrocytes participated in the formation of SPs but GFA-positive reactive astrocytes associated with SPs and in other regions were Tau-2-negative. In neuronal perikarya, tau immunoreactivity first appeared as excessive granules representing stained ribosomes and later as NFTs. In full-blown cases, the most prominent patterns of selective neuronal vulnerability were: a) a horizontal laminating cortical pattern, with layer V containing most of the NFTs and swollen neurites, layers II and III containing most of the SPs, layer I being the least affected, and the depths of sulci being more severely involved than the crests of gyri; b) the primary sensory areas were the least affected neocortical regions; c) the subiculum, CA1 and CA2 were the most and the CA3, CA4 and presubiculum were the least involved regions of the hippocampus; d) SPs were frequently associated with vessels; e) the basomedial region of the amygdala contained the most and the lateral region the least number of SPs; f) cholinergic, noradrenergic, serotonergic and dopaminergic neurons were equally affected; and g) efferent-afferent associations did not correlate with the patterns of vulnerability. Controls were negative.

These findings are best explained by a hypothesis which includes: 1) a diffusible factor that originates from blood vessels and damages both vulnerable neurons and astrocytes, and 2) known patterns of regional brain vascular densities and angio-architecture.

EM immunostaining of formalin-fixed tissues showed that NFTs and swollen neurites were made of mostly straight tubular structures with intensely stained walls, unstained lumens and occasionally a visible protofilamentous substructure with helical arrangement of protofilaments. The relationship between fixation in glutaraldehyde and the number of paired helical filaments is under further investigation. These findings suggest that straight or twisted tubules are a more appropriate name for the filamentous structures that comprise the NFTs than paired helical filaments.

230.12 UBIQUITIN CONJUGATES: A NEW COMPONENT OF ABNORMAL NEURONAL FILAMENTS IN NEURODEGENERATIVE DISEASES. V. Manetto*, P. Gambetti, M. Tabaton*, P. Mulvihill*, V. Fried*, H. Smith*, L. Autilio-Gambetti, G. Perry. Institute of Pathology, Case Western University, Cleveland, OH 44106 and St. Jude Children's Research Hospital, Memphis, TN 38101

A distinctive feature of several neurodegenerative diseases is the presence of neuronal cytoplasmic inclusions. Neurofibrillary tangles (NFT) of Alzheimer disease, Pick bodies (PB) of Pick disease, Lewy bodies (LB) of Parkinson disease and neurofibrillary tangles of Progressive Supranuclear Palsy (NFTPSP) are all inclusions composed primarily of filamentous structures. Although the chemical nature of these inclusions has not yet been established, immunocytochemical studies have shown that all share epitopes with phosphorylated neurofilaments and, except for LB, with tau proteins.

Recently, it has been reported that ubiquitin, a protein involved in non-lysosomal proteolysis and other cell functions, forms stable conjugates with NFT of AD. An alteration in the ubiquitin dependent proteolytic system has been postulated in this condition. In order to test if stable ubiquitin conjugates are unique to AD, we investigated the presence of such conjugates in inclusions characteristic of other neurodegenerative diseases, known to share epitopes and insolubility properties with NFT of AD. Using eight monoclonal antibodies (Mab) directed against different ubiquitin conjugates, we carried out an immunocytochemical study at the light and electromicroscopic level. We found that five of the eight Mabs immunostained all these inclusion and that the filamentous constituents of these inclusions, i.e. the paired helical filaments in NFT of AD, straight filaments in PB and in NFT of PSP, as well as filaments radiating from the core of LB in Parkinson disease, were decorated at the ultrastructural level. No other intracytoplasmic organelles were immunodecorated, indicating that ubiquitin is conjugated with the filamentous components of these inclusions. These findings suggest that the ubiquitin system is involved not only in AD, but also in Parkinson disease and other neurodegenerative diseases. Ubiquitin's role in the formation of these inclusions remains to be elucidated.

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231.1 **REAL-TIME VIDEO IMAGING OF ELECTRICAL EVENTS IN THE OLFACTORY BULB USING A POTENTIOMETRIC DYE.** J. S. Kauer. Depts. of Neurosurgery and Anatomy and Cell Biology, Tufts-N.E.M.C. Boston. MA. 02111.

Optical recording methods offer the opportunity to observe global responses from neural circuits after a variety of experimental manipulations. The use of diode arrays has permitted analysis of rapidly changing dye-related activity at as many as 144 simultaneous sites. An improvement in spatial resolution was achieved by Blasdel and Salama (Nature 321:579-585, 1986), who used averages of many video frames to image static dye absorption changes related to ocular dominance and orientation columns in monkey striate cortex. In the present paper sequences of real-time video images of changes in a fluorescent voltage-sensitive dye have been generated after a shock to the olfactory nerve (ON). The frame sequences may be displayed as short, animated 'movies' and can thus describe the temporal progression of ensemble activity in identifiable bulbar layers.

Series of 16 sequential, real-time (30 frames/sec) video images (128 x 128 pixels x 8 bits) of fluorescence changes in dye RH414 (kindly provided by Dr. A. Grinwald) were generated after stimulation of the ON. Images were acquired using a Dage Newvicon TV camera, and were digitized and enhanced using computerized processing. The time course of the fluorescence changes correlated well with simultaneously recorded field potentials. Signals were not seen in unstained tissue nor in the absence of electrical stimulation, and were abolished after treatment of the bulb with 10 um. tetrodotoxin. Stimulation of the entire ON elicited widely distributed fluorescence changes, first in the glomerular layer, then within the deeper bulbar layers, and subsequently in the medial olfactory tract. Signals were observed in the contralateral bulb with delays appropriate for activation via centrifugal fibres stimulated by the ipsilateral bulb. Stimulation of widely separated medial and lateral ON fascicles also elicited widespread fluorescence changes in the bulbar layers, suggesting that small ON fascicles have influence over broad regions of the bulbar ciruitry. The onset latency of the fluorescence signals was increased after stimulation of the peripheral fascicles, reflecting the increase in propagation delay.

This study has provided the first global view of the temporal and spatial characteristics of olfactory bulb activity after electrical stimulation. The method holds promise for permitting high resolution analysis of the distribution of potentials generated by physiological, odor stimulation as well as for allowing investigation of global processes in other CNS regions.

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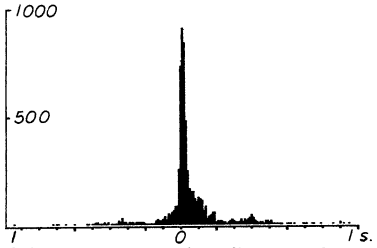
231.2 **THE ALL-INTERVAL PARADIGM AND ANALYSIS OF THE STRUCTURE OF NEURONAL SPIKE TRAINS.** R. Lestienne* and B.L. Strehler* (Sponsor: S.W. Bottger), CNRS, Paris (France) and Molecular Biology, ACBR, U.S.C., Los Angeles, CA., 90089

It has recently been demonstrated that large numbers of precisely replicating patterns of discharge (<1/7000th s. difference between copies) are imbedded in neuronal pulse trains (PNAS, 83,(1986), 9812) and that the capacity to produce such replicas decays is a direct function of the number of pulses present in each copy of the replica (to be published, Brain Res.) The above suggested to us that the generation of such specific patterns results from the facilitation of specific synaptic pathways to a common output neuron and that the decay rate is determined by the probability that a given involved synapse loses its ability to generate one or another pulse in a complex pattern. This in turn implies that the occurence of a given complex and replicating pattern (e.g a triplet of pulses) should be followed by copies of less complex patterns (e.g. doublets) whose intervals correspond to those present in the more complex associated pattern. As shown in the accompanying figure, we found that indeed there is a close association in time between the occurence of replicating triplets and the separate emission of component doublets as seen in outputs from neurons of the monkey visual cortex in response to Heubel and Wiesel stimuli. Doublets are emitted in the immediate vicinity (+/- 20 ms) of both the first and the second copy of the triplet. An interpretation of these findings is as follows: When an appropriate input is presented, specific (coded) patterns of output in time are generated that are decodable by receiving cells that possess appropriate delay line networks for decoding such inputs; and that a temporary record of the decoding of such patterns is stored in specific patterns of synapses of the responding cells, perhaps through Hebb-like selective postsynaptic facilitation. The use of the All-Interval Paradigm makes the detection of such patterns of events possible and capable of being evaluated through appropriate computer analysis.



Histogram of time between onset of the first copy of a replicating triplet and separate doublets identical to those present in such triplets. Abscissa: Time between occurence of triplet and related doublet; Ordinate: Number of doublets/0.01 s. Only triplets whose copies are < 0.5 sec apart are included. (# of triplets: 1482)

231.3 **SEROTONERGIC PRIMARY SENSORY NEURONS FEED BACK TO MOTOR NEURONS IN THE CRAB STOMATOGASTRIC GANGLION.** P.S. Katz and R.M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

We have previously reported that serotonergic muscle receptor cells innervate the gastro-pyloric region of the stomach in crabs (Katz and Harris-Warrick, Neuroscience Abstr., 12:1207, 1986). These Gastro-Pyloric Receptor cells (GPR cells) are the sole source of serotonergic fibers to the stomatogastric ganglion (STG) in the crabs *Cancer irroratus* and *Cancer borealis*. In these crabs there are 2 bilaterally symmetric pairs of GPR cells that innervate stomach muscles and project centrally to the STG and higher ganglia. One cell (GPR1) innervates a muscle (gm8b) that is responsible for closing the lateral teeth of the gastric mill. This muscle is controlled by two motor neurons (MG and LG). The other GPR cell (GPR2) innervates two different muscles (gm9a and cpv3a) that in turn receive motor input from 2 different motor neurons (MG and LP) that are members of the gastric and pyloric Central Pattern Generators (CPGs), respectively.

In this study, we examined the response properties of the GPR cells as well as their effects on the motor neurons. Both cells fire increasing numbers of action potentials in response to increases in muscle tension generated either by imposed stretch of the muscle or by motor neuron stimulation (isometric tension); however their response properties are different. GPR1 responds phasically to increased tension and adapts to repeated muscle stretches. GPR2 responds more tonically, generating continuous spiking at a constant rate if the muscle remains stretched for long periods of time.

Both cells have effects on motor neurons in the STG. GPR1 causes one-for-one EPSPs in the LG motor neuron, but inhibits the MG. We have not yet determined whether these are direct effects or are mediated through other cells. Exciting GPR2, by stretching gm9a, can have modulatory effects, including prolonged activation of the pyloric rhythm. Bath applied serotonin can mimic the activation of the pyloric CPG.

The different response properties of the 2 GPR cells may be related to the cells' different sensory feedback roles. We have shown that GPR1 is phasically active and excites the LG motor neuron in what may be an example of a classical resistance reflex feedback loop. In contrast to a classical reflex, the tonic excitation of GPR2 may function to activate or modulate the pyloric CPG under certain conditions of food entering the pylorus. The two muscles innervated by GPR2 are located at the border between the gastric mill and the pylorus and thus may be in a position to sense the passage of food between the two stomach regions.

In conclusion, we have demonstrated that: 1) Serotonergic input to motor systems can arise from peripheral sensory cells and therefore need not be considered only to descend from higher control centers in motor systems. 2) Proprioceptive feedback can be modulatory in addition to having direct excitatory or inhibitory effects.

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231.4 SENSORY INPUT OR SPONTANEOUS CENTRAL RHYTHMICITY CAN "SWITCH" A NEURON FROM ONE NEURAL NETWORK TO ANOTHER. S.L. Hooper and M. Moulins, Lab. de Neuro. et Physiol. Comp., 33120 Arcachon, France

The pyloric (cycle period 1-2 s) and the cardiac sac (CS, period 30-90s) are two different central pattern generators (CPGs) of the stomatogastric nervous system (SNS) of the lobster, Palinurus vulgaris. Brief electrical stimulation of the pln, a SNS sensory nerve, or stretch of the stomach region innervated by the pln, induces 1) a CS CPG burst; 2) excitation of the ventricular dilator neuron (VD) of the pyloric CPG during the CS burst, followed by a long lasting (av. 36s) silence in which the VD no longer fires with pyloric activity; and 3) inhibition during the CS burst of another pyloric neuron, the inferior cardiac (IC), followed by a long lasting phase shift of the IC activity in the pyloric pattern. The same changes in VD and IC activities occur during spontaneous CS activity, and thus the VD functionally "switches" from the pyloric to the cardiac sac CPG when the latter is rhythmically active.

To characterize the cellular bases of these changes we used pharmacological agents and Lucifer Yellow photoinactivation (Miller & Selverston, Science, 1979) to isolate the VD and IC from pyloric synaptic input. Isolated VDs still respond to pln stimulation or CS activity as noted above. The VD is excited during a CS burst by known cardiac sac CPG elements, the IV neurons, but its ensuing silence is due to the long lasting effects of as yet unidentified modulatory neuron(s) that likely act by suppressing the VD's regenerative membrane properties. The effects of pln stimulation or spontaneous CS activity on the IC in the intact pyloric CPG, however, are indirect; isolated ICs are unaffected by pln stimulation or spontaneous CS activity. These effects instead result from an inhibitory synapse from the VD to the IC. During a CS burst (either spontaneous or triggered by pln stimulation), the IC is inhibited because of the VD excitation during CS bursts. After the CS burst, the VD is silent and so no longer inhibits the IC; thus the IC phase changes from that observed during normal pyloric activity, in which the VD participates. This conclusion is supported by photoinactivating only the VD, which results in IC phase shifts similar to those caused by pln stimulation or spontaneous CS activity.

We conclude: 1) Both sensory and rhythmic neural activity can induce a long lasting quiescence in the VD, and since the VD normally participates in the generation of the pyloric pattern, its absence results in a functional rewiring of the pyloric CPG. 2) When thus removed from the pyloric CPG, the VD can express the activity of another CPG, and then uses its synapses to pyloric CPG neurons to modify the pyloric output during cardiac sac CPG bursts. Supported by a NSF/CNRS exchange fellowship to S.L.H.

231.5 POTASSIUM CHANNEL BLOCKADE INDUCES RHYTHMIC ACTIVITY IN A CONDITIONAL BURSTER NEURON IN THE LOBSTER STOMATOGASTRIC GANGLION. R.M. Harris-Warrick and B.R.Johnson , Sect. Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853

The anterior burster (AB) neuron is a conditional burster in the stomatogastric ganglion of the spiny lobster, Panulirus interruptus . When isolated from all synaptic input, it loses its burst-generating abilities. However, unpatterned neuromodulatory input reinitiates rhythmic bursting. This induction of bursting could occur by two general classes of mechanisms: 1) activation of the conductances that support bursting, or 2) removal of inhibitory conductances that prevent the cell from bursting. We have found that several treatments which inhibit potassium currents can uncover bursting in quiescent AB cells, supporting the second mechanism.

The AB cell was isolated in situ from all detectable synaptic input by a sucrose/TTX block of descending inputs from other ganglia and by 6-carboxyfluorescein photoinactivation of the three cells within the STG that synapse on the AB. Addition of 1-2mM 4-aminopyridine (4-AP), which selectively blocks a current similar to IA in STG cells (Hartline et al, Neurosci. Abst. 12 :358,1986), induced regular and rhythmic bursting in quiescent AB cells. Tetraethylammonium chloride (TEA:5-10 mM), which blocks several other potassium channels (but not IA) in STG neurons, also induced bursting; these bursts were of larger amplitude than those seen with 4-AP.

Rhythmic bursting can also be induced by a reduction of calcium entry into quiescent AB cells. This was accomplished by superfusion with 0-Ca2+-high Mg2+ saline or addition of 0.1-0.5 mM Cd2+ or 10 mM Co2+ to the normal saline. With all three treatments, bursting began within 2 min. and was stable for several minutes. However, the bursting mechanism itself depends on calcium entry (Gola and Selverston,J.Comp.Physiol., 145 :191,1981), and with time the cell stopped rhythmic activity. One possible explanation for this induction of bursting by reduced calcium entry is that a tonic calcium-activated potassium conductance contributes to the inhibiton of bursting in the quiescent AB cell. Supporting this hypothesis, we found that apamin, a polypeptide toxin from bee venom which blocks one class of calcium-activated potassium channel, can also induce slow bursting in quiescent AB neurons.

These results suggest that in the non-bursting state of the AB cell, the conductances that underlie bursting are present but their expression is actively blocked by the simultaneous presence of several potassium conductances. Thus, in this cell, the induction of bursting may be more properly described as a disinhibition. Supported by NIH NS17323 and NRSA NS 07859.

231.6 NEURAL CONTROL OF FEEDING IN BASOMMATOPHORAN SNAILS. A.D. Murphy and M.M. Lu.* Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60680.

The neural control of feeding has been studied extensively in the basommatophoran snails, Helisoma and Lymnaea. Though feeding behavior in the two snails appears very similar, reports suggest that the neural organization of feeding is fundamentally different (e.g. Benjamin, P.R. in Neural Origin of Rhythmic Movements; Roberts A., & Roberts B., eds., Cambridge Univ. Press, 1983). Comparative studies in our laboratory suggest that some reported differences are more apparent than real, though real differences exist. We have shown in Helisoma that the pattern generator is composed of three subunits, S1,S2, and S3. Each subunit is comprised of a group of interneurons which can generate bursts of action potentials (APs) that evoke characteristic post-sunaptic potentials on identified motoneurons. Bursts of APs can occur sporadically or rhythmically. Subunits can be either phase-linked or independently active, and the relative timing of activity of subunits within a "feeding cycle"is variable, i.e. they can be phase-linked with different temporal relationships. These characteristics allow a variety of qualitatively different motor patterns to be produced.

Morphological and physiological studies suggest that the S1 subunit of Helisoma includes homologs of the N1 interneurons described in Lymnaea by Benjamin. It also includes an apparent homolog of the slow oscillator neuron of Lymnaea and several additional interneurons for which no counterpart in Lymnaea has been described. The S2 subunit corresponds to the "cyberchron network" described by S.B. Kater (Am. Zool. 14, 1017-1036, 1974) and appears to be homologous with the N2 interneurons of Lymnaea. The S3 subunit in Helisoma includes a pair of interneurons located on the dorsal surfaces of the buccal ganglia (BG) adjacent to the commissure. It also includes the VB1 cluster of neurons with small cardioactive peptide B (SCP_B)-like immunoreactivity located on the ventral surfaces of the BG. The S3 subunit is totally different from the N3 interneurons described in Lymnaea. S3 inputs are excitatory to motoneuron B19 and inhibitory to neuron B5 in Helisoma. No comparable inputs have been described onto the apparent homologs, B4 and B2, in Lymnaea. However, immunocytochemical staining with a monoclonal antibody to SCP_B (from A.O.D. Willows) shows a cluster of neurons with SCP_B-like immunoreactivity on the ventral surface of the BG of Lymnaea. They have similar axonal projections to those of the VB1 cluster of Helisoma. This suggests that the missing S3 components that impart tremendous flexibility to Helisoma may exist in Lymnaea and account for variability in its motor patterns that has not previously been addressed. Direct comparisons and new findings in both systems may show that the neural organization of feeding may be fundamentally very similar in the basommatophoran snails.

231.7 EVOLUTION OF BEHAVIOR: AN HOMOLOGOUS PEPTIDERGIC ESCAPE SWIM INTERNEURON IN SWIMMING GASTROPOD NUDIBRANCHS (TRITONIA, HERMISSENDA) AND A RELATED NON-SWIMMING SPECIES (AEOLIDIA). R. D. Longley* and A. J. Longley. Pacific Sciences Institute, P.O. Box 835, Friday Harbor, WA 98250 and Friday Harbor Laboratories, Friday Harbor, WA 98250.

Electrophysiological mechanisms responsible for behavioral change during the evolution of neurons have not been described, in part because of the small number of known neuron homologues with a clearly defined function. We have searched for homologues to an interneuron, C2 in Tritonia diomedea, which is necessary for escape swimming in the semi-intact animal (Taghert, P.H., Willows, A.O.D., J. Comp. Physiol., 123:253, 1978) and for the neural correlates of escape swimming in the isolated ganglia of this species (Lennard, P.R., Getting, P.A., Hume, R.I., J. Neurophysiol., 44:165, 1980).

Using lucifer yellow fills of visually similar cells and immunohistochemical staining of these cells (Longley, R.D., Longley, A.J., Soc. Neurosci. Abstr., 11:943, 1985), we have identified presumptive homologues to C2 in Hermissenda crassicornis and Aeolidia papillosa. We found, in their somata, a unique dual immunoreactivity to an antibody to SCP_B (from Boris Masinovsky and Steve Kempf, Univ. Wash.) and an antibody to FMRFamide (AB-232 from John Bishop, NIH). This homologue, in all three species, belongs to a class of cells with FMRFamide-like immunoreactive axons that enter the pedal commissure contralaterally, an axon pathway not previously reported for C2 (Getting, P.A., Lennard, P.R., Hume, R.I., J. Neurophysiol., 44:151, 1980; Snow, R.W., J. Neurobiol., 13:251, 1982).

Intracellular recording from these cells shows short-latency excitatory monosynaptic input on pedal nerve stimulation, as described for C2 in T. diomedea (ibid.). Both T. diomedea and H. crassicornis also receive long lasting excitatory input and tend to burst, but in A. papillosa, which does not do escape swimming, these inputs do not occur after nerve stimulation. Instead, in A. papillosa we usually see long lasting inhibition.

In the interneuron homologues described here, we find no evidence for a change in neurotransmitter or general morphology, but rather an electrophysiological change in the character (excitatory or inhibitory) of their synaptic input. This could result from a change in synapse number or type and/or a loss or gain of presynaptic neurons. The former possibility, where synaptic contacts vary but neuron number and axon morphology remain similar to an ancestral nervous system, could be a more labile evolutionary mechanism, occurring late in development, that alters a specific behavior without gross modification of a developmental program.

231.8 PROPRIOCEPTIVE INPUT DETERMINES THE TIMING OF ELEVATOR ACTIVITY IN INTACT FLYING LOCUSTS. H. Wolf* and K.G. Pearson, Department of Physiology, University of Alberta, Edmonton, Canada.

In intact, tethered flying locusts intracellular recordings were obtained from flight motoneurons and interneurons. The depolarizations occurring in elevator motoneurons at high wingbeat frequencies were qualitatively different from those generated in deafferented preparations. The main difference was that deafferentation slowed the rising phase of the depolarizations and thus delayed the onset of elevator activity relative to the preceding depressor activity. This resulted in a phase shift of elevator activity in the depressor cycle. The following observations demonstrated that the initial rapid depolarizations in elevator motoneurons of intact animals are produced by phasic input from a group of wing proprioceptors, the hingwing tegulae: 1) hindwing tegula afferents are excited during the wing downstroke and the onset of their activity precedes the onset of depolarizations in elevator motoneurons by a few milliseconds, 2) hindwing tegula afferents make monosynaptic excitatory connections to hindwing elevator motoneurons and strong disynaptic excitatory connections to forewing elevators, 3) removal of the hindwing tegulae slows the rate of depolarization in elevator motoneurons, and 4) electrical stimulation of tegula afferents in animals with tegulae surgically removed restores the initial rapid depolarizations in elevator motoneurons. From these observations we conclude that the timing of the onset of elevator activity depends on phasic input from the hindwing tegulae.

In animals flying with low wingbeat frequencies the depolarizations occurring in elevator motoneurons were characterised by two distinct components. The rapid depolarizations typical of intact flight at high wingbeat frequencies were prolonged by the addition of discrete, late depolarizations resembling the depolarizations occurring in elevator motoneurons of deafferented preparations. We investigated the effect of stimulating the wing stretch receptors on these two distinct depolarizations. Stretch receptor stimulation suppressed the late depolarizations but had no effect on the rapid early depolarizations. These observations demonstrate that in intact flying animals stretch receptor input functions to limit the duration of the depolarizations in elevator motoneurons. The results further indicate that the centrally generated depolarizations produced in deafferented preparations are suppressed by stretch receptor input. Thus at high wingbeat frequencies elevator depolarizations may be generated entirely by phasic input from the tegulae.

231.9 LOCAL INTERACTIONS AMONG INTERNEURONS IN THE LOCUST FLIGHT SYSTEM
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Interneurons in the flight system of the locust are located in the three thoracic ganglia (prothoracic, mesothoracic and metathoracic). The circuit of interconnections between flight interneurons which has been described as contributing to burst generation in the system spans the meso- and metathoracic ganglia, but it has been reported that each of these two ganglia is capable of generating rhythmical motor activity in isolation. Furthermore, a delayed excitatory connection has major importance in the operation of the central circuit and has been modelled as disynaptic disinhibition with tonic and graded release of transmitter at the second synapse (i.e. incorporating a local interaction). Recently I have investigated the synaptic interactions among flight interneurons within single ganglia to determine the extent to which local interactions contribute to rhythmicity. I used a deafferented preparation of *Locusta migratoria* which is capable of expressing the centrally generated flight rhythm. Simultaneous recordings from different flight interneurons were made by penetrating their processes in the neuropil with glass microelectrodes containing Lucifer Yellow.

Delayed excitatory connections were found to have a widespread occurrence. In all cases the properties of the connections were consistent with the idea that each was produced by a similar disinhibitory process. Spikes in some interneurons were followed by short latency ipsps as well as delayed epsps in the same follower neuron to produce inhibitory/excitatory potentials. The relative sizes of the two components were variable and depended on the level of polarization of the follower neuron and other undetermined factors (possibly the level of polarization of the intercalated interneuron). A subthreshold depolarization of interneuron 301 induced a plateau-like potential which outlasted the stimulus. Evidence indicates that this did not result from an intrinsic property of the neuronal membrane but resulted from feedback *via* a local synaptic interaction, thus demonstrating subthreshold graded release of transmitter from the dendritic region of 301. Such a mechanism may contribute to the rapid depolarizing phase of the membrane potential oscillation of 301 during expression of the flight rhythm. The results show that local and graded interactions have a greater role in the operation of the central circuit underlying locust flight than previously suspected.

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231.10 **INTERACTIONS BETWEEN CUTANEOUS FLEXION REFLEX PATHWAYS AND SCRATCH CENTRAL PATTERN GENERATORS IN THE TURTLE.**
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An immobilized, low-spinal turtle displays a fictive scratch reflex in response to mechanical stimulation of specific regions of the shell (J. Neurophysiol. 53: 1501-34, 1985). Turtles exhibit three forms of the scratch reflex: the rostral, pocket and caudal scratches. Each form exhibits a rhythmic alternation between hip protractor (flexor) activity and hip retractor (extensor) activity. Cutaneous stimulation of parts of the hindlimb elicits a fictive flexion reflex, characterized by a robust activation of hip protractor nerves (J. Comp. Physiol. 146: 401-9, 1982). In the present study, we elicited fictive flexion reflexes by mechanical or electrical stimulation of the dorsal surface of the foot.

Stimulation of the contralateral foot during a flexion reflex caused a strong crossed inhibition of hip protractor nerve activity and often a weak crossed excitation of the hip retractor nerve. Intracellular recordings from hip protractor motor neurons exhibit a long-lasting depolarization during a flexion reflex (ibid.). We found that contralateral foot stimulation during a flexion reflex caused a transient repolarization of hip protractor motor neurons. This repolarization may be a direct inhibition of motor neurons via the crossed inhibitory pathway, an inhibition of pre-motor interneurons (removed excitation), or a combination of both effects.

Foot stimulation perturbed on-going scratch motor patterns in a phase-dependent manner. Stimulation of the ipsilateral foot during the hip protraction phase of a fictive rostral, pocket or caudal scratch lengthened the duration of hip protractor activity; stimulation during the hip retraction phase shortened the duration of hip retractor activity. Stimulation of the contralateral foot during the hip protraction phase of a rostral or pocket scratch shortened the duration of hip protractor activity; stimulation during the hip retraction phase lengthened the duration of hip retractor activity. Cutaneous stimulation of the ipsi- or contra-lateral foot could cause permanent resets of fictive scratch motor rhythms. These results demonstrate strong central interactions and suggest shared circuitry between cutaneous flexion reflex pathways and scratch central pattern generators. We are currently developing criteria for interneuron identification based on activity during (1) flexion, (2) crossed inhibition of flexion and (3) scratch reflexes.
Supported by NIH Grants NS07850 to S.C. and NS15049 to P.S.G.S.

231.11 SPINAL INTERNEURONS THAT MAY MEDIATE THE CROSSED INHIBITION DURING A MAUTHNER-INITIATED ESCAPE BEHAVIOR IN GOLDFISH.
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During the relatively simple motor behavior initiated by the Mauthner cell (M-cell) in goldfish, the M-axon monosynaptically excites ipsilateral motoneurons and descending spinal interneurons, producing an abrupt, forceful bend toward the side of the active M-axon. While the ipsilateral neurons are excited, contralateral motoneurons and descending interneurons are chemically inhibited. Earlier studies, in conjunction with our recent data, provide several predictions about how the inhibition is accomplished: 1) Neither the M-axons nor the inhibited contralateral motoneurons and descending interneurons have crossing processes; therefore the inhibition must be mediated by interneurons that cross the spinal cord; 2) The short latency of the inhibition indicates that the M-axon-inhibitory interneuron connections are probably electrical; and 3) The IPSPs in motoneurons and descending interneurons are most easily inverted by chloride injection near the location of the excitatory inputs from the ipsilateral M-axon, suggesting that the inhibitory contacts are located near the excitatory ones.

We have identified neurons with these expected features in the spinal cord of goldfish. We recorded intracellularly, simultaneously from both M-axons and spinal neurons in anaesthetized, paralyzed goldfish, and found neurons that were excited by the M-axon at very short latency (m=.11 msec, s.d.=.02, N=8) with excitatory postsynaptic potentials that were resistant to fatigue by high frequency stimulation (50 Hz). These neurons received a longer latency (m=.56 msec, s.d.=.16, N=4), Cl^--dependent inhibition from the M-axon contralateral to the one that excited them. Intracellular HRP injections (N=4) show that they have a relatively small cell body located dorsally, above the central canal, and ipsilateral to the excitatory M-axon. A thin process from the cell body runs ventrally to the ipsilateral M-axon where it enlarges, passes dorsal to the M-axon and crosses the spinal cord between the two M-axons. After crossing, it runs longitudinally, parallel to the contralateral M-axon for approximately one segment. Terminal branches of this contralateral process show a striking correspondence with the collaterals of the M-axon that form the excitatory contacts with motoneurons and descending interneurons. Each branch arising from the longitudinal process ramifies distal to the end of a single M-axon collateral, with as many as ten branches from one interneuron associated with an equal number of collaterals. Thus, as predicted, these neurons are electrically coupled to one M-axon, cross the spinal cord, and have terminals in the regions where contralateral neurons receive their excitatory input from the other M-axon. Supported by an NIH Postdoctoral Fellowship (JRF) and NIH Grant NS-15335.

231.12 SELF ORGANIZED PATTERN FORMATION IN COORDINATED MOVEMENT BEHAVIOR: THE SYNERGETIC APPROACH.
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Synchronization, entrainment, frequency- and phase-locking are commonly observed features of neuronal patterns generated by many species [e.g., A. I. Selverston, (1985) Ed. Model neural networks and behavior. Plenum, New York]. Their relation to macroscopic behavioral patterns, however, is less clear. We report the results of joint experimental and theoretical work showing that similar intrinsic patterns can arise also in human multilimb coordination tasks, in a purely self-organized fashion. Such pattern formation may be understood as follows [Kelso, Schöner, Scholz & Haken, (1987) Physica Scripta, 35, 79-87]: 1) movement patterns are characterized by a collective variable, relative phase, which serves as an order parameter [H. Haken (1983) Synergetics: An Introduction, 3rd Edition, Springer, Heidelberg]; 2) stable patterns are modelled as attractors for the collective variable; 3) the stability and stationarity of the patterns is measured and interpreted in terms of the stochastic dynamics of relative phase with certain time scales relations; 4) switching among patterns is due to loss of stability of the attractor (a nonequilibrium phase transition); 5) the switching process is governed by the stochastic dynamics of relative phase; 6) the dynamics of the collective variable are derived from a cooperative (nonlinear) coupling among the individual components, thus linking levels of observation. Schoner and Kelso [Biol. Cybernetics, submitted] have elaborated this approach to include recent observations on environmentally elicited and learned movement patterns. Again, these patterns can be derived from the component level, analytically and computationally. We conjecture that our approach has the potential to relate macroscopic behavioral levels to more microscopic physiological levels (e.g., neural networks), by linking functionally specific dynamics at different scales of analysis.

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231.13 SYNERGETIC THEORY OF MOVEMENT COORDINATION: RECOGNITION AND LEARNING OF ENVIRONMENTALLY ELICITED PATTERNS. G. Schöner* and J.A.S. Kelso* (SPON: R. K. Berntson). Center for Complex Systems, Florida Atlantic University, Boca Raton, FL 33432.

The synergetic approach to the study of coordination [Kelso & Schöner, In Graham, R. and Wunderlin, A. (Eds.) Lasers and Synergetics, Springer, 1987] is applied to the coordinated rhythmic patterns formed: a) in the continuous presence of environmental information (periodic stimuli specifying a required relative phase); and b) when the periodic stimuli are removed after the patterns have been learned. Using the collective variable, relative phase, the dynamics of these patterns are modelled as arising from the competition between external (environmentally specified) and intrinsic dynamics. The intrinsic dynamics have been derived in earlier work based on observations of phase transitions in movement coordination [Haken, Kelso & Bunz, Biol. Cybern., 51, 347 (1985)]. Recent experimental results on human movement coordination by two groups of investigators are explained and new predictions are presented. The well-documented learning of environmentally specified movement patterns is modelled as a dynamic transformation of external into intrinsic dynamics. The formulation includes the process of pattern recognition as a learned pattern is elicited. Learning and pattern recognition processes are driven by: (1) a match between the learned patterns (as attractive states) and the environment; and (2) a competition among learned patterns. The solutions of the learning model and experimental implications, including a possible phase transition in learning, are presented. The relation of our theory, which is formally related to theories of nonlinear reaction networks by M. Eigen and P. Schuster, [The Hypercycle, Springer-Verlag, Berlin, 1979] to recent neural network models of learning and pattern recognition is discussed.

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231.14 THE PHASE TRANSITION CHARACTER OF SWITCHING BETWEEN RHYTHMIC PATTERNS OF HUMAN BIMANUAL COORDINATION: TEST OF A DYNAMICAL MODEL. J.P. Scholz*, J.A.S. Kelso* and G. Schoner* (SPON: N. F. Capra). Dept. of Physical Therapy, Georgia State Univ., Atlanta, GA 30303; Center for Complex Systems and Dept. of Psychology, Florida Atlantic Univ., Boca Raton, FL 33431

Transitions among patterns of intra- and interlimb coordination are a common feature of animal behavior. Yet little is known about the processes underlying such transitions. Recent work of Kelso and colleagues has addressed this issue in human movement. Principles from the interdisciplinary field of synergetics (Haken, H., Synergetics: An Introduction, Springer, Berlin, 1983) have been employed to develop a detailed stochastic model (Soc. Neurosci Abstr. 385.18, 1986; Schoner, G., Haken, H. & Kelso, J.A.S., Biol. Cybern., 53:247, 1986) of switching between patterns of bimanual coordination. This model provides an analogy between movement transitions and the spontaneous formation of patterns in non-equilibrium systems treated by synergetics, in which stability considerations and self-organizing processes play a decisive role. The research presented here attempted to test specific predictions about the bimanual system's behavior that derive directly from the model, specifically, critical slowing down (CSD) and switching time. Another important prediction of the stochastic model, critical fluctuations, has been addressed elsewhere (Kelso, J.A.S., Scholz, J.P. & Schoner, G., Physics Letters A, 118:279, 1986).

In the present experiment, subjects (N = 5) rhythmically flexed and extended their index fingers in synchrony with a metronome while producing either an in-phase (ϕ = 0 deg) or an out-of-phase (ϕ = 180 deg) pattern of finger coordination. Metronome frequency was increased in 0.2 Hz steps every 10s, 9 times/trial (N = 70). CSD was evaluated by measuring the time taken for the fingers to return to their previous coordinative state (i.e., relaxation time) after a perturbation was administered to the right finger. In all cases, a transition from the out-of-phase to the in-phase pattern (but never in the opposite direction) occurred at a critical value of oscillation frequency. The time taken for the switch to occur was measured from the onset of the transition frequency until attainment of the first stable cycle in the new state (ϕ = 0 deg).

The mean and distribution of the experimental switching times were remarkably similar to those estimated from the stochastic model. Relaxation times increased significantly with frequency (CSD) in the out-of-phase but not the in-phase pattern of finger coordination. Thus, the model and results were self-consistent suggesting that movement transitions can be understood as non-equilibrium phase transitions, and providing supportive evidence for the self-organizational nature of motor pattern generation.

SPINAL CORD AND BRAINSTEM II

232.1 SPINAL NETWORK DEVELOPMENT AND ITS RELATION TO HINDLIMB MOVEMENT: AN *IN VITRO* ELECTROPHYSIOLOGICAL STUDY IN NEONATAL RAT. R. Navarrete, K. Walton and R. Llinas. Dept. Physiol. & Biophys., New York Univ. Med. Sch., 550 First Ave., New York, NY 10016.

Unraveling the factors relevant in governing the development of the nervous system is an essential step toward understanding CNS function. We have approached this problem by focusing on the role of electrical neuronal activity studying the spinal cord (SC) of neonatal rat (P0-P10) using *in vitro* techniques. Previously, we studied motoneuron membrane properties (Fulton & Walton, 1986, J. Physiol. 370,651) and spontaneous oscillations, the latter using a SC brainstem-cerebellum preparation (Walton & Llinas, 1986, IUPS. 16:118). Recently we have investigated the development of lumbar circuits in the contex of motor activity using a hemisected SC-hindlimb preparation. Electromyograms (emg's) and the electrical activity in ventral roots (L4 or L5) and motor nerves were used as measures of motor output. This preparation has allowed intracellular recordings from flexor and extensor motoneurons [identified by their antidromic invasion from common peroneal (c.p.) or tibial (tib.) nerves, respectively]. Some cells were further identified as innervating predominantly slow (soleus) or fast (EDL-TA) muscles. Spontaneous rhythmic activity is present in this preparation as indicated by emg's and corroborated by extracellular ventral root and intracellular motoneuron recordings. These oscillations were readily triggered by dorsal root or by peripheral nerve stimulation. Synaptic potentials were evoked by muscle nerve stimulation. For example, EPSP's were recorded from extensor motoneurons after tib., soleus and c.p. nerve stimulation. Differences in the time-to-peak of compound EPSP's evoked by agonist (tib.) or antagonist (c.p.) nerve stimulation (16.4 $\pm$ 6.9ms, n=3) and (45.5 $\pm$ 5.1ms, n=6), respectively (P7-10, room temp.) are consistent with their identification as extensor motoneurons. Emg and intracellular recordings show that, in contrast to the adult, the latency for activation of extensor motoneurons was similar for stimulation of agonist and antagonist nerves. The latency for EPSP's was 11.7 $\pm$ 1.0 ms, (n=7) for tib. and 16.9 $\pm$ 2.2ms, (n=7) for c.p. stimulation. Recurrent and reciprocal inhibition was also present. IPSP's were evoked in soleus motoneurons by tib., soleus and by c.p. nerve stimulation. Thus, intracellular recordings have demonstrated that all elements of synaptic integration are present, but immature, in *in vitro* neonatal SC. Further, their molding into mature spinal circuits is amenable to study *in vitro*. Supported by NS22975, NS13742 from NINCDS and The Irma T. Hirschl Trust.

232.2 REFLEX ORGANIZATION IN A LONG TERM *IN VITRO* TURTLE PREPARATION. K.A.S. Shirley, V. Boss, and P.R. Lennard. Dept. of Biology, Emory Univ., Atlanta, GA 30322.

An *in vitro* spinal cord-nerve-muscle preparation provides numerous advantages for the study of reflex organization and modulation. The advantages are accessibility for recording and stimulating, stability for intracellular penetrations, and ease of application of pharmacological agents by rapid perfusion. We have developed a preparation which remains viable for 4-5 days so that neuroanatomical tracing methods can be used in conjunction with physiological and pharmacological procedures.

The limb enlargement of the spinal cord along with various combinations of muscles and their respective peripheral nerves were dissected from an euthanized turtle *(Pseudemys scripta)*. Following gross dissection, the preparation was transferred to Hepes-buffered reptilian Ringer's solution where the dissection was completed over a 3-5 hour time period using sterile technique. The limb enlargement and 5-8 spinal segments were exposed except for a small strip of vertebral bone immediately surrounding the spinal roots ipsilateral to the chosen muscles. The preparation was pinned out over nylon mesh above wells formed in a sylgard-coated chamber which held a volume of 15-20 ml. The preparation was perfused continuously with culture medium at a rate which turned over the chamber volume at least twice per hour. The medium was Dulbecco's modified Eagle (GIBCO 430-1600), to which 44 mM bicarbonate and 15 ml/l of antibiotic-antimycotic (GIBCO 600-5240AE) were added. Sterile 95% O_2, 5% CO_2 was bubbled constantly into the medium. The whole system was placed on a vibration isolation table in a laminar flow hood. Bipolar hook electrodes for recording and/or stimulating were placed around the spinal cord and each muscle nerve. Two sets of bipolar EMG electrodes were implanted in each muscle. Intra- and extracellular electrodes were positioned in the spinal cord using hydraulically driven remote-controlled micromanipulators.

A typical preparation consisted of the hindlimb enlargement plus 2 rostral and 1 caudal spinal segments, appropriate peripheral nerves, and the muscles iliofibularis and ambiens. Viability of the preparation was assessed by EMG activity evoked either by spinal cord or peripheral nerve stimulation, or by stretch reflexes induced with a muscle vibrator. The EMG response declined only slightly over three days, and to a greater extent during the fourth and fifth days. This *in vitro* system is being used to determine the organization of reflexes in various combinations of synergistic and antagonistic muscles. The effects of descending monoaminergic and serotonergic pathways thought to be involved in modulating spinal reflexes and locomotion are being examined using bath applied pharmacological agents and electrical stimulation of spinal tracts. (Supported by USPHS grant NS17732).

232.3 NEURAL CONTROL SYSTEM FOR MAMMALIAN LOCOMOTION *IN VITRO*. J. C. Smith and J. L. Feldman. Systems Neurobiology Lab, Dept. of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

Motor patterns for locomotion are generated by *in vitro* preparations of neonatal rat brainstem and spinal cord (Smith et al., Soc. Neurosci. Abs. 12: 386, 1986; J. Neurosci. Methods, 1987, in press). We have now established that these preparations retain functional neural circuitry for major components of the mammalian locomotor control system, including brainstem locomotor command generating networks, spinal motor pattern generating circuitry, and spinal sensory afferent input pathways. Activation of any of these components *in vitro* generates rhythmic locomotor activity. We developed methods for selective stimulation of each of these components and the locomotor output patterns were analyzed. The neuraxis was isolated from neonatal rats and maintained *in vitro* as previously described (*ibid.*). Motor patterns were characterized from recordings of cervical and lumbar spinal ventral root discharge and recordings of limb muscle (flexor, extensor) EMG in preparations retaining innervated hindlimbs. Brainstem and spinal networks were selectively activated by specific neurochemicals (*ibid.*). Excitatory amino acids [L-aspartic acid or L-glutamic acid (0.1-0.5 mM) with amino acid uptake inhibitor dihydrokainic acid (0.2 mM)] were typically used to activate the spinal circuitry; substance P (1-10 μM) was typically used to active brainstem networks. Sensory pathways were activated by dorsal root stimulation/ exteroceptive stimuli (e.g. tail pinch) in preparations with hindlimbs and tail. Similar patterns of nerve and muscle activity during the locomotor cycle were generated in all cases. Characteristics of the motor output patterns include: (1) multi-joint, stepping-like movements of limbs; (2) alternating flexor-extensor activity during the step cycle characteristic of quadruped locomotion; (3) interlimb coordination (all four limbs) resembling quadruped walking. Using selective pharmacological antagonists and uptake inhibitors for candidate neurotransmitters (*ibid.*), we established that the generation of the locomotor activity critically depends on synaptic release of endogenous excitatory amino acids in spinal networks. Excitatory amino acids activating N-methyl-D-aspartic acid (NMDA) receptors play a central role as previously established (*ibid.*); NMDA receptor block with 2-amino-5-phosphonovaleric acid (AP5) suppresses the locomotor activity in all cases. The data suggest that the descending locomotor command and sensory input pathways converge on a common set of spinal locomotor rhythm generating neurons activated by the endogenous excitatory amino acids. These results indicate that key networks of the locomotor control system can be selectively activated *in vitro* to produce appropriate motor behavior. The *in vitro* brainstem-spinal cord preparations offer the opportunity for investigation of several major neural networks of the mammalian locomotor control system. SUPPORTED BY NIH GRANTS HL-37941 & NS-24742. JCS is a P. B. FRANCIS FOUNDATION FELLOW.

232.4 **REGENERATION OF DESCENDING COMMAND PATHWAYS FOR LOCOMOTION IN AN IN VITRO PREPARATION OF THE LAMPREY CNS.** A.D. McClellan, Dept. of Physiol., Univ. of Iowa, Iowa City, IA

Larval lampreys that have received a complete spinal cord transection regain voluntary control of locomotion within about 8 weeks (@ 25°C). An unresolved issue, however, is whether locomotor activity below the healed transection site is due to regeneration of descending command pathways for locomotion or to other mechanisms, such as mechanosensory coupling, that bypass the transection site. Therefore, the role of regenerated descending command pathways for locomotion was tested in an in vitro brainstem/spinal cord preparation (McClellan, 1984), in which mechanical factors and sensory feedback were eliminated.

Control animals: EMG activity obtained during episodes of locomotion in larval lampreys indicated that cycle times (0.24 - 1.39 s), burst proportions (0.1 - 0.4), and segmental phase-lags (0.003 - 0.012) were similar to those described in adult lampreys (McClellan, 1984). In curarized in vitro brainstem/spinal cord preparations, electrical stimulation of brainstem "locomotor command regions" (McClellan and Grillner, 1984) could elicit well-coordinated locomotor patterns in spinal ventral roots similar to the muscle patterns observed in intact larval animals.

Acute spinal-transected animals: Larval lampreys with an acute mid-body transection could still locomote, but muscle activity was not usually present below the transection site. Presumably the undulatory locomotor movements in the rostral half of the body are passively conducted along the body caudal to the acute transection site.

Spinal-regenerated animals: In spinal-regenerated animals locomotor activity in body muscles could be recorded along the entire body above and below the healed transection site. These patterns were similar to those observed in control animals. In curarized in vitro preparations, stimulation of brainstem "locomotor regions" and sometimes sensory stimulation of the head could elicit well-coordinated locomotor patterns in ventral roots above and below the transection site. Moreover, with a low-calcium Ringer's solution bathing only the spinal cord above the transection site, locomotor activity could still be elicited caudal to the transection suggesting that descending command pathways had regenerated across the transection site and made connections with motor networks. This is the first demonstration of coordinated locomotor activity elicited below a healed transection site by regenerated descending command pathways in an in vitro preparation in which nonspecific factors, such as sensory feedback, have been eliminated. However, locomotor activity was only present up to 3.0 cm caudal to the transection site in animals that had recovered for 8-20 weeks. Thus, regenerated descending command pathways alone appear capable of initiating locomotor activity just below the transection site, but the muscle activity patterns and movements which occur over more caudal parts of the body may depend on a combination of factors. These results set the stage for a cellular analysis of the role of regeneration of descending locomotor command pathways in behavioral recovery.

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232.5 **REPETITIVE FIRING PROPERTIES OF NEURONS WITHIN THE NUCLEUS AMBIGUUS OF ADULT GUINEA PIGS USING THE *IN VITRO* SLICE TECHNIQUE.** S.M. Johnson* and P.A. Getting, Dept. of Physiol. Biophys., Univ. of Iowa, Iowa City, IA 52242.

Our goal is to understand how neurons within the medulla shape the form and timing of the rhythmic respiratory motor pattern in mammals. The purpose of this study was to determine the repetitive firing properties of neurons in the region of the Nucleus Ambiguus (NA), a heterogenous nucleus which contains neurons belonging to the Ventral Respiratory Group (VRG). The *in vitro* slice technique was used to obtain stable intracellular recordings (n=48) in the current clamp mode from transverse brainstem slices (400-500 microns thick, 0.0-1.5 mm rostral to the obex) of adult guinea pigs.

Approximately 13% of the cells displayed delayed excitation (DE). DE is a prolonged delay in the onset of firing following a hyperpolarizing prepulse. The maximum delay that could be elicited from cells within the NA was 200-250 msec. These findings differed from those found in the Dorsal Respiratory Group (DRG), a group of respiratory neurons found in the region of the ventral Nucleus Tractus Solitarius. In the DRG, 40% of the cells display DE with a delay ranging from 380-760 msec (Dekin and Getting, *Brain Res.*, 324:180-184, 1984).

All cells displayed spike frequency adaptation (SFA) which was quantified as the ratio of the steady state frequency to the peak firing frequency during a two second depolarizing stimulus. Cells which displayed DE had a broad range of SFA ratios from 0.4-0.8. In contrast, the cells which did not display DE could be separated into two major groups based upon the degree of SFA. Cells with SFA ratios of 0.6-0.8 were considered to have mild SFA and cells with SFA ratios of 0.0-0.4 were considered to have strong SFA. Using this criteria, 56% of the cells exhibited mild SFA and 44% of the cells exhibited strong SFA.

Post-inhibitory rebound (PIR) was found in 13% of the cells in which DE was not expressed. PIR was never found in a cell which exhibited DE and there was no obvious correlation between PIR and either mild SFA or strong SFA. PIR is present in NA cells but not in DRG neurons (Dekin *et al., J. Neurophysiol.*, in press).

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232.6 RESPIRATORY INTERNEURON LOCATIONS AND PHOTOINACTIVATION IN LAMPREY. D.F. Russell. Dept. of Anesthesiology, Box 8054, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The study of the lamprey, a simple stem vertebrate, may clarify basic mechanisms of neural control of respiration. The first aim of this work was to localize the somata of neurons that generate the respiratory rhythm. HRP was applied to sites in the medulla that are known to contain the axons of respiratory interneurons, in both adults and larvae. (1) Premotor interneurons were localized by making small HRP injections into the respiratory motor nuclei (nuc.X; as in other fish, respiratory motorneurons are in the medulla). An ipsilateral and ventral axon tract passed rostrally near cranial nerves VIII and V. In adults, an ipsilateral ventral cell group was labelled near the exit zone of n.V. In larvae, an ipsilateral and dorsal group of ca. 50 neurons with ca. 5 μ somata was labelled rostral to nuc.V. (2) Since the respiratory generator is duplicated bilaterally, a search was made for coordinating neurons that synchronize the two sides, by making local injections of HRP at the midline at the level of rostral nuc.V, the site of a respiratory commissure in adults. This gave bilateral labelling of dorsal populations of neurons located rostral and lateral to nuc.V, extending dorsal of the sulcus limitans, although some cells were more ventral.

The second aim was to dissect the respiratory generator using the photosensitizing dyes sulforhodamine and rose bengal and 546 nm illumination from a compound fluorescence microscope. Intracellular injection of dye into Muller cells and illumination caused loss of the input resistance and of resting, action, and synaptic potentials, as in other systems. Two new methods were developed: photoinactivation after axonal backfilling with dye, and local illumination after bath application of dye. The first method was tested on the Müller (reticulospinal) neurons by backfilling their axons with dye applied to the rostral spinal cord, while monitoring the reflex excitation of these cells by vestibular inputs; illumination of backfilled cells was effective in abolishing most of the vestibular-evoked descending volleys. The second method was used to test whether neurons rostral to nuc.V are needed for respiratory pattern generation in larvae. Illumination with 200 μ spots of light abolished the respiratory rhythm, supporting Homma's (J. Comp. Physiol. 104:175) conclusion and the HRP results above that certain respiratory interneurons are located in this region.

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232.7 LOCALIZATION OF LAST-ORDER SPINAL INTERNEURONS PARTICIPATING IN THE PRODUCTION OF LOCOMOTION IN THE CAT. B.R. Noga, S.J. Shefchyk, and L.M. Jordan. Dept. Physiol., Univ. Manitoba, Winnipeg, CANADA, R3E 0W3.

The wheat germ agglutinin-horseradish peroxidase (WGA-HRP) transneuronal labelling technique (Jankowska, Brain Res., 341: 403, 1985) was used to localize last order spinal interneurons which participate in the production of hindlimb locomotion in intact cats. Wheat germ agglutinin conjugated HRP was unilaterally injected in the proximal part of the cut nerve branch to the anterior biceps muscle. After injection, the cats were walked on a treadmill (belt speed 0.3 m/sec) or overground for periods of 1.5 to 2 hours/day for 3 days.

Anterior biceps motoneurons located in the motor nuclei of lumbar (L) and sacral (S) segments (L7-S1) were heavily labelled. In addition to spinal motoneurons, interneurons were transneuronally labelled with this technique. Labelled interneurons were localized throughout L5-L7 but were not found in the S1 segment or caudal to the motor nuclei. Ipsilateral to the injection, the interneurons were primarily localized to Rexed's lamina VII (L7 > L6 > L5), although some labelled cells were also found in lamina V-VI (L6). Contralateral labelled interneurons were restricted to lamina VIII (L5-L7). The cells located in ipsilateral lamina VII also overlap those areas in which Renshaw cells and Ia inhibitory interneurons have been localized (Jankowska and Lindstrom, Acta physiol. scand., 81: 428, 1971; J. Physiol., (Lond.), 226: 805, 1972). Previous work has demonstrated that these cells are rhythmically active during locomotion induced by stimulation of the mesencephalic locomotor region (Noga, et al, Exp. Brain Res., 66: 99, 1987). It is expected that some of these lamina VII cells belong to a different functional group which participate in the production of the rhythmic excitatory drive to motoneurons during locomotion (Shefchyk and Jordan, J. Neurophysiol., 53: 1345, 1985). Previous work (Harrison, et al, J. Physiol. (Lond.), 371: 147) has demonstrated that contralateral lamina VIII interneurons may be involved in crossed reflex interactions. The present results indicate that these cells are also active during overground locomotion. It is suggested that these cells may comprise at least part of the crossed spinal module for walking movements revealed in fictive locomotion experiments (Jordan, et al, Soc. Neurosci. Abstr. 12, p. 877). Transneuronal labelling experiments in paralyzed cats induced to walk by stimulation of the mesencephalic locomotor region are presently underway.

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232.8 ACTIVITY OF L4 INTERNEURONS WITH GROUP II INPUT DURING FICTIVE LOCOMOTION IN THE MESENCEPHALIC CAT S. J. Shefchyk, D. A. McCrea, D. J. Kreillaars*, B. R. Noga and L. M. Jordan. Dept. Physiol, Univ. of Manitoba, Winnipeg, CANADA R3E 0W3.

Experiments were performed to determine the activity during fictive locomotion of the population of interneurons in the midlumbar spinal cord previously described by Jankowska and coworkers (Edgley and Jankowska, J. Physiol. 385:393-414, 1987). These interneurons receive input from cutaneous, joint and muscle afferents as well as several descending pathways (Edgley, Jankowska and Shefchyk, unpublished), but their most striking features are a strong group II input from quadriceps, sartorius and deep peroneal nerves and the observation that they project monosynaptically to motoneurons in the lower lumbar segments where they can have either excitatory or inhibitory actions (Jankowska and coworkers, unpublished). It was suggested that these interneurons may play a role in determining hip position, and perhaps influence the production of the step cycle. This is supported by the fact that stimuli applied to the cuneiform nucleus, or mesencephalic locomotor region (MLR) could activate the L4 group II interneurons in the anesthetized preparation (Edgley et al., unpublished).

We have conducted experiments using extracellular recordings from L4 interneurons to examine the activity of these cells during fictive stepping induced by electrical stimulation of the MLR in decerebrate cats. The interneurons were identified using the following criteria: they received group II afferent input from the quadriceps nerve, they were not ascending tract cells, and they were antidromically activated by stimulation in the posterior biceps-semitendinosis or gastrocnemius motor nuclei.

None of the interneurons meeting these criteria were rhythmically active during fictive stepping. In addition, the responses evoked from peripheral nerve stimulation (at group II strengths and greater) in these neurons were inhibited during fictive stepping. These evoked responses returned immediately upon cessation of the fictive locomotor activity. These findings are consistent with the fact that these cells are inhibited in animals treated with l-DOPA (Edgley, et al. unpublished). We suggest that these cells form a component of the short latency flexor reflex afferent pathway shown by Lundberg and collaborators (Jankowska, et al, Acta physiol. scand. 70:369-388, 1967) to be depressed in spinal animals following treatment with L-DOPA.

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232.9 MODULATION OF SHORT LATENCY CUTANEOUS EXCITATATION IN FLEXOR AND EXTENSOR MOTONEURONS DURING FICTIVE LOCOMOTION IN THE CAT. B.J.Schmidt, D.E.R.Meyers, M.Tokuriki*, J.W.Fleshman and R.E.Burke
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We previously showed that low threshold afferents in the cutaneous superficial peroneal (SP) nerve produce short-latency (minimally disynaptic) EPSPs in flexor digitorum longus (FDL) motoneurons (MNs) that are markedly facilitated during the early flexion phase of fictive stepping, when FDL is normally active (Neurosci. Abstr. 12:242.9 1986). This suggests that interneurons in this pathway receive convergent excitation from the central pattern generator (CPG) for locomotion and could mediate some fraction of the excitatory CPG locomotor drive to FDL MNs. The present study was designed to examine whether the same general pattern of modulation might hold true for excitatory effects from distal skin regions (nerves: SP, sural, saphenous, and plantar) in other hindlimb flexor and extensor alpha-MNs.

Fictive stepping was induced in 19 decerebrate, unanesthetized cats, either by administration of nialamide and l-DOPA in acutely spinalized (T13) cats (n=17), or by electrical stimulation of the mesencephalic locomotor region (n=2). Each cutaneous nerve was stimulated repetitively (2 x thr; 10 Hz) while recording intracellularly from lumbar MNs during fictive stepping. MNs were identified either by antidromic activation from hindlimb muscle nerves or by phase of activity during stepping. Current pulses (CP; 15-25 ms duration, up to 15 nA) were injected intracellularly between the recurring PSPs to monitor fluctuations in background input conductance. PSPs and CP responses were averaged during the early, mid and late phases of extension or flexion during fictive stepping (3 to 30 steps), limiting attention to cases with initial excitatory components. All EPSP amplitudes in a given stepping sequence were evaluated at the same reference latency.

Input conductance systematically increased in extensor MNs during the phase of flexor activity, and vice versa in flexor cells. Evoked EPSP amplitudes were therefore normalized with respect to input conductance in order to estimate the contribution of premotoneuronal mechanisms to EPSP modulation. In extensor MNs, the earliest EPSP component (n=35; normalized for input conductance) was consistently increased (mean 2-fold) during the flexion phase, irrespective of skin nerve stimulated. In flexor MNs, SP (n=16) and saphenous (n=5) EPSPs exhibited inconsistent phasic modulation, while all sural (n=4) and plantar (n=4) EPSPs were increased (mean 1.4-fold) during the extension phase. The patterns of facilitation do not support the generalization that last-order excitatory interneurons in these cutaneous reflex pathways also mediate CPG excitatory drive to hindlimb motor nuclei. Rather, phasic modulation of these excitatory pathways may simply control specific reflexes during the step cycle.

232.10 ACTIVATION OF LOCOMOTION BY MICROINJECTION OF NEUROTRANSMITTER AGONISTS AND ANTAGONISTS INTO THE AVIAN BRAINSTEM. G.N. Sholomenko and J.D. Steeves. Dept. of Zoology, University of British Columbia (UBC), Vancouver, B.C., V6T 2A9.

The bulbospinal pathways subserving the initiation of locomotion in birds strongly resemble those found in lower vertebrates and mammals (Steeves et al., 1987. Brain Res. **401**:205-212; Sholomenko & Steeves, 1987. Exp. Neurol. **95**:403-418). Electrical microstimulation (25-50uA at 30-60Hz) of several areas within the avian brainstem, including the ventromedial gigantocellular reticular formation (Rgc) and a mesencephalic region near the lateral spiriform nucleus (SpL), activate walking and runnning, as well as flying (at slightly higher stimulating current strengths). Since electrical stimulation activates both neuronal cell bodies and fibers of passage within the region of effective current spread, a more specific means of activating only neuronal cell bodies was sought.

Neurotransmitter agonists and antagonists were infused into the brainstem of acute decerebrate adult birds (Canada geese or White Pekin ducks) via multi-barrel glass micropipettes. Injection of the GABA antagonist, picrotoxin (0.3M,1.5ul), near the SpL produced bouts of locomotor activity (treadmill walking and wing flapping) in animals that did not spontaneously locomote in response to peripheral afferent input from a moving treadmill belt. Injection of smaller amounts of picrotoxin decreased the threshold for activating locomotion via electrical stimulation. Microinjections of GABA blocked both electrically activated and picrotoxin induced locomotion.

Direct injection of the ACh agonist, carbachol (20-100mM,2.0ul), into the Rgc produced prolonged periods of walking. The carbachol induced locomotion was blocked by infusion of an ACh antagonist, atropine (20mM,1.0ul), from another barrel of the micropipette.

Our results in the bird agree with similar findings in rats and cats and indicate that GABAergic and cholinergic components modulate the excitability of brainstem locomotor regions involved in the activation of locomotion.

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232.11 INVOLVEMENT OF SUPRASPINAL REGIONS IN THE PHRENIC-TO-PHRENIC INHIBITORY REFLEX. Dexter F. Speck, Department of Physiology and Biophysics, University of Kentucky, Lexington, KY 40536-0084.

Previous experiments (Brain Res., in press) suggest a role for supraspinal involvement in the production of a phrenic-to-phrenic inhibitory reflex (PIR). A plausible hypothesis is that phrenic nerve afferents project to a brainstem interneuron which has a descending inhibitory effect on phrenic motoneurons. Since expiratory neurons in the Botzinger Complex (BotC) have been demonstrated to have an inhibitory effect on phrenic motoneurons, electrolytic and chemical lesion studies were used to examine the BotC involvement in the phrenic-to-phrenic inhibitory reflex.

All experiments were conducted in decerebrate, paralyzed, artificially ventilated, thoracotomized and vagotomized cats. After routine surgery the brainstem was exposed to permit insertion of microelectrodes into the BotC. Phrenic nerves were isolated bilaterally for recording and/or stimulation. In the first study, tungsten recording microelectrodes were inserted into the region of the BotC. After mapping the expiratory-modulated population, the microelectrode was positioned in the middle of the BotC. The right phrenic nerve was stimulated (3 Hz, 0.2 msec, 0.01 - 15 mA) and the threshold current for eliciting the bilateral PIR was determined. Phrenic neurogram responses to threshold and supramaximal (15 mA) stimulation were recorded before lesioning the BotC (20uA for 2 min) through the recording electrode. After lesioning, the PIR was examined for changes in threshold, duration and efficacy. The microelectrode was then moved to the contralateral BotC and the protocol was repeated. After each experiment, brainstems were removed and the lesions were histologically verified. In most animals the inhibitory reflex was attenuated by bilateral BotC lesions suggesting that the lesioned areas played a role in the reflex.

Six experiments involving chemical lesions (kainic acid - KA) were performed to determine if BotC neurons were important in the PIR. Micropipettes filled with KA (1 ug/ul) and a dye were inserted into the BotC. While recording expiratory-modulated activity, 30-50 nl of KA was ejected. Unilateral KA injections caused transient, severe hypertension and 1-2 hours of apnea and/or apneusis. Once normal rhythmicity resumed, the PIR was compared to control values before making a contralateral injection. This second injection usually disturbed respiratory rhythm for 3-12 hours. When normal rhythmicity returned, the response to phrenic nerve afferent stimulation was unaltered. These results suggest that axons important in producing the phrenic-to-phrenic reflex pass through the region of the BotC, but that BotC neurons are not necessary for this reflex. (Supported by HL 34568)

232.12 EFFECTS OF RETICULAR FORMATION ACTIVATION ON RHYTHMICAL TRIGEMINAL BEHAVIOR IN THE GUINEA PIG. S.M. Gurahian, L.J. Goldberg and S.H. Chandler. Depts. of Oral Biology, Kinesiology, Anatomy and Brain Research Institute, UCLA, Los Angeles, CA 90024

It has been shown that rhythmical jaw movements (RJMs) evoked by 40 Hz stimulation of the masticatory area of the cortex in anesthesized guinea pigs are produced by the potentiation and depotentiation of a short-latency, cortico-trigeminal pathway. This pathway terminates on motoneurons innervating jaw closer and opener muscles. Stimulation of the pontis nucleus caudalis (PnC) of the reticular formation was shown to completely suppress the cortically induced RJM behavior. We hypothesized that this suppression was not due to a direct effect of PnC stimulation on the motoneurons themselves. In order to test this hypothesis, the effect of PnC stimulation on the excitability of trigeminal motoneurons was investigated.

In ketamine anesthesized guinea pigs, bipolar electromyographic (EMG) electrodes were placed in the left anterior belly of the digastric muscle and in the left deep masseter muscle. Jaw movements were monitored utilizing a photoelectric position sensor which tracked a tungsten light source fixed to the mandible. Jaw opener and closer motoneuron field potentials were activated by stimulation of reflex pathways. Repetitive stimulation (40 Hz) of the masticatory area of the cortex was used to evoke RJMs. Stimulation of the same cortical area at 2 Hz (3 pulses, 500 pps) was used to activate the short-latency, cortico-trigeminal pathway (2 Hz stimulation does not evoked RJMs). The PnC was stimulated at 100 Hz, at a current sufficient to abolish cortically evoked RJMs. Recordings of jaw opener and closer motoneuron field potentials and intracellular recordings of closer motoneurons were obtained during stimulation of the PnC alone or in conjuction with cortical stimulation.

It was found that PnC stimulation had no observable effect on the reflex evoked opener or closer motoneuron field potentials. The activation of trigeminal motoneurons by short train pulses to the masticatory area of the cortex was also not affected by PnC stimulation. This same PnC stimulation, however, completely blocked cortically evoked RJMs. In this case the inhibitory postsynaptic potentials produced in jaw closer motoneurons during cortically evoked RJMs were significantly reduced as were the digastric motoneuron field potentials. PnC stimulation itself did not affect the resting membrane potential of jaw closer motoneurons. These findings suggest that PnC stimulation exhibits no direct effect on trigeminal motoneurons and that the suppression of cortically evoked RJMs by PnC stimulation must result from the PnC effect on brainstem networks responsible for the rhythmic potentiation and depotentiation of the cortico-trigeminal pathway. Supported by NIH grants DE4166 and DE06193

NEUROENDOCRINE CONTROLS: PITUITARY V

233.1 VOLTAGE DEPENDENT POTASSIUM CHANNELS IN TELEOST FISH PITUITARY CELLS. C. R. Fourtner and C. A. Loretz* , Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York, 14260.

Hormone-secreting paraneuronal cells of the goby (Gillichthys mirabilis: Teleostei) pituitary gland were studied using the cell-attached and excised-patch variants of the patch clamp technique to investigate the regulation of membrane ion channels in relation to changes in membrane potential. Cells of the rostral pars distalis (RPD) were dissociated using hyaluronidase (0.1 mg/ml) and plated in normal Ringer solution onto plastic petri dishes. Isolated cells of the RPD exhibited spontaneous action potentials of slow duration (ca. 20ms) in cell-attached recordings, resembling the Ca^{2+}-based action potentials described for prolactin-secreting GH3 clonal rat pituitary cells (Dufy, B., et al. Nature 282:855, 1979.) and prolacting-secreting cells of the teleost RPD (Teraskevich, P.S., and Douglas, W. W. Nature 276:832, 1978.); the spontaneous rate of firing was about 2 Hz. In excised, inside-out membrane patches bathed on both sides with defined solutions, two channels have been identified. One is a voltage-dependent K^+ channel with a single-channel conductance of ca. 20-40 pS. Channel activity (as the open probability, P_+) is inversely related to membrane polarization, i.e., depolarization increases P_+ over the range, roughly, of -80 mV to 0 mV. The other is a slow-inactivating K^+ channel of conductance 25-30 pS. Depolarizing voltage steps induce channel opening followed by channel inactivation over the course of a few seconds. Inactivation occurs even after substantial depolarization to membrane potentials in excess of +50mV. The rate of inactivation is proportional to the magnitude of the voltage step. This slow-inactivating channel may be part of a pacemaker mechanism for the rhythmic action potentials.

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233.2 STIMULATION OF DOPAMINE D-2 RECEPTORS ACTIVATES POTASSIUM CHANNELS IN MAMMOTROPHS: EVIDENCE FOR A FUNCTIONAL TARGET OF DOPAMINE ACTION. M. Memo, C. Missale, L. Castelletti*, A. Valerio* and P.F. Spano. Inst. Pharm. Exp. Ther., School of Medicine, University of Brescia, Italy.

The effect of dopamine (DA) and some DAergic agonists (RU 24213, bromocriptine and SKF 82526) were examined for their ability to modify potassium permeability in enriched population of rat prolactin-secreting cells.

Measurement of fluxes of Rb was used to study the properties of voltage-regulated potassium channels. Enriched population of mammotrophs was achieved by discontinous density gradient of Percoll.

We found that DA dose-dependently increased potassium efflux from pituitary cells. The calculated EC-50 was about 3 nM. The maximal effect was achieved by 50 nM DA. This effect was mimicked by different DA D-2 receptor agonists, such as RU 24213 and bromocriptine. On the contrary, the selective DA D-1 agonist SKF 82526 (1 μM) was unable to change potassium permeability. DA-stimulated potassium efflux was antagonized by DA D-2 antagonists such as (-) sulpiride (1 μM) or haloperidol (1 μM). Both (+)sulpiride (the pharmacologically inactive isomer) and SCH 23390 (the DA D-1 antagonist) were inactive in blocking DA-induced potassium efflux.

The increase of potassium permeability was significantly evident after a very short period (30 sec) of exposure of the cells to 10 nM DA.

We previously found that DA, by a D-2 receptor interaction, inhibits calcium entry into pituitary cells. In order to establish whether the reduced calcium channel activity was responsable for an enhanced potassium efflux, cells were exposed to an inorganic calcium entry blocker (500 nM Cadmium) and then DA was tested for its ability to modify potessium channel permeability. The results showed that in this experimental conditions DA was still capable to increase potassium efflux.

Taken together, these data suggest that the primary site of action of DA which may be determinant in cousing iperpolarization is an opening of potassium channel.

233.3 SECONDARY REGULATION OF POLYPHOSPHOINOSITIDE METABOLISM: DOPAMINERGIC ATTENUATION OF PHOSPHOINOSITIDE PHOSPHORYLATION. W.D. Jarvis[1], A.M. Judd*, P.C. Stock*, and R.M. MacLeod[1]. Departments of Internal Medicine and Neuroscience[1], University of Virginia, Charlottesville, VA 22908.

Prolactin release by the anterior pituitary lactotroph is regulated primarily by the inhibitory action of dopamine (DA). Previously, we reported that DA and the D-2 receptor agonist bromocriptine (Br) also produce substantial inhibitions of both basal and stimulated [^{32}P]orthophosphate incorporation into anterior pituitary membranal phosphoinositides; however, receptor-mediated increases in inositol phosphate formation persist unimpaired in the presence of DA or Br, demonstrating that phospholipase C (PhLC) activity is insensitive to dopaminergic influence.

We therefore have examined the influence of D-2 DA receptor activity on the basal turnover rates of phosphatidylinositol (PtdIns), phosphatidylinositol-4-phosphate (PtdIns(4)P), and phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2). Briefly, primary cultures of normal female rat anterior pituitary cells were labeled with [^{3}H]inositol ([^{3}H]Ins) for varying times in the absence or presence of Br (50 nM). Extracted radiolabeled phosphoinositides were deacylated under alkaline conditions and then assayed by conventional anion-exchange resin chromatography.

Incorporation of [^{3}H]Ins into cellular phosphoinositide was linear, reaching isotopic equilibrium; exposure to Br resulted in a continuous (6-36 hr) and significant ($P < .01$) reduction in the rate of [^{3}H]Ins incorporation into all phosphoinositides. Disparate attenuations in the respective rates of radiolabeling of individual phosphoinositide species were also observed. The relative proportions obtained at 36 hr were as follows:

	% total cellular phosphoinositide		
	Control	50 nM Br	
PtdIns	76	87	$p < .01$ vs. control
PtdIns(4)P	19	13	$P < .01$ vs. control
PtdIns(4,5)P_2	4	1	$P < .01$ vs. control

Consistent with our earlier findings, these data indicate that activation of the D-2 DA receptor attenuates the activity of those systems associated with the tandem phosphorylations of PtdIns and PtdIns(4)P processes that give rise to PtdIns(4)P and PtdIns(4,5)P_2, respectively. We therefore propose that DA exerts an inhibitory influence on the phosphorylation, rather than the hydrolysis, of anterior pituitary membranal phosphoinositides, as one of the component mechanisms of the dopaminergic inhibition of prolactin release. [Supported by NIH Research Grant CA-07535 and grants from the American Parkingson Disease Association.]

233.4 DOPAMINE WITHDRAWAL: AN IMPORTANT SIGNAL FOR PHOSPHOINOSITIDE HYDROLYSIS IN LACTOTROPHS. G. Martinez de la Escalera*, T.F.J. Martin* and R.I. Weiner. Reprod. Endo. Center, UCSF, San Francisco, CA and University of Wisconsin, Madison, WI.

Prolactin (PRL) release is tonically inhibited by dopamine (DA). Extensive evidence indicates that withdrawal of the inhibitory influence of dopamine is an important physiological trigger for the stimulation of PRL secretion. Increased activity of adenylate cyclase and intracellular Ca^{++} have been implicated as post receptor mechanisms involved in mediating the action of DA. However, the acute administration of DA has yielded conflicting results regarding the hydrolisis of phosphoinositides, as an early event in the activation of the Ca^{++} pathway. We have tested the effect of withdrawal of tonic DA inhibition on the concentration of phosphoinositides and inositol phosphates. Dispersed anterior pituitary cells from estrogen-treated rats, composed of 70 - 80% lactotrophs, were plated on Matrigel-coated 35 mm dishes. Cells were labeled with (^{3}H)-myoinositol for 36 hs, in the presence or absence of DA (500 nM). The effect of removing or adding DA (500 nM) to the incubation medium for 5 min were tested in the presence of 10 mM LiCl. Labeled inositol phosphate metabolites (IPx) were extracted from the cells and quantitated by anion exchange chromatography. Phosphoinositides (PIx) were quantitated after KOH deacylation. The concentration of IPx was moderately but significantly ($p<0.05$) lower in cells incubated with DA for 36 hs vs untreated cells. The removal of DA for 5 min induced a 77% stimulation of IPx (19,114 ± 981 vs 10,800 ± 388 dpm/1.5X 10^6 cells, n = 3) and a reciprocal 27% decrease in PIx concentrations (11,526 ± 524 vs 15,818 ± 617 dpm/1.5 X 10^6 cells, n = 3). On the other hand, the acute administration of DA to cells incubated without DA for 36 hs, induced no change in the concentration of IPx, although the concentration of PIx was 50% higher than in the controls. These results confirm previous observations showing no decrease in the concentration of IPx in response to the acute administration of DA to cultured anterior pituitary cells. However, the withdrawal of DA was an effective stimulus of phospholipase C mediated hydrolisis of phosphoinositides, consistent with the involvement of this pathway in mediating the action of DA. Furthermore, these results suggest that the important biological signal for PRL secretion is the removal of DA inhibition, which is in fact the normal physiological occurrence.
(Work supported by NIH Grant HD08924 and the Mellon Foundation, fellowship G.M.E.).

233.5 EFFECTS OF TRH AND THE CALCIUM CHANNEL AGONIST BAY K 8644 ON IP_4 AND OTHER INOSITOL PHOSPHATES AND ON PROLACTIN RELEASE. J.A. Pachter*, G.J. Law* and P.S. Dannies* (SPON: S.F. Basinger). Dept. of Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

TRH (thyrotropin-releasing hormone) stimulates prolactin release from anterior pituitary lactotrophs by a mechanism which is thought to involve stimulation of phosphoinositide metabolism and opening of membrane Ca^{2+} channels, both of which elevate cytosolic free calcium levels ($[Ca^{2+}]_c$). Both 1 uM TRH and the calcium channel agonist BAY K 8644 (1 uM) stimulated prolactin release from perifused GH_4C_1 anterior pituitary tumor cells. When added simultaneously, these agents were synergistic. To investigate the mechanism of this synergism, we measured the effects of TRH and BAY K 8644 on $[Ca^{2+}]_c$ levels, $^{45}Ca^{2+}$ uptake, and inositol phosphate production. Measurements with the fluorescent Ca^{2+} indicator indo-1 in a perifusion system as previously described (Law et al., 1987, Endocrine Soc. Abstract) showed that each agent was capable of elevating $[Ca^{2+}]_c$ above the level stimulated by the other agent. This enhancement of $[Ca^{2+}]_c$ does not result from the collective actions of TRH and BAY K 8644 on measurable Ca^{2+} influx, since the net stimulation of $^{45}Ca^{2+}$ uptake by BAY K 8644 was inhibited by 60%, rather than facilitated by TRH. Although these $^{45}Ca^{2+}$ data do not rule out the possibility that synergistic effects of TRH and BAY K 8644 on Ca^{2+} influx might be masked by a simultaneous increase in the rate of Ca^{2+} efflux, they suggest that the collective effects of TRH and BAY K 8644 on $[Ca^{2+}]_c$ may result, at least partly, from intracellular Ca^{2+} mobilization. Thus, we predicted that BAY K 8644 and TRH might cooperatively stimulate the production of inositol trisphosphate (IP_3) which is known to mobilize intracellular Ca^{2+}.

To measure inositol phosphate production, GH_4C_1 cells were preincubated in monolayers for 48 hours with media containing [^{3}H]-myoinositol. The dishes were treated with 1 uM TRH or 1 uM BAY K 8644 in the presence of 10 mM LiCl, followed by acid extraction, neutralization and separation of inositol phosphates on dowex columns. TRH induced 1.9-, 3.3-, 4.8- and 1.6-fold rises in IP_1, IP_2, IP_3 and IP_4, respectively within the first 15 seconds. BAY K 8644 had no effect. When given together, however, BAY K 8644 enhanced the effect of TRH on IP_3 and IP_4 by 62% and 45%, respectively over a 20 minute period, with lesser enhancements of TRH-stimulated IP_1 and IP_2 levels. These results suggest that the synergistic effects of TRH and BAY K 8644 on prolactin release may be mediated, at least partly, by enhancement of TRH-stimulated inositol polyphosphate production by the Ca^{2+} channel agonist. Whether this effect of BAY K 8644 is a consequence of its action on Ca^{2+} channels remains to be determined.

233.6 EFFECTS OF SRIF AND DA ON BAY K 8644-INDUCED PROLACTIN AND GH RELEASE FROM PITUITARY CELLS IN VITRO ; MODULATORY ACTION OF 17 β-ESTRADIOL. S.V. Drouva, C. Berthelier*, E. Rérat*, E. Laplante*, H. Clauser*, C. Kordon U. 159 INSERM, 2 ter rue d'Alésia, 75014 Paris, France.

Dopamine (DA) as well as SRIF are both effective inhibitors of prolactin (PRL) and the latter of Growth hormone (GH) release from rat anterior pituitary ; their potency is however modulated by sex steroids. Different cellular mechanisms have been proposed to mediate their inhibitory action. In the present study we attempted to investigate their possible interactions with the pituitary Ca2+ voltage dependent channels and evaluate sex differences or modifications after 17 β-estradiol (E2) treatment. BAYK8644 (BK) a calcium voltage dependent channel agonist, stimulated in a dose (10^{-9}-10^{-7} M) and time (15-120 min) dependent manner PRL and GH release in primary cultures of pituitary cells obtained from male or female rats. The effect on PRL secretion was however more pronounced than that on GH release with any dose, time and experimental model tested and both were blocked by nifedipine, a calcium channel antagonist. DA (10^{-7} M) inhibited BK-induced PRL release from male or female pituitary cells treated or not with E2 (10^{-9} M for 72 hours). SRIF (S-14, 10^{-9}-10^{-8} M) although without effect on BK-induced PRL release, inhibited significantly the GH responses to BK from male as well as female pituitary cells cultured in the absence of E2. Administration of E2 to female pituitary cells not only enhanced the stimulatory action of BK on PRL and GH release, but revealed the inhibitory effect of SRIF on BK-induced PRL secretion and increased SRIF inhibition of BK-induced GH release, effects which may be related to the E2-induction of SRIF receptors. Interestingly, BK was unable to stimulate LH release from male pituitary cells ; however, it was effective after long incubations with female pituitary cell cultures and became more potent in cultures receiving E2. BK stimulation of pituitary hormones secretion does not implicate inositol phospholipids hydrolysis, since inositol phosphates estimation by anion-exchange chromatography showed no significant difference between control and BK-treated cells at any time and experimental model tested. In addition, involvement of phospholipase A2 in BK effect, can be excluded since 5, 8, 11, 14-eicosatetraynoic acid (ETYA) was unable to counteract the BK-induced hormones secretion. These data indicate that the distribution as well as the activity of calcium voltage dependent channels are different in different pituitary cell populations and might be modulated by sex steroids. In addition, they suggest that a possible site of the inhibitory effect of SRIF and DA may be the calcium channel, gating calcium entry into pituitary cells.

233.7 EFFECTS OF GENDER ON FORSKOLIN-ASSOCIATED LUTEINIZING HORMONE RELEASE BY PERIFUSED RAT ANTERIOR PITUITARY FRAGMENTS. M.J.Sollenberger*, L.Rajan* and W.S. Evans* (SPON: C.Desjardins). Department of Internal Medicine, University of Virginia, School of Medicine, Charlottesville, VA 22908.

We have previously shown that forskolin (FORSK), a compound which increases intracellular cyclic AMP (cAMP), causes a concentration-dependent release of luteinizing hormone (LH) by continuously perifused anterior pituitary cells from female rats (AJP 12:E392,1985). We have also observed that cAMP causes a gradual rather than acute release of LH and have postulated that cAMP mediates the process whereby non-immediately-releasable LH becomes available for release. We have further hypothesized that the non-immediately-releasable LH pool is estrogen dependent and that the amount of LH accessable by cAMP is greater in female vs. male pituitary tissue. To investigate this thesis we have extended our original studies to compare the concentration-response relationships between tissue from female (at random stages of the estrous cycle) and male rats. Anterior pituitary fragments were perifused for 4 h with medium (Medium 199) alone and then exposed to medium or various concentrations of FORSK for 4 h. LH in the eluate was measured by radioimmunoassay. Mean (±SEM) LH secretory rates (pg/min/mg pituitary tissue), expressed as change from baseline, were as follows:

	Concentration of Forskolin (uM)						
	0.01	0.03	0.1	0.3	1.0	3.0	10.0
Male (n=4)	1.4 ±1.4	1.8 ±1.2	5.3 ±5.3	5.0 ±2.3	8.7 ±3.9	8.1 ±6.0	10.2 ± 3.8
Female (n=3)	3.1 ±1.5	9.9 ±3.1	4.9 ±2.6	21.3 ± 6.0	25.0 ± 5.4	27.5 ± 4.8	26.3 ± 3.2

The concentration of FORSK necessary to elicit a half-maximal response (EC_{50}) was approximately the same in tissue from female vs. male rats (0.15 vs. 0.13 uM). However, the maximal secretory rate achieved using tissue from female rats was 3-fold higher than that obtained with tissue from male rats (28.1 vs. 9.3 pg/min/mg pituitary tissue). This gender related difference in the ability of FORSK to release LH is particularly interesting since it is well described that pituitaries from female rats contain less total LH than those from male rats. These results are consistent with the above hypothesis that increased LH secreted by female glands may be the result of increased cAMP activation and/or increased amounts of hormone which can be accessed by cAMP. Supported by HD19170 and HD00711.

233.8 HUMAN RELAXIN STIMULATES CYCLIC AMP ACCUMULATION IN CULTURED RAT ANTERIOR PITUITARY. M.J. Cronin and C. Bakhit. Genentech, Inc., Pharmacological Sciences, South San Francisco, California 94080.

Relaxin is a hormone of human pregnancy that promotes restructuring of connective tissue, induces a quiescent myometrium until late gestation, and facilitates cervical ripening at term. Recently, it has been shown with native porcine relaxin that oxytocin and vasopressin neurons in the hypothalamus may be functional targets of relaxin (Nature 309:372, 1986 & 325:813, 1987). Following the structural identification and synthesis of pure human relaxin, the scope of its action and the response of second messenger system(s) activated or inhibited by the relaxin receptor can be ascertained. We studied anterior pituitary cells to determine if there was a change in cyclic AMP levels that would suggest both an important target for relaxin and a potential intracellular mediator for the relaxin receptor. Primary cultures of anterior pituitary cells from randomly cycling female rats were utilized. Intracellular cyclic AMP was measured by radioimmunoassay. We found that human synthetic relaxin stimulated cyclic AMP accumulation in a graded manner (Table). In cells treated with a phosphodiesterase inhibitor (i.e., isobutylmethylxanthine, 1 mM), the half-maximal concentration of relaxin required for stimulation was 100-300 pM, and maximal stimulation was 8.3 ± 2.2-fold above baseline at 15 min (4 independent experiments). It was of interest that somatostatin inhibited by about 90% relaxin-stimulated cyclic AMP levels, indicating that a somatostatin-sensitive endocrine cell(s) responds to relaxin. These data imply that anterior pituitary cells express relaxin-like receptors that can directly or indirectly stimulate adenylate cyclase activity. With the necessary involvement of the pituitary in reproduction, we suggest that this gland is a component of a relaxin endocrine axis.

Treatment	Concentration (nM)	Cellular Cyclic AMP* (pmoles/well/15 min)
Vehicle	-	0.88 ± 0.12 (5)
Relaxin	0.003	0.93 ± 0.15 (3)
	0.010	1.18 ± 0.06 (3)
	0.030	1.96 ± 0.08 (3)
	0.100	3.55 ± 0.16 (3)
	0.300	9.83 ± 1.0 (3)
	1.0	10.8 ± 1.2 (3)
	3.0	11.5 ± 2.2 (3)
	10.0	11.0 ± - (2)
Somatostatin + Relaxin	100. 1.0	1.88 ± 0.04 (3)

* mean ± SEM (n wells of representative study)

233.9 WHOLE-CELL PATCH PIPETTE RECORDINGS OF MEMBRANE CAPACITANCE CHANGES IN GH3 PITUITARY CELLS EXPOSED TO SECRETAGOGUES. B. Dufy and J.L. Barker, Lab. Neurophysiologie, CNRS UA 1200, University of Bordeaux II, France and Lab. Neurophysiology, NINCDS, Bethesda, MD.

Clonal "GH3" pituitary cells that secrete prolactin and growth hormone express chemically and electrically regulated forms of membrane excitability. We have attempted to resolve the relationship between membrane excitability and exocytotic secretion by utilizing the patch pipette recording technique in the whole-cell recording (WCR) mode in conjunction with a lock-in amplifier system similar to that described by Neher and Marty (PNAS, 79 (1982) 6712). The coupling of secretagogue stimulation at the membrane with exocytosis of cytoplasmic granule hormone contents is thought to involve fusion of granules with plasma membrane followed by an increase in surface area and, in consequence, membrane capacitance (C_m). When WCRs were made from 64 15-25 micron GH3B6 cells with a simple intracellular electrolyte in the pipette (140mM Kgluconate, 2mM $MgCl_2$, 5mM HEPES (pH7.3) and 1.1mM EGTA (estimated intracellular Ca^{2+}-about 10nM)), the resting properties were -45 (±8)mV membrane potential, 2 Gohms input resistance and 19(±5) pF C_m. Under these conditions C_m fluctuated over a 0.3pF range with smaller excursions superimposed on the larger fluctuations. Applications of 30mM K^+ sufficient to depolarize cells by about 40mV for 30 seconds had typically modest effects on C_m, usually involving 10-20-second increases of about 0.2-0.3pF. Invariably, these disappeared after 15-20 minutes of WCR. When WCRs were made with pipettes containing an electrolyte formulated using an aqueous lysate of the GH3B6 clone as the diluent (Dufy, et al BBRC 137 (1986) 388), resting properties were ostensibly similar. However, pulses of K^+ now evoked C_m increases of 0.6-0.8pF that often lasted over 120 seconds. If such K^+ pulses were repeatedly applied the increase and duration of the C_m response decreased and eventually disappeared. In cells dialyzed with lysate C_m did not detectably change in response to depolarizing current injections sufficient to generate 60-second trains of Ca^{2+}-dependent action potentials at -30mV. Applications of thyrotropin releasing hormone (TRH), which induces prolactin secretion from GH3B6 cells, evoked changes in membrane excitability and C_m that rapidly disappeared when simple electrolyte was used, but could be sustained if lysate was included. When present, C_m often increased during the period of TRH-induced hyperpolarization. The results indicate that soluble components in the cytoplasm are essential for secretagogue-mediated changes in C_m and that C_m responses induced by TRH can start without activation of voltage-dependent Ca^{2+} conductance mechanisms.

234.1 ADRENERGIC INFLUENCES ON APOMORPHINE-INDUCED STEROTYPED MOVEMENTS IN PIGEONS. D. C. Fisher* & I. J. Goodman
Department of Psychology, West Virginia University, Morgantown, WV 26506

A variety of sterotyped responses may be induced in birds by apomorphine (APO), a dopamine (DA) agonist, with dosage affecting the type, frequency and duration of these responses (Goodman, 1981). Agents affecting other brain transmitter systems (e.g. cholinergic, serotonergic) interact with the DA system to alter APO-induced repetive pecking rates, for example; whereas, adrenergic agents are reported as ineffective (Cheng & Long, 1974). Because the latter report is based on limited testing and because norepinephrine (NE) is implicated in repetitive pecking induced by a feeding intervention (FIBS) (Goodman et al, 1983), the present study attempted to further test the possible influence of adrenergic systems on APO sterotypy.

Observations of adult male pigeon behavior were made by raters via a video monitor. The procedure of visual-hand counting has the disadvantage of requiring vigilant viewing, but permits measurement of non-wall or -floor pecks (e.g. air and body pecks) and non-pecking sterotypies (e.g. headshakes, mandibulating, and swallowing), which an automated sensing system might misinterpret or ignore. Birds were tested with a fixed dose (1.5 mg/kg, im) of APO alone during one session and with the systemic administration of adrenergic test drugs preceding that of APO in another session.

Clonidine, an alpha agonist acting at pre- and postsynaptic receptors, slightly increased pecking rates at low doses (below .03 mg/kg), but markedly decreased pecking at higher doses, as did yohimbine, a presynaptic alpha antagonist, (.1 - 1.0 mg/kg). Prazosin, a postsynaptic alpha antagonist, on the other hand, increased pecking (.1 - .5 mg/kg). The beta antagonist, propranolol, reduced pecking slightly at 3 mg/kg and below, and markedly above this dosage. Isoproterenol, a beta agonist, markedly increased pecking at .05 mg/kg.

Future work should more directly test the effects of centrally administered adrenergic agents to separate out peripheral contributions and localize their central site(s) of action.

234.2 ACCUMBENS DOPAMINE MODULATES AMYGDALA SUPPRESSION OF SPONTANEOUS EXPLORATORY ACTIVITY IN RATS. C.Y. Yim and G.J. Mogenson. Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada. N6A 5C1

Previous electrophysiological experiments showed that stimulation of the basolateral nucleus of the amygdala (AMY) inhibits ventral pallidal (VP) neurons and that the inhibition was modulated by accumbens (NAcc) dopamine (DA) (J. Neurophysiol. 50(1983)-148). VP neurons, on the other hand, project to the midbrain mesencephalic locomotor region and it has been shown that GABAergic inhibition of neuronal activity in the VP suppresses locomotor activity. These observations suggest that AMY may have an inhibitory influence on ambulatory activity mediated by way of the projection to the NAcc and subsequently to the VP. Furthermore, as suggested by the electrophysiological findings, the influence of AMY on locomotor activity may be modulated by NAcc DA. To investigate these possibilities, the effects and interactions of injections of NMDA into the AMY and DA into the NAcc on the spontaneous ambulatory activity of rats were tested in a series of behavioral experiments. Male Wistar rats implanted with injection guide cannulae into the AMY, NAcc and/or VP were placed in an experimental cage partitioned into 3 chambers with communicating holes between the chambers. Animals remained spontaneously active in the experimental cage for over 30 min even for repeated trials. It was found that NMDA injection into the AMY produced a 35%-72% (n=48) reduction in this spontaneous exploratory activity. The suppression of the exploratory behavior was enhanced by an injection of nipecotic acid (.5-1.0 ug), a GABA uptake inhibitor, into the VP but was attenuated by injection of GDEE (5 ug), a glutamatergic receptor blocker, or DA (5 ug) into the NAcc. None of these injections by themselves produced any significant change in locomotor activity. These findings indicate that the AMY to NAcc to VP projection has an inhibitory role in the behaving animal, a hypothesis which is consistent with electrophysiological findings and earlier reports in the literature that stimulation of AMY produces "arrest" and "attentive" responses in animals. Furthermore, these results also illustrate that dopamine released in the accumbens, perhaps by other competing behavioral processes, can indirectly activate the motor system by suppressing the inhibition from the amygdala by a presynaptic modulatory action shown in previous electrophysiological experiments.
(supported by MRC of Canada)

234.3 DIFFERENTIAL EFFECTS OF DOPAMINE D_1 AND D_2 AGONISTS (SKF 38393 AND QUINPIROLE - LY 171555) AND ANTAGONISTS (SCH 23390 AND SULPIRIDE) ON THE ACOUSTIC STARTLE REFLEX: INTERACTIONS WITH APOMORPHINE AND COCAINE. M. Davis, Dept. Psychiat., Yale Univ. Sch. Med., 34 Park St. New Haven, Ct 06508

The acoustic startle reflex is known to be increased by drugs that increase dopamine transmission such as d-amphetamine, apomorphine, and cocaine. The present study evaluated the effects of more selective D_1 and D_2 agonists on this behavior.

Groups of 10 rats each were injected subcutaneously with a selective agonist or its vehicle and startle was elicited with a 105-dB burst of white noise presented once every 20 sec for a 30-60 min period immediately after injection. The D_2 agonist quinpirole markedly and potently (ED_{50} = 0.30 mg/kg) depressed acoustic startle monotonically over a dose range of 0.07 to 10 mg/kg. This effect was blocked by the D_2 antagonist sulpiride (20 mg/kg), which at this dose did not alter startle. In contrast, the D_1 antagonist SCH 23390 (0.25 mg/kg) depressed startle baseline but still did not block the inhibitory effect of quinpirole. On the other hand, the D_1 agonist SKF 38395 (5 - 40 mg/kg) increased acoustic startle. The excitatory effect of 40 mg/kg was blocked by the D_1 antagonist SCH 23390 (0.25 mg/kg). Similarly, excitatory effects of cocaine (10 mg/kg) were blocked by SCH 23390. In contrast, this same dose of SCH 23390 did not block excitatory effects of other drugs such as strychnine (1.0 mg/kg) or 8-OH-DPAT (1.25 mg/kg) which increase startle by non-dopaminergic mechanisms.

These data suggest that D_2 agonists depress acoustic startle whereas D_1 agonists increase acoustic startle. Given this antagonist relationship between D_1 and D_2 selective drugs, the effects of mixed D_1,D_2 agonists like apomorphine on acoustic startle may result from a balance between D_2 mediated inhibition and D_1 mediated excitation. Consistent with this hypothesis, a dose of 0.4 mg/kg of apomorphine, which increases acoustic startle in vehicle-pretreated rats, actually depresses startle after pretreatment with the D_1 antagonist, SCH 23390. Conversely, the excitatory effect of 0.2 mg/kg of apomorphine was augmented by pretreatment with sulpiride (20 mg/kg).

These data suggest that activation of D_1 and D_2 receptors can have opposite effects on a simple reflex. Moreover, acoustic startle provides the advantage that the same behavioral measure could be used to evaluate functional changes in D_1 vs. D_2 receptor sensitivity following denervation or chronic treatment with dopamine antagonists, a subject currently being investigated.

234.4 **A COMPARISON OF INTRASTRIATAL AND PERIPHERAL AMPHETAMINE STEREOTYPY.** J.M. Wallace* and P. Winn* (SPON: P. Dean). Dept. Psychol., Univ. St. Andrews, St. Andrews, Fife, Scotland KY16 9JU.

Lesion studies suggest that (d-) amphetamine (AMP) stereotypy is mediated by dopamine (DA) in the dorsal striatum (DS) while AMP-induced locomotion is mediated by the DA in the ventral striatum (VS) (Kelly P., Seviour P.W. and Iversen S.D., Brain Res., 94: 507-522, 1975). However, attempts to induce stereotypy by injection of AMP into DS have not been wholly successful. It might be suggested that to produce stereotypy equivalent to that induced by i.p. AMP one needs to stimulate both DS and VS. If this is the case, simultaneous injection of AMP into DS and VS should produce responses similar to i.p. AMP; but if stereotypy can be produced by stimulation of DS alone, and locomotion by VS, it would suggest striatal dissociation. Twelve male rats were implanted with 2 pairs of bilateral guide cannulae, one aimed at DS the other at VS. Rats were given 3 habituation sessions of 1 hr each. After this i.p. injection of 5mg/kg AMP and saline were given in a randomised order, followed by 4 intracerebral AMP conditions: VS 160ug AMP, DS saline; VS saline, DS 160ug; VS 80ug, DS 80ug; VS saline, DS saline. Each injection was of 2ul, infused over 4min and cannulae were left for a further 2min to allow for diffusion. A minimum of 48hr was allowed between treatments. Behaviour was categorised using a checklist and a modified Creese-Iversen stereotypy rating scale, observations being made in a 10sec window every 2min for 40min. Infusion of AMP into DS, VS or DS/VS produced stereotypy no different to that of 5mg/kg i.p.. Chekclist data revealed some AMP-saline differences: infusion into VS produced more sniffing, rearing and locomotion than control; infusion into DS produced no increase in rearing or locomotion but did increase head down movements and sniffing; simultaneous infusion into DS and VS increased locomotion and sniffing but not rearing. Rating scale data suggests that infusion into VS or DS/VS produces a stereotypy with locomotion but that infusion into DS produces stereotypy in a fixed position. These data suggest that it is possible to elicit stereotypy from infusion of AMP into the striatum indistinguishable from that produced by 5mg/kg i.p., but that the dissociation of DS and VS is not as clear as has been thought.

234.5 IN VIVO DIALYSIS MEASUREMENTS OF STRIATAL DOPAMINE AND DOPAC IN THE TRAINED CIRCLING RAT. K.E. Sabol and C.R. Freed, Univ. of Colo. Health Sciences Center, Denver, CO 80262.

Previous reports from our laboratory have shown that trained circling rats have increased dopamine (DA) and DOPAC levels in striatum contralateral (contra) to the direction of the circling. (Yamamoto and Freed, Nature 298: 467, 1982; Freed and Yamamoto, Science 229: 62, 1985). These results were obtained using direct tissue assay and in vivo electrochemistry. We have now used the technique of in vivo dialysis to directly measure release of striatal DA and DOPAC during movement. Male Sprague-Dawley rats 300-350 gm were trained to run in place on a circular treadmill. The treadmill apparatus was made of 2 concentric plexiglass tubes, the outer 8" and the inner 2" in diameter. The rats ran in the circular path between the 2 cylinders. One quarter of the chamber was closed off to the rat and contained a tail holding device. The floor was detached from the rest of the chamber and was free to rotate. A liquid feeder was attached to the inner cylinder. Rats were water deprived and placed in the chamber with the tail clamped. As the animal walked in place with its anchored tail, the floor moved under him. The animal received a drop of water for each 360 degree turn of the floor disk. The circular shape of the chamber and running path led to an asymmetric arc posture. The apparatus was designed so that the rat could circle both to the right and left and turning was monitored by a VIC-20 microcomputer. When stable performance was achieved in both directions, rats were implanted with 17 gauge guide cannulae which extended 3 mm below dura overlying striatum. After one week of surgical recovery and additional training, animals were tested with an in vivo dialysis probe lowered into medial anterior striatum (A 2.4 mm anterior to bregma, L 2.0 mm, H 7.5 mm below dura; nosebar set at +5). The concentric design dialysis probe was made of 300 μ diameter dialysis tubing (ENKA). Ringer's solution was perfused through the cannula at 0.52 μl/min and the effluent was directly injected into an on-line HPLC system. Every 10 min, a 5 μl sample was automatically injected onto a 100 x 1 mm microbore column (Keystone Scientific: 3 μ C18 Spherisorb packing) by a pneumatically activated valve under the control of a VIC-20 microcomputer. An ISCO uLC-500 HPLC syringe pump and BAS LC-4B EC detector were used. Mobile phase consisted of 0.05 M trichloroacetic acid, 0.2 M H_3PO_4, 0.1 mM EDTA, 3% MeOH, 0.22 mM octyl sodium sulfate, and pH = 4.4. Under these chromatographic conditions, DOPAC and DA retention times were 3.0 and 5.8 min respectively. Three hours after the cannulae were lowered into the brain baseline DOPAC values were 14.4 ± 1.8 pmole/5 ul/10 min; and dopamine values were 30.5 ± 4.4 fmole/5 ul/10 min. The effects of inplace circling on dopamine and DOPAC values will be discussed.

234.6 MEASUREMENT OF SEROTONIN RELEASE BY IN VIVO DIALYSIS IN HYPOTHALAMUS OF FREELY-MOVING RATS. M. Minzenberg* and S. Auerbach. Biological Sciences, Rutgers Univ., Piscataway, N. J. 08855.

A controversy exists concerning the role of serotonin (5-HT) in physiology. Pharmacological studies indicate that increased 5-HT release in the CNS mediates sleep onset. In opposition is evidence from single-unit studies that the discharge rate of 5-HT- neurons declines during the transition from waking to slow-wave sleep. An important factor to consider when interpreting single-unit studies is that release of 5-HT at terminal areas may be locally altered independent of changes in neuronal discharge. We are using in vivo dialysis to determine if there are significant dissociations between neuronal activity and 5-HT release that could account for the disparate conclusions.

Rats were stereotaxically implanted with guide cannulae aimed at the lateral hypothalamus. Two weeks later, a concentric-model dialysis probe was inserted into the hypothalamus through a guide cannula. The probe was perfused with artificial CSF through a fluid swivel. Rats were free to move within the experimental chamber. Analysis of 5-HT in the perfusate was accomplished via HPLC-EC.

Initial experiments were carried out to determine if 5-HT release reflects predicted effects of drugs that alter monoamine release. Fenfluramine is a 5-HT releasing agent that produces an acute behavioral "5-HT syndrome". Fenfluramine (10 mg/kg ip) stimulated 5-HT release to 2.1 ± 0.8 times the mean baseline value (at 1.5 h post drug; mean ± s.e.; n=5). Duration of the elevated 5-HT release closely paralleled the 2.5 h time course of the 5-HT syndrome. Local injection of fenfluramine (1 ug in 1 ul saline) into the hypothalamus had no apparent behavioral effects but produced a 2050% (mean; n=3) increase in 5-HT release. Peak release was obtained at 15 min post-drug.

We next examined 5-HT release across the light-dark cycle. Levels of 5-HT in the perfusate collected during the dark period was 260.5 ± 25.4% (mean ± s.e.; n=10) of 5-HT baseline levels before lights-off. Since rats are inactive for a significant portion of the light hours and very active in the dark, these results support previous electrophysiological studies in freely-moving cats that suggest decreased 5-HT activity at sleep onset. (supported by NSF grant BNS-8708014)

234.7 METAPHIT PREVENTS LOCOMOTOR ACTIVATION BY DOPAMINE UPTAKE BLOCKERS AND INCREASES HOMOVANILLIC ACID. M.E.A. Reith, P. Berger*, A.E. Jacobson*, K.C. Rice* and H. Sershen*. Ctr. for Neurochemistry, N.S. Kline Inst., Ward's Island, NY 10035, Clinical Neuroscience Research Unit, Yale Univ. Sch. of Med., New Haven, CT 06508, and NIADDK, NIH, Bethesda, MD 20892.

Metaphit (1-(1-(3-isothiocyanathophenyl)-cyclohexyl)-piperidine), a phencyclidine (PCP) analog and a proposed PCP receptor acylator, inactivates the carrier involved in the neuronal uptake of dopamine in in vitro experiments with mouse striatal preparations (Berger et al., Neuropharmacol. 25: 931, 1986; Schweri et al., J. Neurochem. 48: 102, 1987). The present experiments were carried out to assess whether in vivo administered metaphit (20 mg/kg i.v.) 1) produces a long-term blockade of dopamine uptake sites, 2) antagonizes the hyperactivity induced by various psychostimulant drugs, and 3) affects the concentrations of dopamine metabolites in the striatum and olfactory tubercle of young adult male BALB/cBy mice.

In ex vivo experiments 2 h and 24 h after i.v. metaphit-pretreatment, no changes were observed either in [^{3}H]cocaine binding to striatal membranes or in [^{3}H]dopamine uptake into synaptosomes or slices. In in vivo experiments 24 h after metaphit-pretreatment, selective labeling of dopamine uptake sites in the mouse striatum with [^{3}H]GBR (1-(2-(diphenylmethoxy)-ethyl)-4-(3-phenylpropyl)-piperazine) was unaffected.

Metaphit-pretreatment 24 h prior to locomotor testing, antagonized the hyperactivity induced by i.p. administration of dopamine uptake blockers (methylphenidate 20 mg/kg, mazindol 12 mg/kg, cocaine 25 mg/kg, GBR 20 mg/kg) but not that induced by drugs that affect locomotion by different mechanisms (amphetamine 6 mg/kg, phencyclidine 5 mg/kg). Metaphit did not affect the baselines of locomotor activity. No differences were observed between the pretreatment groups in the distribution of postinjection activities over 10-min blocks during the 30-min period after injection of methylphenidate, mazindol, or cocaine.

Twenty-four h after treatment with metaphit there was an increase in homovanillic acid in the striatum and olfactory tubercle. There was no effect of metaphit on the disappearance rate of 3,4-dihydroxyphenylacetic acid (half-life 8 min) and homovanillic acid from the striatum during monoamine oxidase inhibition with pargyline (100 mg/kg i.p.). If the increase in homovanillic acid reflects a higher rate of dopamine catabolism in metaphit-pretreated animals, it could explain the lack of locomotor stimulating effect of dopamine uptake blockers in these animals resulting from a rapid breakdown of extracellularly accumulated dopamine.

Supported by USPHS grant DA 03025 and a Cocaine Research Grant from New York State to M.E.A.R.

234.8 **SPARING FROM SENSORIMOTOR IMPAIRMENTS IN RATS DEPLETED OF BRAIN DOPAMINE DURING DEVELOPMENT.** **F.B. Weihmuller* and J.P. Bruno. Psychobiology Program, Dept. of Psychology, Ohio State University, Columbus, OH 43210.**

We have reported that rats given near-total depletions of brain DA on Day 3 do not exhibit the severe deficits in ingestion seen in comparably lesioned adults. The present experiments compared sensorimotor integration in rats depleted of striatal DA as neonates, weanlings, or adults, and determined whether exposure to stress can undermine these functions. Animals were injected with 6-HDA (150-250 ug, ivt) on Day 3, Day 15, or as adults 30 min after treatment with the NE uptake blocker desmethylimipramine (25 mg/kg, ip). Weanlings and adults were also given the MAO inhibitor pargyline (40 mg/kg, ip) to potentiate the actions of 6-HDA. Sensorimotor function was assessed using a comprehensive battery of tests in each group of animals soon after the lesion and once the pups reached adulthood. Rats sustaining ≥ 90% depletions of striatal DA as adults (n=6) exhibited akinesia, catalepsy, and tremor for up to 49 days after the lesion. These animals also exhibited sensory neglect, particularly of the hindlimbs and flanks, as 3-8 times more force was required to elicit orientation to a Von Frey hair than was needed in controls. Three aspects of the behavior of adult-treated animals were noteworthy. First, while the sensorimotor deficits in rats with ≥ 90% depletions persisted for up to 7 weeks postlesion, the initial ingestive deficits were only apparent for 7-9 days; thus the appearance of ingestive and sensorimotor deficits can be temporally dissociated. Second, deficits in sensorimotor integration were obvious even in rats with depletions as small as 50-80% (n=6), although they recovered more quickly. Third, even after these sensorimotor impairments had disappeared they could easily be reinstated by forcing the animals to swim in cold (13°C) water for 25 sec. In marked contrast, rats sustaining ≥ 90% depletions on Day 3 (n=4) or Day 15 (n=6) did not exhibit any signs of akinesia, catalepsy, tremor, or sensory neglect either soon after the lesion or once they reached adulthood. Moreover, the stress of a cold-water swim revealed no deficits. These results extend our original observations by demonstrating that the behavioral sparing seen in rats depleted of DA as neonates is also seen when lesions are made during weaning and that the nature of this sparing includes sensorimotor functions as well as ingestive behavior.

234.9 MAPPING OF DOPAMINE LEVELS IN THE MESO-CORTICAL DOPAMINE TERMINAL FIELD: AN IN VIVO ELECTROCHEMICAL STUDY. G. Gerhardt, M. Parrish* and A. Gratton, Depts. of Psychiat. & Pharmacol., U. of Colorado Hlth. Sci. Ctr., Denver, CO 80262.

There is increasing evidence that the meso-cortical dopamine (DA) system may be an important substrate for the euphoretic properties of psychomotor stimulants. While the anatomy of the DA cortical terminal field has been characterized with various histochemical techniques, there is little data describing, in vivo, the anatomical distribution and temporal dynamics of DA release in cortex. In the present study we measured, with in vivo high speed chronoamperometry, the release of cortical DA following local applications of potassium (K^+).

Male Sprague-Dawley rats were anesthetized with urethane and placed in a stereotaxic apparatus. A glass capillary filled with 123 mM K^+ and a Nafion-coated graphite/epoxy capillary (GEC) electrode were fastened together and lowered into the brain. The cortical region explored extended from 1.5 to 4.0 mm anterior to bregma and from 0.8 to 2.5 mm from the midline. Chronoamperometric measurements were performed by applying square-wave pulses (from -0.2 to +0.45 V) to the GEC at a rate of 5 Hz. The current flow resulting from the oxidation and subsequent reduction of electroactive species at the GEC tip was digitized and integrated over the last 60% of the positive and negative going pulses. The ratio of the recorded oxidation to reduction currents was used as an index for the identification of the released electroactive species. Figure A is an example of a K^+-evoked release of DA in the medial prefrontal cortex and Figure B shows variations in the magnitude of evoked releases as a function of the cortical sites tested. The anatomical distribution of K^+-evoked releases as well as the ratio of the recorded oxidation to reduction currents suggest that DA is the predominant monoamine in the area of cortex we studied. Interestingly, the magnitude and temporal dynamics of K^+-evoked releases of cortical DA appear to be similar to those previously reported in nucleus accumbens.

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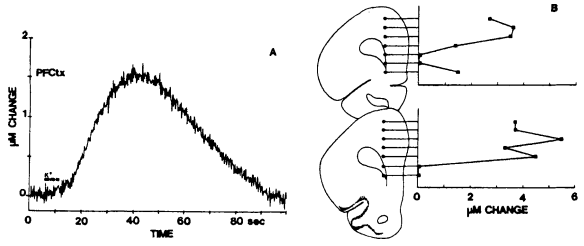

234.10 PHARMACOLOGICAL AND BEHAVIORAL STIMULATION PRODUCE CHANGES IN STRIATAL DOPAMINE ACTIVITY MEASURED BY IN VIVO DIALYSIS. J.D. Salamone*, E.D. Abercrombie, R.W. Keller, M.J. Zigmond, E.M. Stricker. (SPON: Shang Yao). Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

In order to monitor central dopamine-(DA)-related activity in behaving animals, rats were chronically implanted with dialysis cannulae in the neostriatum. The cannulae consisted of a 4.5 mm loop of dialysis tubing (pore size, 15000 Daltons) that was supported by stainless steel wire and glued to polyethylene tubing. Striatal dialysis perfusates were collected for several days after implantation, with the rats allowed free movement in the test chamber by the use of a fluid commutator. Artificial CSF was pushed through the cannulae at a flow rate of 2.0 ul/min, and samples were collected at 15-30 min intervals. All samples were analyzed by high performance liquid chromatography with electrochemical detection, under conditions that permitted the measurement of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA).

In baseline samples, DA was present in much lower concentrations than DOPAC and HVA. Systemic administration of 5.0 mg/kg amphetamine produced stereotyped behavior in parallel with increases in DA of 300% or greater. Perfusion with a medium that contained the DA uptake blocker nomifensine (10^{-5} M) increased extracellular DA by more than 400%. In contast, food-deprived rats that were feeding or lever-pressing for food reinforcement in 30-min sessions showed no consistent changes in DA. Feeding increased DOPAC and HVA by 8-15% relative to the baseline values. Responding on schedules of reinforcement also increased DOPAC and HVA, with HVA showing consistent and stable increases that reached a peak of 15-50% above baseline 30-120 min after the cessation of the behavioral session.

The increases in HVA demonstrate that behavioral activation can cause modest enhancement of DA turnover. However, it is possible that the high-affinity uptake of DA prevents the detection of small but behaviorally relevant increases in extracellular DA. These results suggest that the use of dialysis perfusion in behaving animals will allow for comparisons between DA activity and behavior across a broad range of conditions.

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234.11 CATECHOLAMINE MEDIATION OF ROOTING AND PROBING IN NEONATAL RATS. L.M. Terry and W.G. Hall. Department of Psychology, Duke University, Durham, NC 27706.

When placed in contact with a dam, infant rats show vigorous rooting and probing into the maternal fur as they search for a nipple. Similar appetitive responding can reliably be elicited experimentally by placing pups on synthetic fur. Our work indicates that such responding is increased by deprivation of nutrition and maternal care (Bornstein et al., 1987). Because appetitive behavior in adult rats is modulated by catecholamine functioning, this study was carried out to assess the role of catecholamine synthesis in the early appetitive responding of rats and, in particular, the enhanced responding of deprived pups.

Six-day-old infant rats were placed in one of three deprivation conditions 22 hr prior to testing: maternally and nutritionally deprived (M&N), nutritionally deprived (N; pups received maternal stimulation from a nonlactating dam), or nondeprived (ND). Four hr before testing 1 pup from each deprivation condition received a s.c. injection of either saline, 100 mg/kg alpha-methyl-p-tyrosine (AMPT), or 150 mg/kg AMPT to inhibit catecholamine synthesis. Baseline activity and behavioral measures were taken just prior to testing. All pups were placed on synthetic fur for 30 min while in a warm incubator. Detailed behavioral observations were made every 5 min.

Baseline activity scores did not vary between drug groups, though, pups in the M&N deprivation group showed more rooting and probing than ND pups or N pups. Probing and activity scores of M&N pups when placed on fur were significantly affected by AMPT. During the first 10 min of fur stimulation all M&N pups showed high levels of locomotion, rooting and probing. Thereafter, pups treated with AMPT showed significant decreases in rooting, probing and locomotion. Untreated pups' activity remained at high levels for the entire 30 min period. Pups in both the N and the ND deprivation groups were unaffected by AMPT throughout the testing period.

Inhibition of catecholamine synthesis significantly inhibits rooting and probing in deprived infant rats, and this effect occurs after an initial bout of high activity. This finding, based on acute manipulations, suggests that the catecholamine system may play a role in mediating nipple search behavior in young rats.

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234.12 SPONTANEOUS ACTIVITY OF STRIATAL NEURONS IS INCREASED IN ADULT RATS GIVEN DOPAMINE-DEPLETING BRAIN LESIONS AS NEONATES. S-P. Onn, J.R. Balzer*, E.M. Stricker, M.J. Zigmond, T.W. Berger. Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Near-total depletions of striatal dopamine (DA), produced by 6-hydroxydopamine (6-HDA)-induced brain lesions in adult rats, cause profound behavioral dysfunctions. In contrast, few such impairments are observed after the same large lesions are made in neonatal rats. We have shown that the spontaneous activity of two types of striatal neurons (Type I and Type II) increases in rats given the lesions as adults (Orr et al., Soc. Neurosci. Abstr. 1986), but returns to normal with recovery of behavioral function (Nisenbaum et al., Brain Res. 1986). The present work examined the spontaneous activity of Type II striatal neurons in adult rats behaving normally despite having received near-total DA-depleting brain lesions as neonates.

Three-day-old rat pups were injected with 6-HDA (150 ug, in 0.9% NaCl and 0.1% ascorbic acid) bilaterally into the lateral cerebroventricles. Three or four months later, the spontaneous activity of single striatal neurons (n = 47) was recorded extracellularly in these rats, using 8% chloral hydrate as anesthesia. Type I and II striatal neurons were identified by their different waveforms and different responses to paired impulse stimulation of the corticostriate pathway. Similar observations in rats not given 6-HDA were used for purposes of comparison. After electrophysiological recording, DA levels in striatal tissue were determined by high performance liquid chromatography with electrochemical detection.

The spontaneous activity of Type II striatal neurons in 6-HDA-treated rats was increased significantly in comparison with control animals. Neurochemical analyses indicated at least 90-95% depletions of striatal DA. These results indicate that the sparing of behavioral function in rats given large DA-depleting brain lesions as neonates is not associated with normal electrophysiological activity in Type II striatal neurons. The elevated activity of these cells instead resembles that seen in Type II striatal neurons of rats given the lesions as adults, in association with profound behavioral impairments.

We conclude that the appearance of normal behavior in rats despite large DA-depleting brain lesions is not invariably linked to normal activity in Type II striatal neurons. The effects of neonatal lesions on the activity of Type I striatal neurons is under investigation.

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234.13 THE ROLE OF MESOLIMBIC DOPAMINE IN THE STIMULANT ACTION OF MDMA. L.H. Gold, C.B. Hubner and G.F. Koob. Div. of Preclinical Neuroscience and Endocrinology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Substituted amphetamines combine hallucinogenic activity with the classical stimulant actions of amphetamine. When administered acutely, methylenedioxymethamphetamine (MDMA) causes a decrease in brain concentrations of tryptophan hydroxylase, serotonin (5-HT) and 5-hydroxyindoleacetic acid. In vitro, MDMA releases ^{3}H[5-HT] from whole brain synaptosomes and striatal slices. Evidence from various laboratories suggests that MDMA may also act on the dopamine system. MDMA has a dose response curve parallel to apomorphine in a drug discrimination test and demonstrates sensitization, like that seen with amphetamine, in a DRL (differential reinforcement of low rate of responding) procedure. Moreover, using in vivo voltammetry, MDMA causes an initial transient dopamine decrease concomitantly with a more long-lasting decrease in 5-HT efflux measured in the nucleus accumbens (N. Acc.). Long term high level use of the parent compounds, methamphetamine and amphetamine, has been found to be toxic to dopamine and 5-HT neurons. Similarly there have been several recent reports which suggest a profound and relatively selective 5-HT terminal degeneration for both MDA and MDMA. Thus, although MDMA does not seem to be toxic to dopamine neurons, alterations in dopaminergic systems may be responsible for some of its acute behavioral effects.

The purpose of the present studies was to investigate the neural substrates for the stimulant properties of the amphetamine analog MDMA by examining the effects of neurotoxin specific lesions to the mesolimbic dopamine system on locomotor activity produced by MDMA. Rats received 6-OHDA or sham lesions of the N. Acc. and were probed on day 10 post-lesion with an injection of amphetamine (0.5 mg/kg subcutaneously) and tested in photocell cages for a two hour session. The number of photocell beam interruptions and crossovers were measured as an index of locomotor activity. On day 14 post-lesion all rats were injected with MDMA (10 mg/kg sc) and tested for a four hour session. MDMA produced an increase in locomotor activity in the sham operated animals that lasted for more than four hours, but this response was significantly attenuated in rats with lesions. Results suggest that like amphetamine, the locomotor activation associated with MDMA may involve the presynaptic release of dopamine in the region of the N. Acc. Determination of whether this depends on a serotonin interaction or serotonin neurotoxicity will require further study. (Supported in part by NIDA Grant DA 04043 and Parkinson's Disease Foundation Summer Fellowship)

234.14 AUTOMATED MEASUREMENT OF BEHAVIOR: TURNING AND WALL-FACING ASYMMETRIES AFTER 6-OHDA INJECTION INTO THE SUBSTANTIA NIGRA. A.E. Bonatz*, H. Steiner*, R.K.W. Schwarting and J.P. Huston. Institute of Physiological Psychology, University of Düsseldorf, 4000 Düsseldorf, Federal Republic of Germany.

Unilateral lesions of the nigrostriatal dopamine system induce sensorimotor asymmetries, usually measured as turning or lateralized sensory responsiveness ("neglect"). The tendency of a rat to move along the perimeter of an open field (called "wall-facing" or "peritaxis") can also be used as a behavioral measure of sensorimotor asymmetries (Steiner, et al., Behav. Brain Res., 22: 283, 1986).

We developed a video image analyzing system (VIAS) for automated measurement of behavior by digitized video images and microcomputer evaluation. This system records the rat's behavior by tracing not only its center point, but also its longitudinal axis. This provides acurate information about the rat's orientation. The VIAS simultaneously measures: (a) locomotion in meters, (b) turning by the number of 1/4, 1/2, 3/4 and complete turns, each classified in different diameter classes, and (c) peritaxis by the time spent facing the wall with either side of the body.

The VIAS was used to measure these three behaviors in rats after unilateral 6-OHDA lesions in the substantia nigra. The results show that: (1) VIAS measures with high validity (correlation with rater recorded data was r=.92 to .98 for the different behaviors). (2) Behavioral recovery from a SN lesion was indicated by decreasing number of narrow ipsiversive turns and an increasing number of turns with wider diameter. (3) Apomorphine- and amphetamine-induced turns differed in diameter. (4) Wall-facing (or peritaxis) was lateralized to the side ipsilateral to the 6-OHDA lesion and was sensitive to apomorphine and amphetamine effects: after amphetamine, wall-facing was predominantly with the side ipsilateral to the lesion and was reversed after apomorphine. (5) Recovery from peritaxis asymmetries was indicated by increased wall-facing with the side contralateral to the lesion.

It is concluded that VIAS provides a useful tool for a detailed measurement of turning behavior and lateralized wall-facing, and these can be used as behavioral indices of sensorimotor deficits after lesions in the basal ganglia.

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234.15 A COMPARISON OF THE EFFECTS OF TYPICAL AND ATYPICAL NEUROLEPTICS ON SEXUAL BEHAVIOR IN MALE RATS. J.G. Pfaus* and A.G. Phillips, Department of Psychology, University of British Columbia, Vancouver, BC, Canada, V6T 1Y7.

Neuroleptic drugs induce a variety of sexual dysfunctions in both animals and humans. In male rats, typical neuroleptics such as chlorpromazine, haloperidol, or pimozide delay or inhibit the initiation of copulation, reduce the number of vaginal intromissions that precede ejaculation, and under some conditions reduce the ejaculation latency and increase the latency to reinitiate copulation after ejaculation. In contrast, atypical neuroleptics such as thioridazine delay the initiation of copulation but increase, rather than decrease, the latency to ejaculate. Other atypical neuroleptics such as sulperide, or peripheral dopamine (DA) antagonists such as domperidone, have no effect on copulation even at high doses. However, differences in methodology, especially concerning the degree of baseline sexual performance among subjects, make it difficult to compare the results of these studies.

In the present experiments, the dose-response effects of haloperidol, pimozide, clozapine, or metoclopramide were assessed on patterns of sexual behavior in intact, sexually active rats with a high level of sexual performance and experience. Following baseline testing for sexual behavior, sexually active rats were randomly assigned to receive a specific neuroleptic drug and received either 0, 0.1, 0.5, 1.0, or 5.0 mg/kg, sc of the drug in a latinized fashion at 4-day intervals. Sexual behavior was assessed following drug administration by placing the male with a sexually receptive female in a testing arena for 30 min. Testing times for each drug were chosen on the basis of previous studies.

Haloperidol and pimozide both significantly delayed the initiation of copulation in a dose-dependent fashion and reduced the number of intromissions that preceded ejaculation. High doses of haloperidol (1, 5 mg/kg) inhibited the initiation of copulation altogether. Clozapine, an atypical neuroleptic that preferentially blocks mesolimbic DA receptors, dose-dependently delayed, and at higher doses inhibited, the initiation of copulation but had no effect on copulatory behavior once it was initiated. In contrast, metoclopramide, an atypical neuroleptic that preferentially blocks mesostriatal DA receptors, dose-dependently reduced the number of intromissions that preceded ejaculation but had no effect on the ability of rats to initiate copulation.

The present experiments suggest that aspects of copulatory behavior are affected differently by neuroleptics depending upon their site of action in the brain. Blockade of mesolimbic DA receptors appears to delay the initiation of copulation whereas blockade of mesostriatal DA receptors decreases the ejaculatory threshold.

Supported by a grant from the Medical Research Council of Canada (PG-23).

234.16 **DIFFERENTIAL EFFECTS OF DOPAMINE ANTAGONISTS ON AMPHETAMINE-INDUCED LOCOMOTOR STIMULATION AND REWARD.** **G.D. Carr and A.G. Phillips. Department of Psychology, University of British Columbia, Vancouver, BC, Canada, V6T 1Y7.**

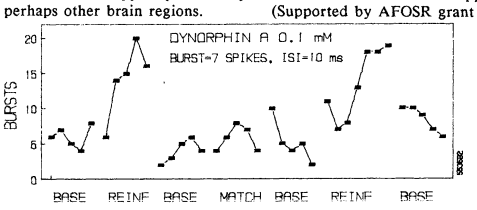
Two well-established behavioral effects of amphetamine are its locomotor stimulation and rewarding properties. Converging evidence has suggested that both of these effects are dependent, to a large extent, on the capacity of amphetamine to increase the release of dopamine in the nucleus accumbens. Both effects can be produced by intra-accumbens micro-injections of amphetamine and the effects of systemic injection are antagonized by lesions of the accumbens.

Previous studies have also reported that dopamine receptor antagonists (neuroleptics) block both the locomotor stimulation and reward. The present study investigated the effects of three atypical neuroleptics on these amphetamine effects in rats. To date, data has been obtained with one dose of each drug; metoclopramide (5mg/kg), clozapine (10mg/kg) or sulperide (20mg/kg) were injected prior to treatment with d-amphetamine (2mg/kg) (all injections s.c.). The animals were tested for the rewarding effects of the drug combinations using the conditioned place preference (CPP) paradigm. Rats received the drugs paired with one of two distinctive compartments and saline was paired with the other. Following six drug and saline pairings over 12 days, a partition separating the compartments was removed and the rats were given a free choice between the compartments. A preference for the drug-paired compartment was taken as evidence of a rewarding effect of the treatment. The pairing compartments were equiped with photocell beam detectors which served to measure the locomotor activity during the drug treatments. Amphetamine alone produced robust locomotor stimulation and CPP as previously reported. However, at the doses tested, the neuroleptics were differentially effective at blocking these effects. Metoclopramide and clozapine compietely blocked the locomotor stimulation but had no effect on the CPP. In contrast, sulperide blocked the CPP but had no effect on the locomotor stimulation.

In contrast to previous findings, this double dissociation of the rewarding and locomotor stimulant effects of amphetamine suggests that they may be mediated by separable dopaminergic substrates. A compiete dose-response curve will be needed to confirm and clarify this possibility.

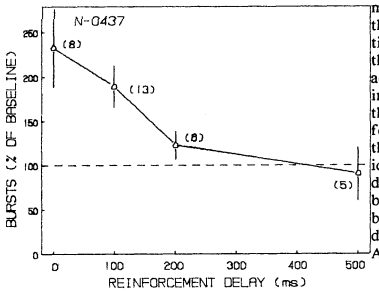
235.1 **DYNORPHIN A: A NEW REINFORCEMENT TRANSMITTER?** L. Stein and J.D. Belluzzi. Department of Pharmacology, College of Medicine, University of California at Irvine, Irvine, CA 92717.

Recent mapping of the distribution of dynorphins in the rat brain (Fallon & Leslie, 1986) has revealed rich concentrations of dynorphin cell bodies and fibers in sites known to support very high rates of self-stimulation. For example, it is now possible to speculate that high rates of self-stimulation in the bed nucleus of the stria terminalis (Olds, 1962), ansa lenticularis (Stein *et al.*, 1973), ventrolateral central gray (Liebman *et al.*, 1973), and particularly in the region surrounding the superior cerebellar peduncle immediately lateral to locus coeruleus (Ritter & Stein, 1973) may be associated with their rich dynorphin innervation. In the latter two sites, self-stimulation is blocked by naloxone (Kelsey *et al* 1984; Loughlin *et al* 1983). In brain slice experiments, we have reported apparent reinforcement of individual CA1 cellular activity by pairing bursts of firing with local applications of dopamine, cocaine, or the selective dopamine D2 receptor agonist N0437 (Belluzzi & Stein, 1983; Stein & Belluzzi, 1987). However, the same dopaminergic drugs failed to reinforce the activity of hippocampal CA3 cells. Because there is a rich enkephalin and dynorphin projection from dentate granule cells to CA3 dendrites, we have attempted to reinforce CA3 activity with leucine-enkephalin and dynorphin A(1-17). Previous attempts to reinforce CA1 activity with these peptides were unsuccessful. In the present experiments, bursts of CA3 activity were recorded extracellularly; pressure microinjections were delivered through the same pipette. The number of spikes required to define a reinforceable burst were set individually for each neuron studied so that bursts occurred, on baseline, at the rate of 2-6/min. The Figure shows a representative positive experiment with dynorphin A (10^{-4}M in pipette) as reinforcement. The frequency of bursts was rapidly increased in two separate periods of operant conditioning (REINF), but not in a control period (MATCH) during which a matched number of dynorphin injections were presented independently of cellular activity. The relatively selective kappa agonist U50488H (10^{-4}M) also reinforced CA3 activity, but leucine-enkephalin (10^{-4} to 10^{-3}M) was ineffective. The results raise the intriguing possibility that dynorphin A may function as a reinforcement transmitter by activation of kappa opiate receptors in the CA3 field of hippocampus and perhaps other brain regions. (Supported by AFOSR grant 84-0325)



235.2 **OPERANT CONDITIONING OF HIPPOCAMPAL CA1 NEURONS REQUIRES IMMEDIATELY-CONTINGENT ACTIVATION OF DOPAMINE D2 RECEPTORS.** J.D. Belluzzi and L. Stein. Department of Pharmacology, College of Medicine, University of California at Irvine, Irvine, CA 92717.

Operant conditioning of individual CA1 cellular activity in slices of hippocampus with local applications of dopamine as reinforcement has been reported (Belluzzi & Stein, *Neurosci. Abst.*, 1983). More recently, we found that dopamine's reinforcing effects are exerted at dopamine D2 receptors (Belluzzi & Stein, *Neurosci. Abst.*, 1986). Here we report (1) that highly reliable neuronal operant conditioning is obtained with the specific dopamine D2 receptor agonist N0437 as reinforcement, and (2) that such operant conditioning is abolished when the reinforcing injection is delayed for 500ms after the neuronal response. A single-barrelled glass micropipette for simultaneous recording and pressure injection was filled with N0437 (10mM in 165mM saline) and aimed at spontaneously active pyramidal cells in the CA1 layer of hippocampal slices. The neuronal response for reinforcement was a "burst" of relatively fast activity. To be eligible for reinforcement, such bursts had to contain a minimum number of spikes; this minimum number was individually established for each neuron studied during a baseline period so that, prior to operant conditioning, reinforceable bursts occurred at a rate of approximately 5/min. In the reinforcement period, a pressure injector was activated for 10-80ms immediately after each burst to deliver a 10 μ-diameter droplet of drug to the vicinity of the cell body. The efficacy of operant conditioning associated with reinforcement delays of 0, 100, 200, or 500 ms was determined in an experiment involving 32 CA1 cells; each cell received operant conditioning at a single reinforcement delay. A delay-of-reinforcement gradient was generated by averaging the peak bursting rates associated with each delay (Figure). The curve indicates that reinforcement delays exceeding 200ms largely eliminate the effectiveness of N-0437 reinforcement in CA1 operant conditioning. The steep gradient of effectiveness of delayed reinforcement makes it unlikely that non-specific stimulation or some artifact of the injection procedure accounts for the increase in neuronal firing. Rather, the stringent requirement for contingency supports the idea that we have identified a neuronal conditioning process that may be closely related to behavioral operant conditioning. (Supported by AFOSR grant 84-0325)



235.3 EFFECTS OF PHLORIZIN ON INHIBITORY AVOIDANCE BEHAVIOR IN RATS AND MICE. J.L. Hall, K.L. Cottrill* and P.E. Gold. Dept. Psychology, University of Virginia, Charlottesville, VA 22903.

Recent evidence indicates that peripheral and central glucose administration enhances memory in several tasks and in a variety of species including rats, mice, and humans. Additionally, circulating glucose levels measured soon after training predict later retention performance, suggesting that circulating glucose levels may regulate memory storage. Glucose gains access to the brain via a carrier-mediated transport mechanism. Phlorizin competes with glucose for binding to this carrier and thus inhibits glucose transport into the brain (Pardridge & Oldendorf, 1975, Biochim Biophys Acta, 382: 377-92). Believing that phlorizin might therefore impair memory, we tested the drug's effects on inhibitory avoidance training in mice and rats.

Mice were trained in a one-trial inhibitory (passive) avoidance task (200 uA escapable footshock). Thirty min prior to training, the animals received an injection of phlorizin (0.3-3.0 mg/kg, IP) or saline. On the test trial 48 hr later, animals which received phlorizin (0.3 or 3.0 mg/kg) had retention scores significantly higher than those of saline-injected animals. Thus, in contrast with our expectations, phlorizin actually enhanced memory storage.

Rats were also trained in a one-trial inhibitory avoidance task (0.5 mA, 0.5 sec). Thirty min prior to training animals received phlorizin (0.0003 - 0.3 mg/kg, IP) or saline. Animals were tested 24 hr later. The dose-response curve was inverted-U in shape. Phlorizin-injected animals (0.03 or 0.003 mg/kg) again had retention scores significantly higher than those of controls.

Since the brain is highly dependent on circulating glucose for its fuel, it is possible that the organism responds to insufficient glucose transport in a compensatory manner by increasing circulating glucose levels. Preliminary studies indicate that this is, in fact, the case. Animals injected with phlorizin (0.03 mg/kg IP) demonstrated elevations in circulating glucose levels of 26 $\pm$ 7 mg/dl near the time at which training would have occurred. Importantly, these increases in plasma glucose levels are comparable to those which predict good retention performance under other conditions as well. Additionally, the blood-brain barrier to glucose is a dynamic system. Thus with insufficient brain glucose, transport of glucose from blood to brain may increase. Future studies will address the issue of the extent to which the increases in plasma glucose effectively compensate for the loss in glucose transport in the presence of phlorizin. [Supported by the Office of Naval Research (N00014-85-K0472) and by the American Diabetes Association].

235.4 FOURTH VENTRICLE ADMINISTRATION OF AN OPIOID ANALOGUE CAN BLOCK THE DEVELOPMENT OF CONDITIONED HEART RATE RESPONSES. G.C. Harris and R.D. Fitzgerald*. Department of Medical Psychology, Oregon Health Sciences University, Portland, OR, 97201.

Previous research has shown that administration of the opioid analogue [N-MePhe^3,D-Pro^4] morphiceptin into the rostral fourth ventricle abolished a previously learned heart rate (HR) conditioned response (CR) (Lavond, Mauk, Madden, IV, Barchas, & Thompson, Pharmacol Biochem Behav, 19: 379-382, 1983). The current study was designed to look at the effects of opioid administration in the rostral fourth ventricle on the development of HR CRs. Classical conditioning training consisted of a discrimination paradigm in which one (6-sec) tone conditioned stimulus (CS+) was paired with a 0.5 sec chest shock unconditioned stimulus (UCS), while a second tone (CS-) was not paired with shock. Three groups of rats were infused with either 0.9% saline (SAL), 10 µgs of D-ala^2-met-enkephalinamide (DALA), or 10 µgs of DALA + 5 µgs of the opioid antagonist naltrexone (DALA-NAL) in the rostral fourth ventricle. A fourth group (DALA-BR.ST.) was infused with 10 µgs of DALA in the brain stem ventral to the fourth ventricle infusion site. Drug infusions were made prior to the conditioning session on two consecutive days. All groups were then left in their home cages for two days after which time they were brought back and tested in a non-drugged state. The results showed that the DALA-NAL and DALA-BR.ST. groups showed the development of a normal HR CR, comparable to that seen in the SAL group. The DALA group, however, failed to develop a HR CR. The absence of a CR in this group was evident even when the rats were tested 48 hrs later in a non-drugged state. A significant reduction in the HR orienting reflex (OR) was seen in the DALA group only on the first CS presentation. No significant effects were seen on the HR UCR in the DALA group when compared to the SAL group. These results suggest that the decremental effects of DALA on the CR were due to the activation of opioid receptors located in the rostral fourth ventricle, possibly in the region of the locus coeruleus or in the periaqueductal/periventricular gray regions. It is thought that DALA may have prevented the development of a learned association by decreasing emotional awareness of stimuli. (Supported by NHLBI #T32-HL07332 and the N.L. Tartar Research Fund)

235.5 EFFECTS OF PHYSOSTIGMINE ON OPERANT SERIAL REVERSAL LEARNING. D.B. Clissold* and G.A. Heise*. (Spon: M.J. Pontecorvo). Nova Pharmaceutical Corporation, and Department of Psychology, Indiana University, Bloomington, Indiana 47401.

The effects of the anticholinesterase physostigmine on performance of repeated acquisition/reversal learning paradigms were examined. In Experiment 1, rats (n = 6/group) were trained to criterion on a series of three stimulus (bright, dim and flashing lights) reversal problems. Subjects remained on the same problem until criterion was met. The stimulus-response relationship was then reversed for two stimuli with the third stimulus constant over two problems. In different groups of rats, physostigmine (or an equal volume of vehicle) was injected either immediately prior to each session (0.03 mg/kg) or immediately following each session (0.5 mg/kg). After 50 sessions, the total number of discrimination problems solved was compared among the four groups. Animals injected with physostigmine either pre- or post-session solved more discrimination reversal problems than did control animals. Experiment 2 utilized a two-stimulus (bright vs dim light) reversal learning paradigm. Animals that were injected with physostigmine in Experiment 1 were injected with vehicle in Experiment 2. Animals injected with vehicle in Experiment 1 were injected with physostigmine in Experiment 2. The time of injection was held constant. Although no between-group differences were found in Experiment 2, a comparison of performance across Experiments 1 and 2 determined that individual animals learned significantly more reversal problems when injected with physostigmine, independent of stimulus condition (experiment). In Experiment 3, animals were trained to perform daily reversals of a light vs tone Go: No Go discrimination. This baseline of performance (trials to criterion) was the dependent variable for contrasting the effects of acute post-session injections of physostigmine (0.5 mg/kg) or vehicle. In this experiment, physostigmine resulted in a decrement in the next session's performance. Results are discussed in terms of cholinergic involvement in learning and memory.

235.6 RATS PERFORM WIN-STAY BETTER THAN WIN-SHIFT IN A TWO-CHOICE WATER ESCAPE SITUATION. L. W. Means. Psych. Dept., East Carolina Univ., Greenville, NC 27858-4353.

In both the T- and radial arm mazes rodents spontaneously make and readily acquire win-shift responses for positive reinforcement. On the other hand, shocking mice in the T-maze results in the development of a perseverative (win-stay) response (D. Mitchell, et al., Learn. & Mot., 1984, 15). In the circular water maze, rats acquire either a win-stay task when the escape platform location is held constant or a win-shift task when the platform is randomly placed in one of 4 positions (C.F. Mactutus & D.L. Murray, Soc. Neurosci. Abstr., 1986).

Three experiments were conducted to compare groups of Sprague-Dawley rats on win-stay and win-shift performance in a 1.4 m circular water maze. Fishline strung above divided the maze into 3 equal pie-wedge sections, a starting section and 2 choice sections. In all 3 experiments, subjects began each trial from the same location and escaped from the water (24+2°C) onto a platform hidden 1.0 cm below the surface. Trials were given in couplets: (1) a reference trial, during which the escape platform was randomly placed in the center of one of the choice sections, and (2) a test trial given 10 m later. For the win-stay task, the platform was placed at the same location on both trials of a couplet. For the win-shift task, the platform was placed in the opposite choice section on a test trial from that of the reference trial. In the first experiment, each group received 20 couplets. In the second experiment, each group received 55 couplets, and the platform was lowered to the bottom of the maze for 60 s following an incorrect first choice on any trial. In the third experiment, each group received 20 couplets, and a barrier separated the 2 choice sections of the maze. Also, the escape platform was lowered for 60 s following incorrect choices.

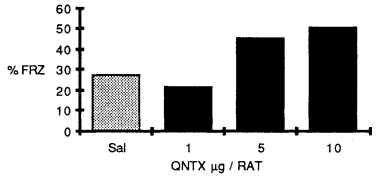
All 3 experiments produced the same results. Animals made significantly more correct test trial choices (entered section containing platform first) on the win-stay task than on the win-shift task. Animals that failed to learn on either task developed a position bias, going to the same section first on a series of consecutive trials. Thus, it appears that it is most natural for a rodent to perform win-stay responses in an aversive situation and win-shift responses in an appetitive situation.

235.7 SELECTIVE DOPAMINE D1 AND D2 RECEPTOR AGONISTS PRODUCE CONDITIONED TASTE AVERSIONS. D.C. Hoffman & R.J. Beninger. Dept. of Psychol., Queen's University, Kingston, Canada K7L 3N6.

Dopamine (DA) receptor agonists (e.g., apomorphine, cocaine and (+)-amphetamine) produce both rewarding and aversive effects in the conditioned place preference and conditioned taste aversion (CTA) paradigms, respectively. With the discovery of DA receptor subtypes, research has focused on determining the possible contributions of D1 and D2 receptors to these DA-mediated effects. Recently, we discovered that selective stimulation of D2 receptors with quinpirole in rats produced a significant place preference, however, treatment with a selective D1 agonist, SKF 38393, resulted in a significant place aversion. These data suggested that the rewarding and aversive properties of DA agonists may be mediated through two different receptor subtypes. If this is true, one might expect SKF 38393, but not quinpirole, to produce a CTA. This hypothesis was tested in the present experiment. The protocol consisted of 3 phases. During the 4-day pre-exposure phase, water-deprived male Wistar rats were presented with 2 flavored solutions for 30 min. The amount consumed was recorded. Conditioning occurred over the following 4 days; on days 1 and 3, rats were treated with drug and presented with one flavored solution. Doses of quinpirole (1.0 mg/kg IP) and SKF 38393 (10.0 mg/kg IP) which were effective in place conditioning were compared to the effects of (+)-amphetamine (2.0 mg/kg IP) and a saline control group. On days 2 and 4, all rats received saline and were given access to the alternate flavored solution. Test days occurred over the next 4 days during which untreated animals were presented with the 2 flavored solutions for 30 min. In contrast to the saline control group, rats treated with (+)-amphetamine, quinpirole or SKF 38393 demonstrated a significant decrease in percent intake of the conditioned flavor from pre-exposure to test indicating the establishment of a CTA. Thus, the hypothesis that the D1 receptor is exclusively involved in mediating the aversive qualities of DA agonists was not supported; both receptor subtypes appear to be involved in taste aversion learning. (Funded by the National Sciences and Engineering Research Council of Canada.)

235.8 CENTRAL BUT NOT PERIPHERAL ADMINISTRATION OF QUATERNARY NALTREXONE ENHANCES FREEZING. F.J. Helmstetter, D.J. Calcagnetti*, and M.S. Fanselow*. Department of Psychology, Dartmouth College, Hanover, N.H. 03755.

Administration of opioid antagonists prior to training in an aversive conditioning preparation using footshock tends to enhance acquisition of conditional defensive responding. We have previously demonstrated that i.p. administration of the tertiary form of naltrexone, which may act at both central and peripheral opioid binding sites, produces an enhancement of freezing at doses that block the analgesia conditioned with this procedure (Physiol Behav,39:501). Two experiments using naltrexone methobromide (QNTX), a quaternary compound that normally does not penetrate the "blood-brain barrier", were conducted to determine the relative contribution of central and peripheral opioid receptors to this effect. In the first experiment 20 female rats were given 1, 10, or 20 mg/ml/kg of QNTX or saline before being placed in a conditioning chamber. Four min later each rat received three 1ma/.75s footshocks spaced 20s apart. Subjects were returned to the chambers 24 h later and the amount of time spent engaged in de- fensive freezing was measured. QNTX administered i.p. had no reliable effect on conditional responding, $F(3,16) = 1.36$, $p > .20$. In the second experiment, 42 rats were prepared with cannula implanted into the right lateral ventricle. The procedure of the first experiment was followed except that now QNTX was administered i.c.v. (4µl/40s) at doses of 0, 1, 5 and 10 µg/rat. As indicated in the figure, QNTX given i.c.v. produced a reliable enhancement of freezing, $F(3,34) = 3.61$, $p < .03$. These results suggest that opioid modulation of aversive conditioning depends on a population of receptor sites within the central nervous system.



235.9 CHOLINERGIC MUSCARINIC BLOCKADE IN THE RAT IMPAIRS STRATEGY SELECTION BUT NOT LEARNING AND RETENTION OF NONSPATIAL VISUAL DISCRIMINATIONS IN A SWIMMING POOL. I.Q. Whishaw and B.F. Petrie*. Dept. of Psychology, Univ. of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

A puzzling feature of the research on cholinergic systems is its apparent bifurcated focus. Although it is known that cholinergic muscarinic receptor blocking agents such as atropine and scopolamine impair a wide range of physiological and behavioral functions, little attention has been directed toward the relationships between the abnormalities in unconditioned behaviors and conditioned behaviors. Ethologists have argued that an animal's ability to perform well on learning tasks is dependent on the existence of innate mechanisms that can be usefully applied to the task. Early learning theorists also suggested that animals experiment with "attempted solutions" or "hypotheses" from which the appropriate behavior pattern is eventually selected. The present study examined whether learning impairments following cholinergic blockade are due to impairments of systems that are in some way necessary precursors of learning or whether they are due to interference with processes of memory storage.

A procedure was developed to study black vs white and horizontal vs vertical pattern visual discriminations in a swimming pool. The effects of central cholinergic muscarinic receptor blockade by atropine sulfate was then evaluated. The drug treatment impaired acquisition but not retention. Behavioral observations showed that the control rats used a number of strategies during the process of problem solving that facilitated acquisition of the discrimination. Through modifications of training procedures, the processes of strategy selection and discrimination learning were dissociated. Cholinergic blockade was found to impair strategy selection but not discrimination learning. The results question the widely held view that cholinergic systems are involved in learning and memory and suggest instead that cholinergic systems are involved in the selection of the movements or strategies that are prerequisite for learning.

235.10 MODULATION OF MEMORY RETENTION BY GUT PEPTIDES IN MICE. J.E. Morley and J.F. Flood, GRECC, VA Medical Center, Sepulveda, CA 91343

Recently, we showed that feeding immediately after learning enhanced retention for footshock avoidance training (Flood et al., Science, 1987). CCK also enhanced memory on this task but its effect could be abolished by vagotomy. The effect of feeding on memory is not blocked by vagotomy. We, therefore, reasoned that other gastrointestinal peptides may also enhance memory by a vagally independent mechanism.

To test this, mice were partially trained on a t-maze footshock avoidance task. Immediately after training mice received an intraperitoneal (IP) or intracerebroventricular (ICV) injection of bombesin, gastrin releasing peptide (GRP) or saline. Retention was tested one week later.

The results indicate that bombesin at 1 ug/kg IP (73% recall score) or GRP at 10 ug/kg IP (80% recall score) enhanced retention relative to a saline control group (13% recall score). Optimal improvement of retention with ICV administration required higher doses than were administered IP; bombesin significantly improving retention at 0.5 ug (as compared to 0.035 ug/mouse IP) and GRP at 2.5 ug (as compared to 0.35 ug/mouse IP). The higher doses needed to improve retention by the central route of administration suggests that bombesin and GRP act through a peripheral mechanism or that the site of action in the central nervous system is not readily penetrated by the ICV administration.

These data indicate that a number of gastrointestinal peptides that inhibit feeding will also enhance memory by a peripheral mechanism. The teleological advantage of memory being enhanced after ingesting a meal is obvious.

235.11 INTRASEPTAL INJECTION OF GABA-A, BUT NOT GABA-B, RECEPTOR AGONISTS PRODUCES DOSE-RELATED WORKING MEMORY IMPAIRMENTS IN THE RAT. J. J. Chrobak, D. F. Emerich, F. Safir-Temple, G. M. Spindler *and T. J. Walsh. Department of Psychology, Rutgers University, New Brunswick, NJ, 08903, USA.

The septohippocampal cholinergic system appears to be a critical neural substrate for memory processes. Alterations of this system induced by drugs, cholinotoxins such as ethylcholine aziridinium ion (AF64A), or neurodegenerative diseases produce impairments in working memory tasks. Manipulation of GABAergic receptors within the medial septum decreases the turnover of acetylcholine in the hippocampus. The following experiments examined whether intraseptal injection of GABA-A (muscimol) or GABA-B (baclofen) agonists would produce working memory impairments similar to those observed following compromise of the septohippocampal cholinergic system.

Twelve male Sprague-Dawley rats were trained to perform a radial-arm-maze task with a one hour delay imposed between the fourth and fifth maze choice. In this task rats have access to 4 out of 8 maze arms during a pre-delay session; the pattern of arms varying daily. After choosing these 4 arms, the animals are returned to their home cages for one hour. Following the delay, they are returned to the maze and allowed to freely chose among all 8 arms. Arms not previously chosen are baited, and entry into arms chosen during the pre-delay session constitutes an error. Following acquisition, a single indwelling canulae was implanted into the medial septal region and animals were allowed one week to recover from the surgery before behavioral testing resumed. A within-subjects design was utilized to examine the effects of intraseptal administration of muscimol (0, 0.75, 1.5 or 3.0 nanomoles) and baclofen (3.0 nanomoles), on RAM performance. All drugs or artificial CSF were administered immediately following the pre-delay session in a volume of 1 ul.

The GABA-A agonist, muscimol, produced a dose-dependent impairment in RAM performance when administered immediately following the pre-delay session. The GABA-B agonist, baclofen, which has been shown not to alter ACh turnover, did not alter performance of this task. Muscimol did not alter motor behavior; following intraseptal injection rats chose arms, consumed food pellets and completed the task with latencies comparable to those observed on control days. Similar muscimol-induced impairments were observed when delays of four hours were imposed between training and testing suggesting that the behavioral deficits represented an inability to store or retain spatial working memories rather than a general performance deficit. These studies demonstrate that pharmacological manipulation of GABA-A receptors in the medial septum modifies working memory processes in the rat. Studies are in progress to determine whether these cognitive deficits are related to an alteration in septohippocampal cholinergic activity.

235.12 STIMULATION OF BRAIN NICOTINIC RECEPTORS ENHANCES MEMORY IN A DELAYED MATCHING TASK BY MONKEYS. K. Elrod, W.J. Jackson and J.J. Buccafusco. Dept. of Pharmacology and Toxicology, and Physiology and Endocrinology, Medical College of Georgia and Veterans Administration Medical Center, Augusta, GA 30912.

Brain cholinergic neurons are known to play an important role in the processes of learning and memory. A selective loss of cholinergic neurons in pathways important for such processes may at least in part underly the symptoms associated with Alzheimer's disease. Such a cholinergic deficiency is readily apparent when measuring enzyme markers selective for cholinergic neurons in autopsy tissue samples. In contrast, the receptive field of muscarinic receptors does not usually exhibit a loss in affinity or number. Several recent reports have, however, confirmed a loss of nicotinic binding sites in relevant brain structures. The role of these nicotinic receptors is not well understood; however, nicotine is known to facilitate acetylcholine release. The purpose of this study was to determine whether nicotine could enhance memory performance, and whether the centrally-acting nicotinic antagonist mecamylamine, could produce the reverse effect. Four young adult macaque monkeys were well-trained in a matching-to-sample paradigm over a period of several months. During the course of this study, the monkeys were maintained at 85% body weight and received food reinforcement pellets for correct responses in the task. Just prior to each set of matching problems a monkey was given an i.m. injection of one of several doses of nicotine. Three of four monkeys exhibited enhanced performance following administration of at least one of the doses of nicotine. The optimal dose, reflected by the most facilitation, was 2.5 or 5 ug/kg. Overall, we observed an approximate 13% improvement in performance at the longer delay periods in monkeys receiving benefit from nicotine. While the dose-response relationship was highly individualized for each animal, the maximal degree of improvement following nicotine occurred at the longer delay intervals. The beneficial effect of nicotine could be repeated in each animal on several occasions. The ability of nicotine to enhance performance in these animals depended upon the dose administered as well as the degree of difficulty of the task presented. In contrast to the facilitating effects of nicotine, administration of 0.25 - 2 mg/kg mecamylamine produced a dose-related impairment in performance. The selective peripheral nicotinic antagonist, hexamethonium, 2 mg/kg, was not as effective in inhibiting matching performance. These results indicate that nicotinic receptors may be exploited pharmacologically to enhance memory processes. If these results have application to humans, patients with cognitive deficits involving learning and memory might receive more benefit than normal individuals from drugs which stimulate brain cholinergic nicotinic receptors. Supported by BRSG 5-07-RR05635-25 and the Veterans Admin.

235.13 REPEATED ACQUISITION OF RADIAL MAZE PERFORMANCE: A NOVEL PROCEDURE FOR ASSESSING CHEMICALLY INDUCED DISRUPTION OF ASSOCIATIVE LEARNING. D.B. Peele and S.P. Baron*, Northrop Services, Inc.- Environmental Sciences, Research Triangle Park, NC 27709.

Deficits in learning, memory and other cognitive processes are commonly reported in humans following exposure to a diverse range of industrial and agricultural chemicals. Despite the existence of a vast number of environmental chemicals whose neurotoxic potential has yet to be assessed, there are virtually no acceptable techniques to rapidly screen for potential chemically induced learning deficits. Further, few tests exist that are capable of longitudinal learning assessments; typically, learning is assessed only once in individual animal subjects. The present experiment was designed to address these problems by examining a repeated acquisition (learning) paradigm with rats performing in a radial-arm maze.

Adult male Long-Evans rats were trained on a radial maze task in which 45-mg food pellets were available in only four of the eight arms of the maze; the particular arms containing food were assigned randomly at the start of each daily session. The automated maze consisted of a covered, eight-arm Plexiglas maze with pellet dispensers located at the distal end of each arm. Access to the arms was permitted by raising or lowering pneumatically controlled guillotine doors, located at the entrance of each arm. Experimental conditions and data collection were arranged by a minicomputer. A trial terminated when the rat obtained all four pellets, or following 300 sec. During each 14-trial daily session, the number of errors (selecting non-baited arms or repeated arm selections) showed a within-session decline and choice accuracy for the first four arm selections showed a positive acceleration across trials for all subjects. Scopolamine (0.03 to 0.3 mg/kg, ip), but not methylscopolamine (0.3 mg/kg, ip), reduced the accuracy of the first four arm selections and increased total errors in a dosage-dependent manner. Session times were also increased by the highest dosages of scopolamine. An examination of within-session error reduction showed only slight signs of improvement (i.e., learning) at the higher dosages of scopolamine. The ease and rapidity with which rats acquired the baseline and the sensitivity of performance to disruption by scopolamine suggests that repeated acquisition of radial-arm maze performance holds promise as a convenient procedure for detecting learning deficits induced by chemical exposure.

235.14 SPATIAL NAVIGATION: DISRUPTION BY MOTOR IMPAIRMENTS? C.F. MACTUTUS, C.R. GUTMAN*, and R.M. BOOZE, Dept. of Medicine (Toxicol.), Jefferson Medical College, Philadelphia, PA 19107 and Dept. of Medicine (Neurol.), Duke University, Durham, NC 27705.

The water maze paradigm, as elegantly demonstrated (Morris, 1981), utilizes place and cue tasks to dissociate treatment effects on spatial localization from motivational or motor performance factors. Similar careful studies have been performed with hippocampal lesions (Morris et al., 1982) and cholinergic agents (Whishaw, 1985). We explored the boundary conditions regarding the susceptibility of spatial localization to disruption by motor impairments induced by a well-characterized tremorogen--harmine.

Adult Fischer-344 rats, bred in our laboratory, had received previous training to find a hidden platform (10 cm^2), positioned 2.5 cm below the milky white, opaque, water surface, in a 1.2 m circular water maze. The animal's prior experience included a total of 40 training trials (with a fixed or random platform location), a probe test, and a transfer test. Presently, all animals received 4 training trials, with a fixed or randomly located platform (ns=24), and were then administered saline or harmine (10 mg/kg). After a delay of 10 or 30 min the animals received 4 additional training trials followed by a probe test trial. Consistent with their extensive experience the animals' training latencies were quite rapid, but asymptotic performance values were significantly shorter for the fixed (7.5 sec) relative to the random (11.3 sec) group ($p < 0.005$). Harmine severely disrupted swimming behavior as observed by the marked and similar increase in response latencies for both the fixed (3.9 vs 26.8 sec) and random (11.6 vs 25.2 sec) platform groups. Post-injection delay did not alter the magnitude of this disruption. Quadrant preference scores during the probe trial suggested the performance of harmine treated rats in the 10-min delay condition was indistinguishable from saline-injected controls; the performance of animals in the random platform groups was similarly unaffected. In contrast, harmine-treated rats in the 30-min delay condition displayed little if any spatial bias for the prior platform location, i.e., their preference scores were indistinguishable from those of both random platform groups but were significantly impaired relative to the saline-injected fixed platform group ($p < 0.005$). A quantitative assessment of whole body tremor indicated that although marked tremor was present in both delay groups, the disruption of spatial localization was associated only with a more intense tremor. In sum, despite the frank motor disturbance and impairment of swimming performance induced by harmine, spatial localization may not be impaired. Such relative resistance to disruption suggests this task may be particularly appropriate for investigating potential cognitive dysfunction produced by toxicants or other chemical agents of unknown action.

235.15 SINGLE AND REPEATED ADMINISTRATIONS OF AF102B, A NOVEL MUSCARINIC AGONIST, IMPROVE COGNITIVE DYSFUNCTIONS IN AF64A-TREATED RATS. N.Nakahara*, Y.Iga*, S.Samuraizono*, T.Sawai*, S.Katayama*, and F.Mizobe, Res.Inst.of Life Sci., Snow Brand Milk Products Co., Tochigi 329-05, Japan

Senile dementia of the Alzheimer type (SDAT) is characterized behaviorally by a general decline in cognitive function. A marked presynaptic hypofunction in cholinergic system is observed in certain brain regions involved in cognitive processes, whereas postsynaptic muscarinic receptors seem to be relatively intact. These findings suggest the possibility to develop a novel treatment approach for SDAT ,i.e., when cholinergic nerve terminals are diminished, a muscarinic agonist directly acting on postsynaptic M1 receptors compensates for loss of ACh and alleviates cognitive dysfunctions. AF64A treatment of rat has been reported to result in a selective and irreversible reduction of presynaptic markers in cholinergic system, and suggested to provide a potential animal model for SDAT. We have investigated the effects of AF102B on performance of AF64A-treated rats (AF64A-rats) in a step-through passive avoidance task and a delayed nonmatching to sample task in T-maze. Electrophysiological and biochemical studies in rabbit SCG and the CNS synaptosomes have suggested that AF102B is a muscarinic M1-type agonist.

Male Sprague-Dawley rats were treated with AF64A (3 nmole/2 ul/side, ICV). We have observed damages in septum, but not in fimbria-fornix, by light microscopic examination of tissues taken 30 days after AF64A treatment. ChAT activity was reduced in hippocampus, but not in frontal cortex and striatum. No significant difference between AF64A-rats and control-rats was found in sensitivity to electric shock and in spontaneous motor activity. AF64A-rats exhibited impairments of performance both in passive avoidance and in T-maze.

A single administration of AF102B to AF64A-rats resulted in a significant improvement in a passive avoidance task at doses of 1.0 mg/kg,i.p. and 1.0 mg/kg,p.o. The performance in a T-maze task was examined to evaluate the effects of chronic administrations of AF102B for 5 weeks. AF102B (5.0 mg/kg,i.p.) significantly improved the performance of AF64A-rats in a T-maze task at the fourth and fifth week. Any side-effects in behavior were not observed in the rats during 5-week periods for chronic administration of AF102B.

In conclusion, AF102B can be considered as a potential candidate for SDAT treatment.

235.16 BEHAVIORAL EFFECTS OF TETRAHYDROAMINOACRIDINE FOLLOWING SCOPOLAMINE PRETREATMENT AND NUCLEUS BASALIS MAGNOCELLULARIS LESIONS IN RATS. C.P.J. Dokla[1], P. Gabos*[1], J.C. Babrowicz*[1], L. Rydelek*[1], D. Pereira*[1], R. Patrignelli*[1], and L.J. Thal [2]. [1]Dept. of Psychology, Fairfield Univ., Fairfield, CT 06430 and [2]Dept. of Neurology, San Diego VA Medical Center, La Jolla, CA 92161.

1,2,3,4-tetrahydro-9-aminoacridine (THA), a potent, reversible, and long lasting anticholinesterase has recently been reported to be efficacious in the long-term treatment of Alzheimer's disease (Summers, W.K. et al. N. Engl. J. Med., 315: 1241, 1986). The behavioral and memory-enhancing effects of THA were examined in a series of experiments following treatment with the cholinergic muscarinic antagonist, scopolamine, and also after lesions of the rat nucleus basalis magnocellularis (NBM).

In Experiment 1, the ability of THA to reverse perseverative peripheral pool swimming induced by scopolamine was assessed. Male Long-Evans rats were given a single 5-min spontaneous swim test (Symons, J.P. et al. Soc. Neurosci. Abstr., 12: 897, 1986) using a black 1.5 m Morris water tank (without escape platform) filled to a depth of 41 cm with clear water. The distance and pattern of swimming was analyzed from videotaped data. Both physostigmine salicylate (1.0 mg/kg,ip) and THA (10 mg/kg, but not 2 mg/kg) reduced total swim distance following scopolamine hydrochloride (0.32 mg/kg) pretreatment but only physostigmine significantly enhanced exploration of central areas of the pool ($p < .01$). In Experiment 2, Morris water-task learning was examined on five consecutive testing days following NBM lesions (6 µg/1 µl, ibotenic acid; bilateral lesions) or scopolamine pretreatment (0.32 and 1.0 mg/kg groups). Both NBM lesions and scopolamine blockade impaired Morris water-task acquisition and induced perseverative peripheral pool swimming. Neither 2 nor 10 mg/kg of THA significantly improved escape latencies when given daily pretesting to the NBM groups; however, spatial search on a probe trial (day 5) was enhanced by 10 mg/kg of THA. THA (2 mg/kg) failed to improve escape latencies after daily scopolamine pretreatment (1.0 mg/kg) but did significantly enhance escape latency on a single test session (day 3) in a group pretreated with 0.32 mg/kg of scopolamine ($p < .001$). In Experiment 3, NBM-lesioned rats were tested on a multiple trial passive-avoidance task which used an ultrasonic tone (30-62 Khz, 120 dB, 3.0 sec) in place of footshock. Sham and unilateral NBM-lesioned Sprague-Dawley rats acquired the task rapidly during the first week, but the bilateral NBM group was severely impaired in acquisition and retention of the task during the first 14 days ($p < .001$). THA (2 mg/kg) given daily immediately after training trials on days 15-19 significantly enhanced retention ($p < .05$), but the deficit re-emerged following the termination of drug treatments (days 20-24).

235.17 EFFECTS OF ORAL SCOPOLAMINE ON MEMORY AND ATTENTION IN MAN. G. C. Preston*, P. Broks*, M. Traub*, C. Ward*, P. Poppleton* and S. Stahl. Merck Sharp & Dohme, Terlings Park, Harlow, U.K.

In the context of the anticholinergic model of dementia, most studies of the effects of scopolamine have emphasised its amnestic properties. Generally, such investigations have employed relatively high drug doses (> 0.4mg subcutaneously or intramuscularly) and a limited range of cognitive tests. Under such conditions it is conceivable that memory loss represents a non-specific consequence of the drug's sedative effects. In a series of studies we investigated the effects of low doses of scopolamine (0.3, 0.6 & 1.2mg p.o.), methylscopolamine (0.6mg p.o.) and placebo on the performance of young, healthy volunteers on a battery of tests of memory and attention. The highest dose of scopolamine impaired performance on a verbal free recall task (Buschke selective reminding procedure), which could be attributed to an effect of the drug on secondary memory. This dose also produced an increase in reaction time using an unsignalled simple and choice reaction time procedure. Performance on a number of tasks was unaffected by these doses of the drug. These included tests of visual selective attention, simple reaction time in which a warning cue was provided (alerting function), tests of semantic memory (letter / category fluency and reaction time to categorize words), verbal and visuo-spatial primary memory (digit span and spatial span) and a visuo-spatial analog of verbal selective reminding. Two tests showed monotonic dose-dependent effects of scopolamine. These were a test of sustained vigilance (Wilkins, Shallice & McCarthy, Neuropsychol., in press) which requires the subject to count low-frequency trains of beeps, and tests of visual contrast sensitivity. Visual acuity was not concomitantly affected by the drug.

In the above studies we reproduced the amnestic effects of scopolamine. However, the tests which were most sensitive to muscarinic blockade were simple tests of attention rather than of memory. At higher effective doses using subcutaneous administration (e.g. 0.4mg s.c.) there is less selectivity in the drug's action: performance on both memory and attentional tests is profoundly impaired. The possibility that attentional tests such as the sustained vigilance test might be useful in the study of dementia itself is now being tested. In addition we are examining the pharmacological specificity of the pattern of results by comparison with the effects of anticholinergics with those of other classes of drug.

235.18 EFFECTS OF PARATHION EXPOSURE ON ACQUISITION OF A FREE-OPERANT AVOIDANCE TASK. F.O. Risinger and W.M. Bourn. School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209-0470.

Parathion is an organophosphate insecticide having anticholinesterase properties. In this study the time course of behavioral effects and cholinesterase inhibition was determined following oral exposure to parathion. Within the time intervals for the greatest behavioral effects and cholinesterase inhibition, the influence of oral parathion exposure on acquisition of a 2-way free operant avoidance task was examined.

Female Sprague-Dawley rats were dosed orally with either parathion (1.5 mg/kg) or a vehicle control. They were tested at 0, 30, 60, 120, or 240 minutes after dosing. Behavioral testing consisted of a 5 minute period in an enclosed activity monitoring device (activity cage) in which two types of motor movement were recorded (whole body movement and head/limb movement). Additionally, the animals were observed in an open field chamber (ambulation and rearing recorded) for a 5 minute period. After the behavioral measures were recorded brain AChE, red blood cell AChE, and plasma ChE activity was determined by a radiometric assay. Significant behavioral changes were seen only 120 minutes after dosing. Activity cage whole body movement, open field ambulation, and open field rearing were significantly ($p<.01$) reduced in parathion exposed subjects. These behavioral effects corresponded with the greatest inhibition of cholinesterase activity.

In the avoidance acquisition experiment female Sprague-Dawley rats were tested from 90 to 150 minutes after receiving various doses of parathion (0, 0.5, 1.0, and 1.5 mg/kg, po). Animals were placed in a shuttle box and subjected to a 2-way shock avoidance conditioning procedure which consisted of non-signaled shock occuring every 20 seconds. Avoidance responses were recorded for 100 trials. Parathion exposed animals at all doses demonstrated lower frequencies of avoidance responding ($p<.01$) with the greatest effect seen with the 1.5 mg/kg dose. Cholinesterase activity paralleled dose levels and behavioral values. The results suggest that free-operant avoidance procedures may be sensitive indicators of parathion exposure.

235.19 INTRA-AMYGDALA INJECTIONS OF β-ADRENERGIC ANTAGONISTS BLOCK THE MEMORY-ENHANCING EFFECT OF PERIPHERALLY-ADMINISTERED NALOXONE. I.B. Introini-Collison, A.H. Nagahara* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717.

Recent findings have suggested that the memory-enhancing effects of naloxone are blocked by treatments interfering with central noradrenergic systems (Gallagher, 1985; Introini-Collison & Baratti, 1986; Izquierdo & Graudenz, 1980). These findings are consistent with evidence that naloxone blocks the inhibitory effect of opioid peptides on the release of norepinephrine. In view of evidence that retention can be modulated by intra-amygdala injections of norepinephrine, the present experiments were undertaken to determine whether the memory enhancing effects of naloxone are blocked by intra-amygdala administration of adrenoceptor antagonists. Sprague Dawley rats (220-250g) were bilaterally implanted with amygdala cannulae. They were then trained on an inhibitory avoidance response and then, two weeks later, on a Y-maze discrimination response. Immediately following the training on each task, they were injected (intraperitoneally, IP, and in the amygdala). Retention was tested one week following the training on each task. Naloxone administered ip (3.0 mg/kg) significantly facilitated retention of both tasks. This effect of naloxone was observed both in unoperated and cannulae-implanted control rats. The memory-enhancing effect of naloxone IP was blocked by propranolol (0.3 or 1.0 μg) injected in the amygdala, but not when this β-noradrenergic blocker (0.3 μg) was injected into either the caudate or the cortex dorsal to the amygdala. Further, when injected into the amygdala, both the β_1-adrenoceptor blocker atenolol (0.3 or 1.0 μg) and the β_2-adrenoceptor blocker zinterol (0.3 or 1.0 μg), in doses which did not affect memory when administered alone, completely blocked naloxone-induced (3.0 mg/kg; ip) enhancement of memory. In contrast, posttraining intra-amygdala administration of α-antagonists prazosin (α_1) and yohimbine (α_2) (1.0 μg) did not attenuate the memory-enhancing effects of systemically-administered naloxone.

These findings support the view that naloxone-induced memory facilitation is mediated by the activation of β- but not α-noradrenergic receptors which are located in the amygdaloid complex.

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235.20 MEMORY-ENHANCEMENT WITH INTRA-AMYGDALA POSTTRAINING OF ADMINISTRATION OF NALOXONE IS BLOCKED BY CONCURRENT ADMINISTRATION OF PROPRANOLOL. A.H. Nagahara*, I.B. Introini-Collison and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717

Previous results from our laboratory indicate that the memory-enhancing effects of posttraining systemic (IP) administration of naloxone on memory are blocked by intra-amygdala injections of β-noradrenergic antagonists. If, as these findings suggest, naloxone affects memory through influences involving β-noradrenergic receptors within the amygdala then the memory-enhancing effect of intra-amygdally administered naloxone should be blocked by concurrent administration of a β-noradrenergic antagonist. The present experiment examined this implication. Sprague Dawley rats (220-250g) were bilaterally implanted with amygdala cannulae. They were first trained on an inhibitory avoidance task (IA) and then, two weeks later, on a Y-maze discrimination task (YMD). Bilateral intra-amygdala injections (1.0 μl) were administered immediately posttraining. Retention was evaluated one week following training on each task. Naloxone (0.1, 0.3 or 1.0 μg) facilitated retention in both tasks. The most effective doses were 0.1 μg for the IA task and 0.3 μg for the YMD task. Naloxone (0.1 μg) did not affect retention when administered via cannulae implanted in either the caudate-putamen or cortex dorsal to amygdala. Thus, the effects of intra-amygdala naloxone does not appear to be due to diffusion of the drug to these brain regions. These results strongly support the view that opioid peptidergic systems in the amygdala are involved in memory modulation. Further, as we observed previously with systemic injections of naloxone, intra-amygdala injections of the $\beta_{1,2}$-adrenoceptor blocker propranolol (0.3 μg) blocked the memory enhancing effects of intra-amygdally injected naloxone (administered concurrently)(IA: 0.1 μg; YMD: 0.3 μg).

We interpret these findings as indicating that the enhancing effects of intra-amygdala naloxone are mediated by the activation of β-noradrenergic receptors within the amygdala. Such effects are presumably due to blocking of inhibitory effects of opioid peptides on the release of norepinephrine.

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236.1 **MODIFICATION OF RUBRAL CELL ACTIVITY UNDERLIES ASSOCIATIVE CONDITIONING INDUCED BY PAIRED STIMULATION OF CORTICORUBRAL FIBERS AND LOCUS COERULEUS.** Y.ODA, H.KISHIDA*, M.ITO* and I.NAGATSU# Dept. Biophysical Engineering, Fac. Engineering Science, Osaka Univ., Toyonaka 560, and #Dept. Anatomy, Fujita-Gakuen Health Univ., School of Medicine, Toyoake 470-11, Japan.

Classical conditioning of forelimb flexion in cat is produced by pairing a stimulus (conditioned stimulus, CS) to the corticofugal fibers with an electric shock (unconditioned stimulus, US) to the forelimb skin (Tsukahara et al.,1981). The pathway mediating the conditioned response is suggested to be the cortico-rubrospinal pathway, since the corticofugal fibers were eliminated just caudal to the red nucleus (RN)(Tsukahara et al.,1981). However, little is known of the US pathway. We consider that the locus coeruleus nucleus (LC) is a candidate mediating the US, since the LC neurons can be activated by noxious stimulus such as skin shock and they are the origin of the central noradrenergic system which may play an important role in synaptic plasticity. In the previous study, noradrenergic innervation of the RN in cat was demonstrated immunohistochemically by using an antiserum against dopamine-β-hydroxylase (Oda et al., 1986). Here, we tested whether paired stimulation of the corticorubral fibers and the LC would induce associative conditioning of forelimb flexion and modification of RN cell activity.

The CS applied to the cerebral peduncle (CP) and the stimulus to the LC were a train of five electric pulses with an interval of *2* msec. The intensity of the CS was adjusted to produce forelimb flexion (flexion angle> *0.06°*) at the probability of *20%* during the pretraining period. Three types of training were employed. In the paired conditioning, the CS was followed by the LC stimulus *120* msec later and *50* trials were applied daily. The probability of forelimb flexion induced by the CS increased during the training period of *8* days ($\overline{X}$ = *72.8%*, on the last day, *n= 10*). Applying the CS or the LC stimuli alone for the same period did not significantly increase the probabilities of the forelimb flexion in response to the CS. The scores on the *8*th day were $\overline{X}$ = *28.6%* (*n= 7*) in the LC-alone group and $\overline{X}$ = *24.0%* (*n= 6*) in the CS-alone group.

We compared the firing responses of single RN cells to the CS among the paired, LC-alone, CS-alone and naive groups. The firing rate of the response was highest in the paired group. The firing rate within *3* msec after a single-pulse stimulus to the CP was higher in the paired group than in others (*p< 0.01*). The response during this period was considered to be monosynaptic, because polysynaptic responses through the cerebellum or the cerebrum do not occur within this period. Neither spontaneous discharge rate nor the monosynaptic responses to stimulation of another excitatory input from the interpositus nucleus were different among the four preparations.

In conclusion, associative conditioning is produced by pairing stimuli to the CP and the LC and its establishment can be explained by the increase of monosynaptic transmission through the corticorubral synapses.

236.2 ELECTROPHYSIOLOGICAL CHARACTERISTICS OF MAGNOCELLULAR MEDIAL GENICULATE NUCLEUS NEURONS DURING PAVLOVIAN DIFFERENTIAL HEART RATE CONDITIONING IN THE RABBIT. W.F. Supple, Jr. & B.S. Kapp, Dept of Psychology, University of Vermont, Burlington, VT 05405

Considerable evidence suggests that a forebrain system involving the amygdaloid central nucleus (ACE) contributes importantly in the rabbit to bradycardia elicited by a conditioned fear-arousing acoustic stimulus (Kapp & Pascoe, 1986). Consistent with this notion are data demonstrating that ACE neurons respond differentially to acoustic CS+ and CS- presentations during differential fear conditioning and that these neuronal responses correlate significantly with CS induced bradycardiac responses (Pascoe & Kapp, 1985). Since the magnocellular medial geniculate nucleus (mMGN) projects to the ACE and contributes to a variety of aversively conditioned responses (Gabriel et al., 1976; Jarrell et al., 1986; LeDoux et al., 1984; Weinberger, 1982), the present study examined the responses of mMGN neurons during differential fear conditioning to determine if they resemble those observed in the ACE and to further understand the contribution of this system to fear-conditioned bradycardia.

Twelve New Zealand rabbits received differential Pavlovian heart rate conditioning trials in which one tone (CS+) was paired with an aversive shock (UCS) and a second tone (CS-) of differing frequency was not. Extracellular single unit recordings were obtained from 60 histologically verified mMGN neurons in response to the CS+ and CS- during subsequent retention testing. The majority of these units demonstrated differential responding to the two stimuli. In 10 neurons with spontaneous rates between 1.4 - 20.0 Hz both the CS+ and CS- elicited an increase in activity. However, as assessed by standard scores the response to the CS+ was greater and of shorter onset latency (22.2 ms ± 4.0 ms) than that to the CS- (71.1 ms ± 9.0 ms). A second group of neurons with spontaneous rates between 4.1 - 18.0 Hz demonstrated a greater increase in activity with shorter onset latency (28.8 ± 8.3 ms) to the CS- compared to the CS+ (48.5 ± 11.1 ms). A third group of neurons demonstrated a differential decrease in activity; some cells showed a greater decrease to the CS+ compared to the CS- while others showed greater decreases to the CS- than to the CS+.

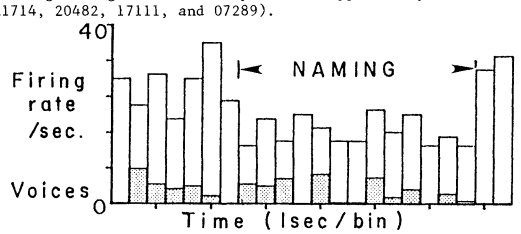
In further support of the contention that the differential mMGN unit responses reflect associative processes are additional unit data recorded in response to unreinforced presentations of the CS+ and CS- tones in naive, unconditioned rabbits. Increased activity in response to both tones was observed in each of six neurons recorded from six rabbits. However, no differential responses were observed and both tones elicited similar increases in activity.

These results suggest that (1) mMGN units do not respond differentially to the CS+ and CS- frequencies in the naive rabbit and (2) the differential mMGN unit responses observed in the conditioned rabbit reflect acquired associative processes. Moreover, unit responses of the mMGN are similar to those recorded in the ACE in response to fear-conditioned acoustic stimuli under similar conditioning and retention procedures. These data suggest that the mMGN and ACE are components of a forebrain system involved in the mediation of fear-conditioned bradycardiac responses to acoustic stimuli in the rabbit. Supported by a Grant-in-Aid from the American Heart Association.

236.3 HUMAN TEMPORAL LOBE NEURONAL ACTIVITY: INHIBITION DURING NAMING IN ONLY ONE OF TWO LANGUAGES. D.F. Cawthon,* E. Lettich,* and G.A. Ojemann. Dept. of Neurol.Surg., Univ. Washington, Seattle,WA 98195.

We recorded extracellular multiunit neuronal activity with tungsten microelectrodes from left anterior middle temporal gyrus dominant for speech (by Wada test) in a bilingual patient, according to institutional human subject standards. The recording site showed no surface epileptic activity and the sampled cell population showed no bursting activity, but at the margin of resection for epilepsy surgery. The patient had a college degree in Spanish but her native language was American English. In naming and reading tasks, she saw 6 slides of objects or words respectively, which she was asked to name or read silently or overtly for each batch of 6 trials either in English or in Spanish, using the identical series of slides.

Multiunit activity in the English naming tasks showed prolonged inhibition compared to Spanish especially in overt naming, as illustrated by a sharp transition to higher firing rates when the task changed from overt naming in English to overt naming in Spanish. In English but not in Spanish, neuronal activity during naming was lower than during reading. Inhibition during naming in English was confirmed in a related task where a prolonged drop in firing coincided with the patient's efforts to retrieve names of animals starting with "d" (Fig.), and higher activity promptly resumed at the end of the task or at interruptions by other voiced instructions. These activity changes in neuronal populations represent independent data to confirm the data from lesions (Stud.Neuroling. 3:65, 1977) and cortical stimulation studies from our surgical author's experience (Arch.Neurol. 35:409, 1978; Behav.Br.Sci. 6:189, 1983) which have demonstrated separation of areas essential for different languages in dominant temporal lobe. Stimulation necessary at surgery in this patient showed no changes at or near the recording site, and the patient had no language changes postoperatively. Therefore, the cell population sampled was not part of a critical area for language processing but may represent activity in an inhibitory surround zone for naming in English but not Spanish. (Supported by NIH Grants NS 21714, 20482, 17111, and 07289).



236.4 AUTO- AND CROSS-CORRELATIONAL ANALYSIS OF THE SEPTO-HIPPOCAMPAL SYSTEM DURING JAW MOVEMENT CONDITIONING IN THE RABBIT. R.A. Swain*, C.G. Oliver* and S.D. Berry. Dept. of Psychology, Miami Univ., Oxford, OH 45056 and SRL, Inc., Dayton, OH 45440.

The temporal parameters of conditioned and unconditioned neural responses in the medial septal nucleus (MSN) and hippocampus were examined in 8 New Zealand White rabbits. Each animal had chronic stainless steel recording electrodes implanted in the MSN and in area CA1 of the dorsal hippocampus under general anesthesia (Ketamine, 50 mg/kg and Xylazine, 10 mg/kg). After 1 week recovery, each rabbit was adapted to restraint and standard classical conditioning apparatus, and was placed on a 22 Hr water deprivation schedule. Classical jaw movement conditioning (CJM) was established using a 350 msec, 85 dB, 1 KHz tone as the conditioned stimulus (CS), and a 1 cc, 100 msec duration, .022% saccharin solution in water as the unconditioned stimulus (UCS). The interstimulus interval was 250 msec, and the intertrial interval averaged 1 min. Six blocks of trials were given in each daily session. Half of the animals were in a control condition, with trials consisting of explicitly unpaired tone or saccharin presentations. Permanent records of the transduced jaw movement, neural activity, and marker pulses denoting stimulus onset times were taken during each trial. In each brain structure, field potential (slow wave or EEG) responses and multiple unit spike activity were recorded from the same microelectrode. Unit activity was band passed filtered (500-5K Hz) and a window discriminator selected the largest spikes (3:1) for computer averaging into 10 msec bins. Slow waves were filtered from 0.5 to 25 Hz, and sampled at 10 msec intervals. Statistical analyses were performed on the responses averaged over the 48 paired trials in a session, or (for the control group) over the 48 tone or 48 saccharin trials. Averages of time-locked unit activity showed the development of conditioned responses to the CS in both MSN and CA1, but responses to the UCS only in CA1. Controls did not display evoked unit responses. Averaged slow wave activity showed significantly larger CS- and UCS-evoked responses in trained rabbits than in controls. In addition, the latency of the CA1 evoked response to the UCS was shorter in trained subjects. Auto and cross correlations of the slow wave, unit, and behavioral averages demonstrated that all CA1 and behavioral responses were periodic, with hippocampal activity occurring prior to behavioral movements. MSN responses, however, were not periodic but occurred at latencies that were consistent with this region being an important source of input in the development of hippocampal conditioned neural responses.

236.5 REFLEX FACILITATION OF THE NICTITATING MEMBRANE RESPONSE AND THE ACQUISITION OF CONDITIONED RESPONSES AS A FUNCTION OF INTERSTIMULUS INTERVAL. T. Scott, C. Walts*, and D. J. Weisz. Dept. of Psychology, Yale University, New Haven, CT 06520

Using the nictitating membrane (NM) preparation in rabbit, Ison and Leonard (1971) demonstrated that the presentation of an auditory stimulus from 160 to 1280 msec prior to the elicitation of an unconditioned NM response could enhance the amplitude of the elicited reflex. Subsequent research has demonstrated that interstimulus intervals (ISIs) that produce the most robust facilitation of the unconditioned NM response support the most rapid acquisition of conditioned responses (CRs) (Young et al., JCPP, 1976; Harvey et al., J. Neurosci., 1985). These findings have led to the hypothesis that there may be an interaction between the circuits that mediate these two behavioral phenomena. The correlation between reflex facilitation and CR acquisition has not been tested, however, at ISIs longer than 1280 msec. Therefore, we examined both reflex facilitation and CR acquisition rate at five ISIs--500, 1000, 2000, 4000, and 8000 msec. The intervals include two (500 and 1000 msec) that support rapid acquisition of CRs and one (8000 msec) that produces relatively few CRs.

All animals (n-25) were trained in two phases. During the first phase, animals received a total of 72 trials consisting of 12 US-alone presentations and 12 CS-US presentations at each of the five ISIs in order to determine the amount of reflex facilitation present at each of the intervals. The CS was a 1 kHz tone (85 db) that ended at the onset of an airpuff US (2.0 psi). During the second phase, animals were randomly assigned to one of five conditioning groups. Each group was then trained at one of the ISIs for eight days (54 CS-US trials/day).

The mean UR amplitudes were 8.2 mm on US-alone trials, and 11.3 mm (500 msec), 11.0 mm (1000 msec), 10.3 mm (2000 msec), 9.8 mm (4000 msec), and 9.7 mm (8000 msec) on the CS-US trials. A one-way ANOVA (randomized block design) revealed a significant effect of trial type ($F(5,120)=17.9$, $p<.01$). Post hoc tests revealed significant reflex facilitation at each of the five ISIs (all p's$<.01$), and significantly greater facilitation at the 500 and 1000 msec ISIs than at 4000 and 8000 msec. In addition, a significant correlation ($r=.9$, $p<.01$) was found between the amount of facilitation and the number of CRs elicited during phase two of training. These results provide further support for the finding that the amount of reflex facilitation is an excellent predictor of CR acquisition rate. Furthermore, they indicate that the physiological events that mediate reflex facilitation should be maximal at relatively short intervals after tone onset (e.g. 500 msec), although their effects may still be present at 8000 msec.

236.6 AN ASSOCIATIVE PROCESS IS INVOLVED IN THE MAINTENANCE OF REFLEX FACILITATION OF THE UNCONDITIONED NICTITATING MEMBRANE (NM) RESPONSE. D. J. Weisz and J. McInerney*. Dept. of Psychology, Yale University, New Haven, CT 06520

Facilitation of the amplitude of the unconditioned NM response by the prior presentation of a neutral stimulus occurs on the very first trial, and, therefore, its initial appearance is considered to involve non-associative processes. We have shown, however, that the ability of an auditory stimulus to maintain its facilitation across trials can be modified by experience with the stimulus (Weisz & LoTurco, Behav. Neurosci., in press). Specifically, non-reinforced presentations of an auditory CS can significantly reduce or eliminate the ability of the CS to facilitate the NM response. On the other hand, repeated pairing of the CS with an airpuff US results in the maintenance of facilitation until animals begin to acquire conditioned responses. Although dishabituation by the US cannot be excluded, we hypothesized that the maintenance of facilitation is due to an associative process by which the neutral stimulus acquires significance during CS-US pairings.

Two experiments were conducted to test this hypothesis. The experiments were identical except for the conditioned stimuli (1 kHz tone and vibrotactile stimulus, counterbalanced, in Expt. 1; and 1 and 4 kHz tones, counterbalanced, in Expt. 2). In each experiment, the experimental group received six blocks of training over two days, with each block consisting of five reinforced trials with the target CS (CS1) intermingled with 25 non-reinforced CS1 presentations. The ability of CS1 to facilitate the NM response was tested at the end of each block by measuring the difference between the amplitudes of unconditioned responses on US-alone and CS1-US trials. The control group in each experiment had identical training except that the conditioned stimulus for the five reinforced trials was CS2.

The results of both experiments showed that there was no amplitude facilitation by CS1 in the control groups. This replicated our earlier finding that non-reinforced presentations of a CS reduces the ability of the CS to facilitate the NM response. On the other hand, facilitation by CS1 in the experimental groups was robust (2.4 mm or 25% in Expt. 1; and 2.7 mm or 23% in Expt. 2), and differed significantly from that in the control groups (Expt. 1, $F(1,18)=9.31$, $p<.01$; Expt. 2, $F(1,11)=6.10$, $p<.05$). Therefore, the presentation of only five reinforced CS1 trials protected CS1 from habituation when it was presented 25 times in isolation. These data indicate that an associative process is involved in the maintenance of reflex facilitation of the unconditioned NM response, and that this associative process occurs with relatively few CS-US pairings.

236.7 PREFRONTAL REPRESENTATION OF SPATIAL AND NON-SPATIAL INFORMATION DURING VISUAL DELAY TASKS. J. Quintana*, J. Yajeya* and J.M. Fuster. Department of Psychiatry and Brain Research Institute. UCLA School of Medicine, Los Angeles, CA 90024.

The extracellular activity of 306 single units was recorded from the dorsolateral prefrontal cortex (PC) of two monkeys performing two visual discrimination tasks with delayed response. One of the tasks, delayed matching-to-sample (DMS), required matching the color of one of two stimuli presented simultaneously for choice to the color of the stimulus presented as a sample 18 seconds before. The other task, delayed conditional position discrimination (DCPD), required the choice of one of two identical (white) side-by-side stimuli (right or left) depending on the color of the cue presented 18 seconds before. At the beginning of task trials, differential unit reactions to the two DCPD colored cues were more common than those to the two DMS samples. During the delay period, 15% of all units showed differential discharge related to the two colors of either task, but only two units (0.6%) showed such differential delay discharge in both tasks. In the DCPD task, a large proportion of the units showing direction-related activity at the time of choice also reacted with a firing frequency change to one or both--spatially identical-- trial-initiating cues. The results indicate that, during visual delay tasks, neurons in the dorsolateral prefrontal cortex (superior convexity and sulcus principalis), may process both spatial and non-spatial information. Because of their protracted differential discharge between cue and response (i.e., during the delay), some units seem involved in the transfer of sensory information (spatial or non-spatial) across time. These findings support the idea of the participation of PC neurons in the representation of multiple attributes of sensory stimuli in a behavioral context and the overlap of the cortical representations of different attributes. They are also consistent with the role of the PC in the cross-temporal mediation of sensory-motor contingencies and, therefore, in the temporal organization of behavior.

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236.8 NEURAL CORRELATES OF CLASSICALLY CONDITIONED BRADYCARDIA IN THE RABBIT: STUDIES OF THE FRONTAL CORTEX. C.M. Gibbs, L.B. Prescott* and D.A. Powell. Neuroscience Lab., WJB Dorn Veterans' Hospital & Dept. of Psychology, Univ. of South Carolina, Columbia, SC 29201.

Repeated pairings of a pure tonal conditioned stimulus (CS) with eye-shock, under appropriate temporal parameters, lead to the rapid development of a bradycardiac conditioned response (CR) in the rabbit that has been shown to be mediated by a rather complex, limbic forebrain system. In this regard, we previously reported (Soc Neurosci Abstr 12:516) that within the cardioactive anterior midline cortex of the rabbit, corresponding to the precentral agranular and anterior limbic regions of Rose and Woolsey, CS-evoked multiple-unit activity (MUA) undergoes associative training-induced changes in a manner consistent with the concomitant development of this heart rate (HR) CR. We now report that such training-induced changes in anteromedial cortical MUA are, in fact, correlated with learned HR changes, a finding that contrasts markedly with the preliminary results of our ongoing studies of the similarly cardioactive insular cortex.

The studies involved experimentally naive, male and female New Zealand albino rabbits, each chronically prepared for recording MUA in either the anteromedial or the insular cortex. Following postoperative convalescence, the animal was adapted to handling/restraint and then received 10 presentations of a 4-sec, 1216-Hz, 75-db tone (pretraining), followed by either 40 classical conditioning trials (tones paired with 0.25-sec, 3-mA eye-shocks) or nonassociative training (unpaired tones and eye-shocks).

We have now confirmed that presentation of a novel tone stimulus elicits reliable increases in MUA at most sites in the anteromedial cortex and that classical conditioning results in a rapid enhancement (<5-10 trials) of this evoked MUA. Moreover, the training-induced changes in the shortest-latency (20-200 msec) component of the CS-evoked MUA are reliably correlated with conditioned decreases in HR ($r=-.51$, $p<.02$). In contrast, pretraining tone presentations appear to have more variable effects on MUA in the insular cortex, since unit subpopulations showing either increased discharge, decreased discharge, or no change in discharge at tone onset have been encountered. Further, conditioning effects on CS-evoked MUA in the insular cortex have been relatively inconsistent, and training-induced changes in MUA, when observed, have shown little or no relationship to conditioned HR changes. We would suggest that the present results are entirely consistent with previous findings in our laboratory, which have indicated that bilateral destruction of the anteromedial cortex profoundly disrupts the development of a discriminative HR CR in the rabbit (J comp physiol Psychol 96:755), whereas insular lesions have more modest decremental effects on this learned response (Behav Brain Res 17:125).

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236.9 SOMATOSTATIN AND CHOLINERGIC TRAITS IN THE BASAL FOREBRAIN-HIPPOCAMPAL SYSTEM: DEVELOPMENT AND BEHAVIORAL FUNCTION. D.R. Kornack and I.B. Black. Div. of Developmental Neurology, Cornell Univ. Med. Coll., New York, NY 10021.

One goal of contemporary neuroscience research is to understand how communication within neural systems generates specific behaviors. In the mammalian brain, the basal forebrain-hippocampal (BF-HP) system is a particularly useful model, having well-defined anatomic interconnections and expressing a variety of known transmitters. However, most attempts to integrate BF-HP behaviors with transmitter functions have focused exclusively on acetylcholine, and ignored the potential roles of other known transmitters in this system. We are beginning to explore two related questions using the BF-HP system in the rat. How do different transmitters interact in the genesis of behavior? How is expression of different transmitter traits regulated in the BF-HP, and how does this regulation relate to organismal behavior? To begin approaching these questions, we have examined the expression of cholinergic and peptidergic transmitter traits during development and after surgical disruption of the BF-HP that alters a specific behavior.

From birth to 8 weeks of age, the activity of choline acetyltransferase (CAT), the acetylcholine biosynthetic enzyme, increased 15-fold in BF and 35-fold in HP. Simultaneously, somatostatin (SS) increased 20-fold in both BF and dorsal HP, and 100-fold in ventral HP, suggesting that very different transmitter traits exhibit similar developmental profiles. SS and CAT are of particular interest, since both are deficient in Alzheimer's Disease, characterized, in part, by disordered memory, and since the BF-HP is critical for normal memory function. To determine whether SS, as well as acetylcholine, is important in BF-HP function, the neural system was disrupted in adult animals. The fimbria were transected bilaterally in rats trained to perform to criterion in a continuous trial, rewarded alternation discrimination T-maze task, a spatial memory behavior. Lesions markedly impaired choice accuracy compared to pre-operative performance and to that of sham-operated controls. HP CAT activity of lesioned rats decreased 50%, while activity in the BF was unchanged. In contrast to the marked cholinergic response, fimbria lesions did not alter SS levels in either BF or HP.

Our observations suggest that SS, which is implicated in degenerative brain disease, and which parallels cholinergic development in the BF-HP, does not play a role in the spatial memory behavior generated by this neural system. (Supported by NIH grants NS 10259 and HD 12108. I.B.B. is a recipient of a McKnight Research Project Award.)

236.10 HIPPOCAMPAL LESIONS CAN IMPAIR MEMORY OF A SHORT-DELAY EYEBLINK CONDITIONED RESPONSE IN RABBIT. E. Akase* and J. F. Disterhoft. Dept. of Cell Biology and Anatomy, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Multiple and single neuron recording studies have demonstrated a substantial involvement of hippocampal neurons during and after learning Pavlovian tasks in cat, rabbit and rat. Biophysical analyses of hippocampal brain slices made from eyeblink conditioned rabbits have demonstrated postsynaptic reductions in a calcium-mediated potassium current which is conditioning-specific and local to the hippocampus (Disterhoft, Coulter and Alkon, PNAS, 83: 2733-2737, 1986). However, several studies have demonstrated no effect of hippocampal lesions, at least on short-delay blink conditioning (e.g., Schmaltz and Theios, J. Comp. Physiol. Psychol., 79: 328-333, 1972). We reexamined this question during the early stage of acquisition. We reasoned that, if the hippocampus is particularly involved in transferring information from the short- to long-term memory store, hippocampal lesions should have maximal effect on retention at this time during learning.

Subjects were 20 young adult male albino rabbits weighing 1.5-2 Kg. Lesion animals (N=11) were anesthetized with i.m. injections of Ketamine (75 mg/Kg) and Xylazine (5mg/Kg). Suction lesions, done in sterile surgical conditions, removed overlying neocortex and at least the dorsal hippocampus bilaterally. Rabbits were habituated and then trained in a short-delay eyeblink conditioning paradigm. A 400 msec, 85 db, 6 KHz tone CS overlapped and coterminated with a 150 msec corneal air puff UCS. All rabbits were trained in daily 80 trial sessions to a behavioral criterion of two successive 10-trial blocks with 80% or more CRs. After 24 hours, two extinction sessions in which the tone CS was presented alone were begun. Four intact and 5 lesioned rabbits were extinguished in the sound attenuated training chamber, 5 intact and 6 lesioned rabbits were extinguished in the laboratory, where the stimulus context was different from that experienced during training.

Differences among the conditions were maximally present in the first 40 extinction trials (values reported here). **Retention effect:** hippocampal lesioned rabbits showed fewer CRs during extinction than did controls (20% vs. 49%, $p<.025$). **Stimulus context effect:** the intact rabbits showed more CRs during extinction in the training chamber than in the changed stimulus context (77% vs. 27.5%, $p<.01$); the hippocampal lesioned animals showed less differentiation between stimulus contexts (31% vs. 12%, n.s.).

Lesioned rabbits extinguish faster than intact rabbits and are not as effected by a change in stimulus context during extinction. Previous studies may not have observed the retention effect because the lesioned rabbits in those studies were more completely trained before extinction commenced. Testing retention of a learned response which is still in the process of consolidation is apparently a more sensitive evaluation of the hippocampal contribution. The stimulus context effect has not previously been tested in eyeblink conditioning. Experiments are continuing to increase the sample size in all groups and to test neocortical lesion control animals.

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236.11 LONG-TERM POTENTIATION IN THE DENTATE GYRUS IS INDUCED PREFERENTIALLY ON THE POSITIVE PHASE OF THETHA RHYTHM. J. WINSON, C. PAVLIDES*, AND Y. GREENSTEIN, Rockefeller University, N.Y., N.Y. 10021 and CUNY.

Hippocampal long-term potentiation (LTP) is considered a model for learning and memory. The physiological conditions under which LTP may occur are poorly understood. Theta rhythm in the hippocampus was hypothesized to be involved in LTP induction. The present experiment was undertaken to test this hypothesis.

In urethane anesthetized rats, a stimulating electrode was positioned in the medial perforant path (PP) to elicit the granule cell field potential and in the midbrain to activate theta rhythm. An electrode was positioned in the dentate gyrus granule cell layer to concurrently record field potentials and hippocampal EEG.

Having reliably produced theta rhythm with midbrain stimulation, the peak-to-peak time interval of the theta rhythm was determined for each individual animal. Baseline for both the initial slope and population (POP) spike of a field response following a PP stimulus were determined. Two control conditions followed: C1- the application of ten, 5-pulse bursts (interspike interval 2.5 ms; pulse width 150 usec) with an interpulse interval equal to the peak-to-peak time interval of the theta rhythm; C2- the application of midbrain stimulation for the generation of 10 cycles of theta rhythm, with no tetanic stimulation. Following C1 and C2, the same tetanic stimulation as in C1 was applied either at the peak (C3) or trough (C4) of ten cycles of theta rhythm. Subthreshold LTP stimulation was used in all conditions. If no LTP was detected, the intensity was increased by small increments and the protocol was repeated.

In the 7 rats in which tetanic stimulation was applied at the peak of theta rhythm there was a significant increase in both the slope ($9.0\%\pm1.6$ SEM, $p<.003$) and POP. spike ($47\%\pm11.0$ SEM, $p<.03$), during C3 relatively to the previous C2. In the 7 rats in which tetanic stimulation was applied at the trough of theta rhythm a significant increase in both slope and POP. spike occurred in C1 before it occurred in C4. In 3 of these rats there was a significant decrease of the slope in C4 ($-8.7\%\pm1.5$ SEM, $p<.02$); in the remaining four, no further change occurred during C4. The POP. spike did not change significantly in C4 in the 7 rats taken as a group. In none of these cases had LTP saturation been reached.

The induction of LTP during theta rhythm and the possible association of LTP with memory suggest that theta rhythm may be involved in mnemonic processes.

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236.12 RED NUCLEUS SINGLE-UNIT ACTIVITY DURING THE CLASSICALLY CONDITIONED RABBIT NICTITATING MEMBRANE RESPONSE. John E. Desmond & John W. Moore. Dept. of Psychology, Univ. of Mass., Amherst, MA 01003.

This study examined the firing patterns of single neurons in the red nucleus (RN) of the awake rabbit during classical conditioning training. The RN has been implicated in the control of the conditioned nictitating membrane (NM) response by the following lines of evidence: (1) HRP administration to the accessory abducens nucleus, the nucleus that controls NM movement, resulted in labeling of cells in the contralateral RN (Desmond et al., *Brain Res Bull*, 10:747, 1983). (2) Lesions of RN disrupted contralateral, but not ipsilateral, NM CRs without affecting URs (Rosenfield & Moore, *Behav Brain Res*, 17:77, 1985). (3) Nucleus interpositus, which is implicated in CR control (McCormick & Thompson, *Science*, 223:296, 1984; Yeo et al., *Exp Brain Res*, 60:87, 1985), sends efferents to contralateral RN.

New Zealand albino rabbits were trained to discriminate between a reinforced CS+ and a nonreinforced CS- (tones of 1200 or 600 Hz, 75 db SPL). This procedure results in both CR and non-CR trial types and thus, helps determine whether changes in neuronal activity are CR-related (Desmond & Moore, *Exp Brain Res*, 65:59, 1985). The UCS was electrostimulation to the periorbital region of the right eye. Rabbits were anesthetized and prepared for subsequent recording. A recording chamber was cemented in place over a small hole on the left side of the skull.

To date, 85 cells have been recorded from the midbrain and 50 of these were located in RN. A total of 26 of these cells displayed CR-related increases in firing. Thus far, there is little evidence of CR-related inhibition of firing. The CR-related cells fell into 2 categories: (1) Cells with very low baseline (pre-CS) firing rates (i.e., < 10 Hz) that increased substantially before and during the CR. These cells were located mostly in dorsal regions of parvocellular RN throughout the rostral-caudal extent of RN. The increase in firing preceded CR onset typically by 50 ms, although some of these cells appeared to lead by ≥ 100 ms. (2) Cells with baseline firing rates of 15-30 Hz or more. Most of these were associated with the dorsal magnocellular division of RN, and were found throughout the rostral-caudal extent of the nucleus. The increase in firing of these cells preceded the CR by 20-100 ms. Many of these cells displayed a characteristic UCS response consisting of excitation followed by 10-60 ms of inhibition.

These results support a role for RN in controlling the CR. The lead times of neuronal activity over the behavior are consistent with causal involvement.

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236.13 HIPPOCAMPAL COMPLEX SPIKE CELL ACTIVITY DURING WAKING AND SLEEPING BEHAVIORS.
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Hippocampal (CA1) complex spike (place) cells of freely behaving rats (8-arm maze) were recorded in pairs over an extended period of time, spanning a number of waking (exploration and still-alert) and sleeping behaviors, including quiet-awake, slow-wave sleep, pre-rapid-eye movement sleep and rapid-eye-movement sleep. Pairs of units were chosen that did not have overlapping place fields. The rats were restricted from entering the place fields of both cells on the night before testing and on the day of recording they were exposed to their diverse place fields independently and in a counterbalanced design. Following exposure of each cell to its place field, recording was made in the succeeding sleeping episodes and the following firing characteristics of both cells were analyzed: rate of firing, rate of bursting, number of spikes within a burst, interspike interval (for spikes within a burst) and the interburst intervals.

Following exposure of each of the place cells to its place field, significant increases in the spiking activity of the exposed cell were observed in the subsequent sleeping states, while the cell that was not exposed to its place field continued to fire at its baseline rate. Similarly, when the second cell was exposed to its place field, its firing was observed to increase in the sleeping states that followed while the first cell's firing subsided to its baseline level. The increased activity was observed in the following parameters: rate of firing (spikes/sec., including all spikes within a burst), the number of spikes within a burst, as well as the number of bursts displaying short interspike intervals (2-4 msec., approximately 400 Hz).

The findings suggest that neuronal activity of hippocampal place cells in the awake states may influence the firing characteristics of these cells in subsequent sleep episodes. The increase in the firing rates along with the greater number of spikes and the shorter interspike intervals within the burst, following exposure to a cell's place field, may speak for possible information processing occurring during sleep.

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236.14 PRIMING AT THETA RHYTHM PERIODICITY FACILITATES THE INDUCTION OF LONG-TERM POTENTIATION IN DENTATE GYRUS. Y. GREENSTEIN, C. PAVLIDES* AND J. WINSON, ROCKEFELLER UNIVERSITY, N.Y., N.Y. 10021 and CUNY.

Long-term potentiation (LTP) in the hippocampus is a long-term enhancement of synaptic efficacy which may be involved in learning and memory . Recent studies have reported that in the CA1 field in hippocampal slices LTP is preferentially induced if a priming stimulus is followed by a tetanic train at an interval of 200 ms., which corresponds to the periodicity of naturally occurring theta rhythm (5 Hz). Theta rhythm is prominent in the hippocampus and one of its generators is located in the dentate gyrus (DG) granule cells. The following study was undertaken to determine if priming at theta rhythm frequency in the IN VIVO preparation could preferentially induce LTP.

In urethane anesthetized rats, a stimulating electrode was placed in the medial perforant pathway (PP). A recording electrode was placed in the granule cell body layer of the DG to record the evoked field potential. The initial slope and population spike were measured. A single pulse (.25 msec width) was delivered to the PP every 30 sec. to evaluate LTP. For LTP induction a priming stimulus was followed by a tetanic train (6 pulses, 100 Hz, .20 msec pulse width) at varying time intervals (100, 200, 350, 500 ms). The order of the time intervals was randomized for each animal. The intensities of both priming pulse and train were subthreshold for LTP induction. If no LTP was induced, the current was increased by a small increment and the sequence of stimulation at different priming intervals was repeated.

The average potentiation (in percent of previous condition) for each of the priming intervals was as follows (n=7):

Priming interval	Spike		Slope	
100	1.20		0.00	
200	28.97	p<.003	3.74	p<.03
350	0.50		0.00	
500	1.47		2.27	n.s.

The findings indicate that LTP in the dentate gyrus in the IN VIVO preparation is preferentially induced at 200 msec, corresponding to 5 Hz. This finding corroborates previous studies done on hippocampal slices in the CA1 field and further supports the possible contribution of theta rhythm to LTP.

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236.15 **A CEREBELLAR CORTICAL CIRCUIT IMPLEMENTATION OF THE SUTTON-BARTO-DESMOND MODEL OF THE CLASSICALLY CONDITIONED RABBIT NICTITATING MEMBRANE RESPONSE.**
Diana E.J. Blazis* & John W. Moore. Dept. of Psychology, Univ. of Mass., Amhers MA 01003.

The Sutton-Barto-Desmond (SBD) model describes many features of rabbit nictitating membrane response (NMR) conditioning, including blocking, higher-order conditioning, interstimulus interval effects, conditioned response (CR) topography and neuronal firing (Moore et al, *Behav Brain Res* 21:143, 1986). Several laboratories have demonstrated that learning and generation of conditioned NMRs may involve cerebellar Purkinje cells (PCs) located in hemispheral lobule VI (HVI) (e.g., Berthier & Moore, *Exp Brain Res*, 63:341, 1986). We considered various schemes by which the cerebellar cortical circuits might be aligned with the computation of synaptic weights as specified by the SBD model. In the SBD model, weights of conditioned stimuli (CSs) are continually altered on the basis of the magnitude and sign of the difference between the model's output and the trace of preceding outputs, $s - \bar{s}$. The relationship between s and $\bar{s}$ can be approximated by an exponential function with a time constant of about 30 ms, thus imposing a constraint on circuit models which might describe how $s - \bar{s}$ is computed.

Observations of CR-related neuronal firing by PCs in HVI suggested that several PC types are involved in NMR production (Berthier & Moore, 1986). These included "lead" and "lag" cells, the firing of which mirrored and preceded or lagged, respectively, the temporal occurrence of the CR, as well as "on" and "off" cells, the firing rates of which increased above or decreased below baseline, respectively, on trials containing CRs. A circuit model which implements the SBD model incorporates these PC types. The SBD model's output, s, is fed back to HVI by mossy fibers (MFs) and climbing fibers which impinge on granule and Golgi cells. A second set of granule cells computes $\bar{s}$. The term $s - \bar{s}$ is computed by Golgi cells, which act as differential amplifiers influencing a third set of granule cells carrying CS inputs to PCs assumed to generate CRs. Learning, defined as changes in synaptic weight, occurs at synapses between MFs carrying CS inputs and the third class of granule cells, to the extent that those synapses are eligible for change.

Time constants of the various components of the SBD model and its circuit implementation are consistent with the empirical literature; for example, the time constant of Golgi cell inhibition is about 30 ms (Eccles et al, *Exp Brain Res*, 3:81, 1967), thereby approximating the relationship between s and $\bar{s}$. The wide range of firing rates observed in Golgi cells (Miles et al, *J Neurophysiol*,43:1437,1980; Schulman & Bloom, *Brain Res*, 210:350, 1981) is consistent with the requirement of a continuum of possible values for $s - \bar{s}$. Furthermore, the circuit model can account for the ratio of on to off cells observed by Berthier and Moore. The alignment of the SBD model with cerebellar cortical circuitry supports the role of cerebellar cortex in learning and performance of the conditioned NMR.

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236.16 SPINAL CONDITIONING EFFECTS ON SPINAL MOTONEURONS. Alvin L. Beggs, Ph.D. and Michael M. Patterson, Ph.D. Ohio University College of Osteopathic Medicine, Athens, Ohio 45701.

Deep peroneal motor nerve responses in spinalized cats show increases when superficial peroneal nerve stimulation (CS) is paired with an ankle skin shock (US). The present experiments were conducted to examine motoneuron activity during acquisition and extinction procedures. In the first experiment, a microelectrode was lowered into the ventral horn motoneuron pools of spinalized cats. Cats then received either paired or explicitly unpaired CS-US presentations followed by CS-alone extinction trials. Responses to single pulse test trials delivered through the microelectrode were recorded. The data indicated that paired trials produced significant acquisition and extinction effects, with no significant changes in the test trial responses. In the second experiment, microelectrode placement and conditioning procedures were the same as in Experiment 1. Multiple unit activity (MUA) was recorded during test trials from the ventral horn. A test trial consisted of a single 1-ms pulse delivered to the sensory nerve. MUA recordings were made for a period of 750 ms on each test trial which represented 250 ms pre-CS, 250 ms beginning with CS onset, and an additional 250 ms which would correspond to US period on a paired trial. Standard scores based on pre-CS spike counts were computed for the remaining two time periods for base-line, acquisition and extinction periods. The conditioning data again showed both acquisition and extinction effects, which were also showed in the standard scores of ventral horn MUA. The first experiment indicated that no motoneuron excitability change occured during conditioning procedures. However, the results of Experiment 2 indicate increased firing rates of spinal motoneurons during stimulus pairing which decreased during extinction trials. These data may suggest that the cells are firing more frequently due to stimulus contiguity effects or that recruitment of more cells occurs due to stimulus pairing.

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236.17 NEURON RESPONSES ELICITED IN MONKEY AMYGDALA BY COMPLEX SENSORY STIMULI AND DELAYED COMPARISON TEST. H. NISHIJO*, T. ONO AND R. TAMURA* Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Toyama 930-01, Japan.

It is suggested that hypoemotionality and some cognitive deficits produced by amygdalar lesion arise from the same underlying dysfunction of sensory processing. In this study, single neuron activity in the monkey amygdala (AM) was recorded during discrimination of various positive and negative stimuli presented in four kinds of operant bar press tasks, and in delayed comparison of pairs (DPC) among four colored lamps. The neuronal responses were analyzed in terms of responsiveness to visual, auditory, somesthetic and oral stimulation. Of 585 neurons tested for sensory responses, 107 responded primarily to a single modality (40 vision, 26 audition and 41 ingestion), 117 responded transiently or tonically to all four modalities, and 14 responded selectively to only one stimulus item. All of these neurons responded to certain rewarding or aversive stimuli. Of the 585 neurons, 423 were also tested in DPC, and 124 responded in one or more phases: 1) 17/30 vision related responded to 1st and/or 2nd cue lamps, and 4/30 responded throughout the 1st and 2nd cue and delay periods; 2) 0/21 audition related responded in DPC; 3) 4/30 ingestion related responded to 1st and/or 2nd cue lamps, and 30/30 responded in the ingestion phase; 4) 21/70 multimodal responded transiently and 47/70 responded tonically to the 1st and/or 2nd cue lamps (9/47 responded also in the delay period); 5) 4/14 selective neurons responded in ingestion phase in DPC only when a specific item was the reward. In summary: No amygdalar neurons, such as those reported in the inferotemporal cortex, responded differentially to color in the delay period. This suggests that the AM is not involved in short term memory in DPC for familiar color matching. However AM neurons that responded in the delay period indicate some involvement of the AM in certain memory processes, possibly internal rehearsal of the stimulus.

236.18 PAIRED-PULSE FACILITATION IS DIFFERENTIALLY AFFECTED BY LTP AND EXTRACELLULAR CALCIUM CONCENTRATIONS. D. Muller* and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717, U.S.A.

These experiments explored the possibility that increases in pre-synaptic calcium fluxes are involved in the hippocampal long-term potentiation (LTP) effect. We reasoned that if this were so, then the induction of LTP should affect various aspects of synaptic transmission in a manner similar to that found after manipulations thought to increase calcium influx into terminals. Studies at the neuromuscular junction have shown that the extent of calcium influx during depolarization is determined in part by the extracellular concentration of the cation and we assumed that this holds for hippocampus. We therefore first assessed the effects of increasing extracellular calcium on one measure of pre-synaptic physiology--paired pulse facilitation--and then tested if the induction of LTP reproduced these effects.

Hippocampal slices were first maintained at 1 mM Ca for one hour and then extra-cellular calcium was increased by steps up to 6 mM. The slope and amplitude of the field EPSP generated in the apical dendrites of field CA1 by stimulation of the Schaffer-commissural system was tested at each concentration as was the degree of paired pulse facilitation using an interpulse interval of 40 msec. As expected the size of the evoked response was a negatively accelerating function of calcium concentration with the shape of the curve closely resembling that described for the neuromuscular junction. Paired-pulse facilitation, expressed as a ratio of the second response to the first, decreased with increasing calcium levels; thus it appears that the degree of facilitation is negatively related to size of the calcium influx occurring on the first pulse.

LTP was tested in different slices at different calcium concentrations. In all cases, the strength of the potentiating stimulation was adjusted to produce a one millivolt population spike. Robust potentiation was obtained in most slices at 2 mM but not at lower concentrations of calcium. Slices incubated at the higher calcium concentrations did not exhibit a greater degree of LTP but the potentiation effect was, if anything, more reliably produced. This suggests, in contrast to earlier reports, that prolonged incubation in elevated calcium by itself does not trigger LTP. The induction of LTP at all levels of calcium did not reduce paired pulse facilitation; i.e., the ratio of the second response to the first was the same in potentiated as "naive" synapses. Thus increasing the amplitude of a given response via LTP does not have the same effect on paired pulse facilitation as does increasing the response by raising extracellular calcium. This discrepancy indicates that LTP is not likely to be due to an increased flux of calcium into pre-synaptic endings.

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LEARNING AND MEMORY: PHARMACOLOGY III

237.1 INHIBITORY AVOIDANCE LEARNING IN DEVELOPING RATS IS IMPAIRED BY NEONATAL 6-HYDROXYDOPA, BUT NOT DSP4. C.A. Cornwell-Jones, J. W. Chang*, B. Cole*, K. Goltz* and J.L. McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717.

Previous evidence suggests that central norepinephrine (NE) may be particularly important for some forms of early learning. For example, although the noradrenergic neurotoxin DSP4 does not impair inhibitory avoidance learning in adult rats, it impairs the ability of early experience to enhance adult learning (McGaugh et al., Neurosci. Abst. 12, 1986). The present experiment compared the effects of two noradrenergic neurotoxins, 6-hydroxydopa (6-OHDOPA) and N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4) on inhibitory avoidance learning in juvenile rats 35-40 days old.

On the day of birth Sprague-Dawley male rat pups assigned to the DSP4 experiment were injected s.c. with 50 μg/g of DSP4 and littermates received water. Rats in the remaining litters were injected with 60 μg/g of 6-OHDOPA on postnatal Days 0 and 2. Control littermates received vehicle. Half the rats in each group were trained in a one-trial inhibitory avoidance task, and tested for retention the following day. Shock threshold values were determined for remaining animals. The frontal cortex (FC), hippocampus (HP), and brainstem (BS) were subsequently assayed for NE, dopamine (DA) and serotonin (5-HT), and their metabolites including 5-hydroxyindole-3-acetic acid (5-HIAA), using high pressure liquid chromatography with electrochemical detection.

Neurotoxin effects on forebrain NE levels were similar: FC NE concentrations were reduced to 35% and 26% of control values by DSP4 and 6-OHDOPA respectively. HP NE concentrations were reduced to 15% and 13% of control values by DSP4 and 6-OHDOPA respectively. Neurotoxin effects on some other values differed however. DSP4 reduced HP 5-HIAA and 5-HT by 30% and 48% respectively, while 6-OHDOPA did not significantly affect such values. The largest differences were seen in the brainstem where 6-OHDOPA elevated 5-HT and 5-HIAA by 54% and 70% respectively, and where NE levels were elevated to 129% and 256% of control values by DSP4 and 6-OHDOPA respectively.

Retention of the inhibitory avoidance task was significantly impaired by 6-OHDOPA, but not DSP4. The deficit did not reflect increased resistance to pain since shock threshold values for controls and 6-OHDOPA-treated animals were not statistically different. The difference in the behavioral effects of these neurotoxins cannot be attributed to differences in forebrain NE. The finding of large differences in brainstem NE strongly suggests that brainstem mechanisms may underlie these behavioral differences.

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237.2 THE ROLE OF ADRENERGIC RECEPTORS IN THE BEHAVIORAL EFFECTS OF HIPPOCAMPAL SYMPATHETIC INGROWTH. A. Peagler* and L.E. Harrell. (Spon: E. Faught) Dept. Neurology and Psychology, VAMC and Univ. Alabama Med. Ctr., Birmingham, AL 35294.

Peripheral noradrenergic fibers, originating from the superior cervical ganglia, grow into the hippocampus following cholinergic denervation. Behavioral studies have suggested that hippocampal sympathetic ingrowth (HSI) is detrimental to the recovery of a spatial/memory task. In order to determine if this effect was mediated through adrenergic activity, we studied the effect of and B adrenergic blockaid on the reacquisition of a spatial/memory task following HSI.

Adult male Sprague-Dawley rats were assigned to one of two groups: Group 1 ultimately treated with the α -blocking agent,regitine (20 mg/kg); Group 2 treated with the B-blocking agent propranolol (20 mg/kg). Initially all animals were reduced to 85% of their body weight and underwent training on a modified version of a radial-8-arm maze task in which 4 of the arms were baited. Training continued until all animals reached a specific learning criterion (visiting 4 baited arms out of the first 5 selections on 4 out of 5 consecutive days). Following this animals in each group were randomly assigned to one surgical/treatment condition: CONV (sham medial septal lesion (MSL), sham superior cervical ganglionectomy (SGx) + vehicle (V)); COND (sham surgeries + Drug (D)); MSLV (MSL + SGx + V); MSLD (MSL + SGx + D); MSLGxV (MSL + ganglionectomy (Gx) + V); MSLGxD (MSL + Gx + D). Two days following surgery, animals underwent reacquisition trainng. This was performed in a similar manner to initial acquisition with the exception that either D or V was administered IP 30 minutes prior to testing. Training was continued until learning criterion was reachieved.

Preliminary examination of our results revealed that attainment of initial learning criterion was similar among animals assigned to both groups and various treatment conditions. As expected MSL produced a deterioration in task performance. In the vehicle treated groups animals without HSI appeared to recover faster than those with HSI. Drug treatment, however, interacted with the lesion effect and the presence or absence of HSI. Propranolol treatment caused a deterioration of learning in lesioned animals without HSI (MSLGx), while producing no changes in behavior of CON or MSL groups. Regitine treatment caused learning to deteriorate in the CON group, had no effect in the MSLGx group and enhanced learning in the MSL animals.

Although preliminary, our results suggest that maintenance of performance of this spatial/learning task in normal animals requires the integrity of α -adrenergic activity and that the detrimental effects of HSI may be mediate through activity within α -adrenergic systems.

237.3 NORDRENERGIC LESIONS BLOCK PHYSOSTIGMINE ENHANCEMENT OF N.BASALIS LESION INDUCED MEMORY DEFICITS: CLONIDINE REINSTATES PHYSOSTIGMINE ENHANCEMENT. V. Haroutunian, G. Tsuboyama, P.D. Kanof, and K.L. Davis. The Mount Sinai Sch. of Med., New York, NY 10029.

Alzheimer's disease(AD) is characterized by forebrain cholinergic deficits,and in some cases dramatic noradrenergic (NE) deficits. Cholinomimetic therapy offers clinically significant symptomatic relief in only a subpopulation of AD victims. Results of recent animal studies suggest that NE lesions can block cholinomimetic enhancement of memory in n. basalis (nbM) lesioned rats and may provided one of the bases for poor response in aproportion of AD patients. Rats received sham lesions, ibotenic acid lesions of the nbM (5ug/ul leading to 32% depletion of cortical CAT), 6-OHDA lesions of the ascending noradrenergic (NE) system (8ug/2ul resulting in 92% depletion of cortical NE), or lesions of both systems. After a two week recovery period each rat was trained in a one trial passive avoidance paradigm. Immediately after training each rat received sc injections of saline or one of several doses of physostigmine. The physostigmine dose was varied between 0.015-0.24 mg/kg. Retention of passive avoidance was assessed 72 hours later and showed that 0.06 and 0.12 mg/kg doses of physostigmine enhanced retention test performance in nbM lesioned rats ($ps<0.01$), but failed to affect the retention test deficits of the nbM + NE lesioned rats ($ps>0.1$). In a second study, sham operated rats and nbM + NE lesioned rats were trained on one trial passive avoidance.and received either a 0.06mg/kg dose of physostigmine alone, or physostigmine plus clonidine. The dose of clonidine was varied between 0.005-0.5 mg/kg in different groups of rats ($Ns=8-10$). Retention test performance was enhanced ($ps<0.01$) in nbM+NE lesioned rats which had received either the 0.5mg/kg dose of physostigmine alone, or in lesioned rats which had received 0.06mg/kg physostigmine plus 0.01 mg/kg clonidine. These results indicate that a)the failure of cholinomimetics to enhance cognition in some AD patients may be due to NE deficits superimposed upon the cholinergic deficit and, b) that the combined administration of cholinomimetics and clonidine may prove to be more efficacious than cholinergic therapy alone.

237.4 THE EFFECTS OF HUPERZINE A, AN ACETYLCHOLINESTERASE INHIBITOR, ON THE ENHANCEMENT OF MEMORY IN MICE, RATS AND MONKEYS[1].
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Huperzine A (Hup-A) is an alkaloid extracted from Huperzia serrata that is a potent and reversible inhibitor of acetylcholinesterase, with greater selectivity and 3 times the potency of physostigmine (Wang et al., Acta Pharmacol. Sin. 7:110-113, 1986). We have studied Hup-A in three species in various tests to assess its activity as a potential cognitive performance enhancing (CPE) agent.

In CF1 mice, Hup-A protected against electro-brain-shock (EBS) disruption in retrieval of an active avoidance response. Hup-A was active at doses of 0.001-0.01 mg/kg, i.p. and exhibited an inverted U-shaped dose-response curve. Physostigmine was active at a single dose of 0.1 mg/kg, i.p. with an equivalent magnitude of effect to that of Hup-A. The duration of action of Hup-A in this procedure was limited to 20 - 30 minutes after intraperitoneal injection. Hup-A was also active after oral administration but only at the single dose of 0.003 mg/kg and only at a 60 minute pretreatment time.

Hup-A was tested in C57BL/10 mice in the Morris water maze, a test which requires an animal to attend to spatial cues to locate the position of a hidden platform. Hup-A significantly decreased the latency to find the hidden platform by these mice at doses ranging from 0.001-0.1 mg/kg i.p. In contrast, physostigmine (0.003-0.1) was inactive in this procedure.

In albino rats, Hup-A was tested for its ability to reverse scopolamine (1 mg/kg) induced amnesia for the retention of a passive avoidance response. Hup-A was active from 0.00003-0.001 mg/kg s.c., whereas the "therapeutic window" of physostigmine was narrow and extended only from 0.005-0.01 mg/kg s.c. Hup-A was also active when administered orally from 0.1-1 mg/kg.

Hup-A also improved the accuracy of retention by 5-13% at doses of 0.003-0.03 mg/kg i.m. in squirrel monkeys on a delayed match-to-sample procedure. At 0.1 mg/kg, Hup-A resulted in a slowing of the animals' performance while accuracy remained high. This decrease in the speed of performance was antagonized by administration of scopolamine methylbromide and thus was the result of a peripheral cholinergic action of Hup-A.

These results demonstrate that Hup-A is a potent CPE agent, with a broad range of activity, especially after parenteral administration.

[1]. Appreciation is extended to Shanghai Institute of Material Medica, Shanghai, China for supplying Huperzine A; these studies are part of a collaborative effort with the Shanghai Institute.

237.5 CONTEXT-CONDITIONED FREEZING IS BLOCKED BY MORPHINE.
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The freezing response, which can be elicited experimentally by electric footshock, is one of the rat's species-specific defence reactions (Bolles,Psych.Review,71:32,1980). Fanselow (Behav.Neurosci,98:269,1984) demonstrated that freezing could be conditioned to contextual stimuli, and that this context-conditioned freezing was enhanced by naloxone administration. Borszcz, Cranney and Leaton (Proc.Eastern Psych.Assoc.,1985) showed that startle stimuli could support context-conditioned freezing, and Cranney (Pav.J.Bio.Sci, in press, 1987) reported that this effect may be mediated by endogenous opioid systems. This study investigated the effects of naloxone and morphine administration on freezing and startle amplitude.

Thirty-six male Wistar rats were matched by weight across four groups. On Day 1, the MOR group was administered morphine sulphate (10 mg/Kg)20 min, and saline (1 ml/Kg)1 min, prior to testing. The MOR-NAL group were administered morphine sulphate 20 min, and naloxone (4 mg/Kg)1 min, prior to testing. The SAL group were administered saline 1 min prior to testing. Testing consisted of ten 110-dB noise bursts with an ITI of 60s. Activity (freezing/no freezing) during the 10s prior to the first startle stimulus of the session was recorded. On Day 2,there was no drug administration but the same startle test was employed.

On Day 1, there were no differences amongst groups in both the prior freezing response and the startle amplitude of the first startle stimulus. No rats froze prior to the first stimulus except for 3 of the 9 MOR rats. On Day 2, the SAL group froze 53 per cent of the 10s prior to the first startle stimulus, and the NAL group froze 42 per cent. This finding does not replicate previous reports of naloxone-enhanced context-conditioning. However, the MOR-SAL Group did not show any context-conditioned freezing. This effect was attenuated by naloxone administration, with the MOR-NAL Group showing 53 per cent freezing to the context. Thus, morphine eliminated context-conditioned freezing. This suggests that either (1) morphine reduced the nociceptive properties of the startle stimulus, and so reduced the capacity of the stimulus to support context-conditioned freezing, (2) morphine interacts with an endogenous opioid system that is activated by startle stimulus presentation, or (3) context-conditioned opiate hyperactivity masked freezing behaviour. The relevance of these findings to theories of fear conditioning and startle modulation are discussed.

237.6 INFLUENCE OF OPIOID PEPTIDES IN MEMORY FORMATION IN THE CHICK. T.A. Patterson, G. Schulteis, J.L. Martinez, Jr., V.J. Hruby*, E.L. Bennett, & M.R. Rosenzweig. Dept. of Psych., Univ. of Calif., Berkeley, CA. 94720 and Dept. of Chem., Univ. of Ariz., Tucson, AZ 85721.

Several studies examined the effects of opioid peptides on memory formation in the chick. Two-day-old male Dekalb-Warren chicks were trained in a one-trial taste-avoidance task. Injections (10.0 µl/hemisphere) of saline or drug were made into the medial hyperstriatum ventrale (MHV) 5 min pretraining and chicks were tested 24 hr posttraining.

Experiment 1 determined that bilateral injection of 0.001-1.0 nmole/hemisphere β-endorphin (β-END) produced amnesia. Bilateral injection of naloxone (50.0 nmole/hemisphere) reversed amnesia produced by the highest dose of β-END. These results suggest that amnesia produced by β-END is an opioid effect.

Experiment 2 showed that bilateral injection of 1.0 nmole/hemisphere [leu]enkephalin (LEU) was amnestic. The dose response curve was U-shaped: 0.001-0.3 and 3.0-10.0 nmole/hemisphere doses were not amnestic. The highly selective delta agonist D-Pen^2-L-Pen^5-enkephalin (DPLPE) was 30 times more potent than LEU in producing amnesia. The dose response curve was again U-shaped: bilateral injection of 0.03 nmole/hemisphere produced amnesia, whereas doses of 0.003-0.01 and 0.1-1.0 nmole/hemisphere were not amnestic. Bilateral injection of the delta selective antagonist, ICI 174,864 (10.0 nmole/hemisphere), reversed amnesia produced by either 1.0 nmole/hemisphere LEU or 0.03 nmole/hemisphere DPLPE. These results indicate that amnesia produced by LEU is mediated through delta opioid receptors and suggest that delta receptors play a role in formation of memory in the chick.

Patterson et al. (1986) showed that memory formation in the chick is lateralized. Experiment 3 compared the effects of bilateral and unilateral injections into MHV of LEU or β-END (1.0 nmole/hemisphere). Only bilateral injections of LEU produced amnesia. Bilateral injection of β-END was amnestic, as was unilateral injection into the right, but not left MHV. These results suggest that the effects of β-END are centrally-mediated, whereas the effects of LEU may be peripherally-mediated or localized to other brain regions. The possible roles of lateralization of opioid effects will be presented.

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237.7 INVOLVEMENT OF OPIOID DELTA RECEPTORS IN ACTIVE AVOIDANCE CONDITIONING IN MICE. G. Schulteis, V.J. Hruby*, and J.L. Martinez, Jr. Psych. Dept., Univ. of Calif., Berkeley, CA 94720, and Chem. Dept., Univ. of Ariz., Tuscon, AZ 85721.

[Leu]enkephalin (LE) impairs acquisition of one-way active avoidance conditioning (1985, Life Sci. , 37:2345). As LE exhibits greater affinity for delta, than for mu or kappa, receptors (1983, Br. Med. Bull. , 39:25), we compared the behavioral effects of the endogenous ligand LE with those of [D-Pen^{2}-D-Pen^{5}]enkephalin, a highly selective delta agonist, and ICI 154,129 (ICI), a delta-selective antagonist.

Male Swiss-Webster mice were tested in a one-way active avoidance task (330 μA shock) 2 min after i.p. injection of drug or saline (SAL). Fourteen training trials were given, with a 20 sec intertrial interval. As compared to SAL, LE mice made significantly fewer avoidances at the 30 μg/kg (t[2,42)]= 2.66, p<.011) and 100 μg/kg (t[2,41] = 3.79, p<.0008) doses. DPDPE (11.62 μg/kg, equimolar to 10 μg/kg LE) also impaired learning (t[2,39] = 4.72, p<.0002), whereas ICI enhanced acquisition at the 30 mg/kg (t[2,27] = 2.59, p<.015) and 100 mg/kg (t[2,28] = 2.22, p<.034) doses.

We also examined the effects of LE and DPDPE on shock-induced locomotor activity levels and analgesia. The low potency and high cost of ICI precluded its inclusion. Activity was measured 2 min after injection of drug or SAL, by giving mice a 2 sec footshock (330 μA) immediately prior to placing them in an open field chamber divided into 16, 3-inch squares. Avoidance-impairing doses of LE and DPDPE had no effect on the number of lines crossed during a 10 min observation period. Analgesia was measured by jump thresholds in a flinch-jump paradigm. Four series of ten, 150 msec shocks, ranging from 75-480 μA in 45 μA steps, were delivered in alternating ascending and descending order. Neither DPDPE (11.62 μg/kg) nor LE (100 μg/kg), given 2 min prior to testing, induced analgesia.

In conclusion, stimulation of delta receptors with the selective agonist DPDPE mimics the impairment of acquisition observed with the endogenous ligand LE, at 3-10 times the LE potency. These effects of LE and DPDPE appear to involve direct modulation of learning, as neither drug alters shock-induced activity or analgesia thresholds. Finally, preventing endogenous ligands from binding to delta receptors with ICI enhances learning, an effect opposite that seen with LE and DPDPE. Taken together, these results suggest that delta receptor activation is normally involved in the modulation of avoidance learning, and that the effects of LE may be mediated by this receptor.
(Supported by NIDA #DA04195 [JLM] and NIMH, NRSA #5 R32 MH15860-08 [GS])

237.8 TWO ENKEPHALIN METABOLITES, TYR-GLY-GLY-PHE AND TYR-GLY, IMPAIR ACQUISITION OF AN ACTIVE AVOIDANCE RESPONSE IN MICE. P.H. Janak*, A. Valedon*, G. Schulteis, B.E. Derrick,*K. Fett*, S.B. Weinberger, J.L. Martinez, Jr. Department of Psychology, University of California, Berkeley, CA 94720.

We previously reported that an enkephalin metabolite, tyr-gly-gly (TGG), impairs acquisition of an active avoidance response in mice (Neurosci. Abs.,1986,12,706). In the present study, we examined the avoidance conditioning effects of the metabolites, tyr-gly-gly-phe (TGGP) and tyr-gly (TG).

Male Swiss-Webster mice were tested in a one-way active avoidance task 2 min following i.p. injection of peptide or saline, as described by Schulteis et al. (Neurosci. Abs.,1987,13). [Leu]enkephalin (LE) (100 μg/kg), TGGP (80 μg/kg), and TG (43 μg/kg) all significantly impaired acquisition of the avoidance response, as compared to saline treatment. The effective dose of both TGGP and TG was equimolar to the effective dose of LE.

We also observed shock-induced locomotor activity in an open field. LE has no effect in this test (Schulteis, et al., Neurosci. Abs.,1987,13). TGGP (80 μg/kg), but not TG (43 μg/kg), significantly decreased activity levels. This effect may contribute to TGGP's, but not TG's, impairing action on avoidance conditioning.

There are several ways to interpret these data: 1) TGGP, TGG, and TG all have low opioid potency as compared to LE (Dewey, In Endorphins: Chemistry, Physiology, Pharmacology, and Clinical Relevance. Malick & Bell (eds), Marcel Dekker, Inc., 1982). It is possible, therefore, that LE and its metabolites act at separate receptor populations, LE acting at opioid receptors, and metabolites acting at non-opioid receptors. 2) The LE metabolite des-tyr-LE has no effect on avoidance conditioning in mice (Neurosci. Abs., 1985,10,383), suggesting that the tyr-gly sequence is necessary for the avoidance impairing effects of LE and its metabolites. With this interpretation, LE and its active metabolites could influence conditioning through a single, although not necessarily an opioid, receptor. 3) Although LE, TGGP, and TG all produce the same action on avoidance conditioning, their spectra of behavioral activity differ, since only TGGP decreases locomotor activity. These peptides may therefore produce similar active avoidance impairments through different receptor mechanisms.

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237.9 **REVERSAL OF LESION-INDUCED SWIM MAZE DEFICITS WITH A CENTRAL ACETYLCHOLINESTERASE (AChE) INHIBITOR.** **J.E. Sweeney, C.F. Höhmann, J.A. Bowersox*, T.H. Moran and J. T. Coyle, Depts. of Environ. Health and Neuroscience. The Johns Hopkins University Schools of Public Health and Hygiene and Medicine, Baltimore, MD 21205.**

Destruction of cholinergic neurons of the basal forebrain (BF) which project to neocortex in rodents results in impaired performance on tasks involving working memory. In this study, we examined whether lesions to and pharmacologic manipulation of the central cholinergic system in mice impair performance on a swim maze task. Further, we examined whether this deficit could be reversed by a centrally-acting reversible acetylcholinesterase (AChE) inhibitor, galanthamine hydrobromide (GHB). GHB has an *in vivo* half life of approximately 6 hours, making its effects longer than most previously tested AChE inhibitors.

Adult male Balb/C mice received bilateral ibotenate lesions to BF and were allowed to recover for two weeks. Working memory was assessed in lesioned animals and age-matched controls on a water maze task. The swim tank (72-cm diameter) contained a platform submerged 1 cm below the surface of the opacified water. Mice were placed into each quadrant of the tank, and latency to find the platform was measured. Following acquisition, the position of the platform was changed daily and the new position demonstrated to the animal before latency was measured. After reaching criteria (< 100 sec), mice received saline injections (0.33 ml / kg, i.p.), and on the following day GHB (5 mg/kg, i.p.) one half hour before testing. Another group of mice were trained to criteria and tested with scopolamine (0.8 mg/kg,i.p.), a centrally-acting muscarinic antagonist, or N-methyl scopolamine (0.8 mg/kg), a peripheral antagonist.

Even though no significant differences were noted for acquisition of the task (either days to acquire or latency when the platform remained in one position), a clear distinction could be noted when the platform was moved on consecutive days; mean latency for lesioned animals was 200± 63 sec, and mean latency for control animals was 60±6 sec. GHB reduced latency in lesioned animals to 100±17 sec, while latency in control animals increased to 169±20 sec. Within 2 days of the drug treatment, performance in both groups returned to pre-injection levels. Scopolamine injections clearly impaired the animals' performance (latency was 206±44 sec), whereas N-methyl scopolamine, the peripheral muscarinic antagonist did not affect latency (51± 3 sec).

We have developed a working memory task for mice which is sensitive to cholinergic interuption, by either lesions to BF or administration of a central muscarinic antagonist. Results suggest that galanthamine can temporarily reverse impaired performance in BF lesioned animals. Further, it appears that optimum levels of acetylcholine (ACh) are necessary for accurate performance of this task; and that either too little or too much ACh is associated with impaired performance. Since galanthamine has a longer half life than many centrally-acting cholinergic drugs, it could be of possible clinical use in patients suffering from central cholinergic losses, for examples, in Alzheimer's Disease.

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237.10 **DOPAMINERGIC MODULATION OF COGNITIVE FUNCTIONS. BEHAVIOURAL AND NEUROCHEMICAL EVIDENCE.** G.Schettini,T.Florio*,E.Landolfi*,O.Meucci*, M.Grimaldi*,G.Magri* and A.Marino*. Department of Pharmacology, II School of Medicine, University of Naples, Via S.Pansini 5, 80131 Naples, ITALY.

Brain dopaminergic transmission is involved in modulating cognitive functions.

In the present study we evaluated the effect of co-dergocrine, a compound used in brain aging, on different behavioural tests and neurochemical parameters related to the dopaminergic transmission, both in young and aged animals.

Co-dergocrine (1-3-10mg/kg ip, 1h before trial, daily for 20 days) improved the acquisition of a conditioned avoidance response (CAR), being more effective at 3mg/kg dose. This latter dose slightly increased the CARs in old animals. The same treatment caused in young rats an increase of NE and DA stimulated adenylate cyclase (AC) activity in the frontal cortex. Co-dergocrine inhibited locomotor activity in old animals in a dose dependent manner, while showed a triphasic pattern of response in young rats: inhibition for low dose (0.03mg/kg), stimulation for middle dose (0.3mg/kg), again inhibition for high dose (10mg/kg). Striatal DA-sensitive AC activity was inhibited in co-dergocrine 3mg/kg 20days treated rats.

Co-dergocrine directly added to membrane preparations of different brain areas did not modify in young animals basal striatal AC activity, while the presence of SCH-23390 (100nM), a D_1 receptor antagonist, it inhibited striatal AC activity. In old rats co-dergocrine dose dependentlystimulated striatal AC activity, while in presence of SCH-23390 the enzyme activity was inhibited. Co-dergocrine reduced the frontal cortex AC activity at high concentrations and in the hippocampus a biphasic pattern of response was present. Thus co-dergocrine inproves cognitive functions in rat, likely acting on dopaminergic transmission. A different response between young and old animals to co-dergocrine is also shown, probably due to the changes in sensitivity and number of the two types of DA receptors during aging.

237.11 ANXIOGENIC EFFECT OF ACTH IN DISCRIMINATION AVOIDANCE LEARNING IN RATS. C. Hara, N. Ogawa* and M. Ishikawa*. Dept. of Pharmacol.,Ehime Univ. Sch. of Med., Ehime-ken 791-02, Japan.

ACTH is known to affect avoidance learning behavior of rats. The behavioral effect of ACTH is based on its extra-adrenal action (Miller, R.E. and Ogawa, N., 1962), and can be attributed to facilitation of consolidation of memory (de Weid, D. and Bohus, B., 1966) or anxiogenic effect (Weiss, J.M. et al., 1970; File, S.E. and Vellucci, S.V., 1978). In order to study the central action of ACTH, the present study examined influences of ACTH4-10 and ACTH4-9 without adrenocortical activity in the acquisition process of discrimination avoidance conditioning, which can be assessed anxiety level of rats (Gomita, Y. et al., 1985).

Male Wistar strain rats with a cannula for intraventricular (i.c.v.) injection were used. The rats were individually housed in the air-conditioned room (23±1°C) with lighting schedule of a 12:12 LD cycle (lights on 7:00-19:00) throughout the experiment. Food and water were supplied ad lib. ACTH4-10 and ACTH4-9 (0.2, 1.0 µg) were injected i.c.v. with 5 µl injection volume 10 min before beginning the discrimination avoidance conditioning. Chlordiazepoxide (CDP; 1.5 mg/kg) as an anxiolytic drug was administered i.p. 30 min before beginning the conditioning. The rats were divided into 4 groups as follows; saline(S; i.c.v.)x S (i.p.), S (i.c.v.)x CDP, ACTH fragments x S (i.p.), and ACTH fragments x CDP. The discrimination conditioned avoidance response was measured in the two-compartment shuttle box. The rats were trained to discriminate between positive and negative conditioned stimuli (CS). Two pure tones with a frequency of 400 Hz for positive CS and that of 800 Hz for negative CS, and visa versa, were used. The positive CS for 6 sec was followed by foot shock (0.7-1.2 mA) as unconditioned stimulus. Transfer of the rat to the opposite compartment in response to the negative CS was regarded to be an incorrect response. Transfer between compartments in the shuttle box during the intertrial period was also recorded as a spontaneous response.

In the results, there was no difference among 4 groups in the acquisition of avoidance learning. However, SxS and SxCDP groups showed significant difference between the acquisition of avoidance learning and the number of incorrect response within 10 days after beginning the conditioning. That is, both groups accomplished the discrimination avoidance learning. On the other hand, ACTH4-10 and ACTH4-9 treatments did not accomplished the discrimination learning, since the incorrect response to negative CS increased. CDP improved the impairment by ACTH fragments. There was no difference among 4 groups in spontaneous response.

Therefore, the results of the present study seem to suggest that ACTH is implicated in anxiety.

237.12 A VASOPRESSIN FRAGMENT MODULATES SELECTIVE ATTENTION. J. RAABE M.D. BUNSEY‡ B. STRUPP‡ D. LEVITSKY‡ and M. KESLER* Dept. of Psychol. and Div. Nut. Sci., Cornell U., Ithaca, N.Y. 14853.

Vasopressin's (VP) putative mnemonic role has recieved a great deal of attention. In contrast, very little research has examined the influence of this neuropeptide on attentional processes. Specifically, several lines of indirect evidence (electrophysiological, neuroanatomical, and behavioral) suggest that VP might narrow the focus of attention. However, only one study has directly addressed this hypothesis (Jennings, et.al.,]986), and it yielded inconclusive results.

The present study further examined this attentional hypothesis. Eighty hooded rats were administered either the VP fragment, AVP 4-9 (1.0, 3.0, or 10.0 ug/kg) or the vehicle solution one hour prior to behavioral testing. The behavioral test assessed the animal's ability to (1) determine which of two sets of tactile cues were predictive of reward and which were irrelevant, and (2) selectively attend to the predictive set of cues and filter out the irrelevant stimuli. On each trial, the animals were presented with two covered boxes, one containing a piece of Froot Loop cereal. For one fourth of the animals, the tactile cues on the boxes predicted reward and the lid cues were non-predictive. For another fourth of the animals, the reverse was true. These two subgroups comprised the distracting condition. The remaining half of the subjects were presented with either box or lid cues, but not both (i.e., the non-distracting condition). The animals were tested in their homecages in a non-deprived state in order to minimize arousal. Analysis of the number of trials to criterion indicated that there was no effect of the peptide treatment in the non-distracting condition. However, in the distracting condition, there was a significant interaction between dose and relevant dimension (i.e., whether boxes or lids were relevant). Specifically, performance of those rats for whom box cues were predictive improved with increasing dose of the peptide, while performance of rats for whom lid cues were relevant deteriorated as the dose increased.

These findings indicate that the AVP 4-9 treatment caused the rats to selectively focus on one aspect of their environment (the box cues). When this focus of attention contained information that was predictive of reward, performance improved. In contrast, when this information was irrelevant, learning rate was impaired. Possible implications for psychiatric disorders characterized by abnormal VP release are discussed.

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237.13 INDIVIDUAL DIFFERENCES IN MEMORY FUNCTION PREDICT VASOPRESSIN'S MNEMONIC EFFECT. B.J. Strupp* (SPON: N. Spear). Div. Nutr. Sci., Cornell Univ., Ithaca, NY 14853.

Although many investigators have reported that administration of vasopressin or analogues improves memory function in animals, two features of these experiments limit their implications for the role of the endogenous neuropeptide. First, the majority of these studies employed systemic administration of arginine vasopressin, the results of which may be artifactual, due to the aversiveness of this treatment. Second, all of the studies that have demonstrated memory facilitation with analogues or routes of administration that are not aversive have utilized aversively-motivated paradigms, rendering the generalizability of such findings questionable (see Strupp and Levitsky 1985). The three studies presented here were designed to determine if memory function could be improved in an appetitively-motivated paradigm by the administration of AVP_{4-9}, a vasopressin fragment that is virtually devoid of pressor effects (and therefore, presumably, aversive effects).

All three experiments assessed memory function in elevated radial arm mazes (8-, 12-, and 16-arm mazes respectively). After each rat had entered half of the arms (selected by the experimenter and hence termed forced choices), it was returned to its homecage. Following an interval of 9-14 hours (depending on the experiment) the rat was placed back into the maze. Only those arms which the rat did not traverse earlier that day were baited. The number of errors committed in retrieving the remaining food was used as the index of memory. In Experiment 1, AVP_{4-9} (0, .2, .67, 2.0, 6.67 µg/kg) was administered 30 minutes prior to the forced choices; all 4 active doses of the peptide significantly prevented the forgetting seen in the control condition. In Experiment 2, AVP_{4-9} (0, .02, .2, 2.0 µg/kg) administered immediately after the forced choice session was ineffective. However inspection of the data suggested (a) that different doses may have been beneficial to different rats, and (b) that the performance of one third of the rats may have been at a ceiling. Therefore in Experiment 3, each rat was tested in a 16-arm maze with the vehicle and the one dose of AVP_{4-9} that had most improved its performance in Experiment 2. Post-trial AVP_{4-9} significantly reduced forgetting. In addition, a significant inverse relationship was found between the rats' baseline performance and the degree of improvement caused by the peptide relative to the vehicle treatment. The most proficient rats were impaired by the peptide treatment, whereas memory of the least proficient animals was improved.

These results support a mnemonic role for vasopressin. Moreover they illustrate the importance of considering individual differences in studying the biology of cognition and in predicting therapeutic utility.

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237.14 ENVIRONMENT-SPECIFIC CONDITIONED ACTIVITY BASED ON SCOPOLAMINE IS NOT BLOCKED BY PIMOZIDE. E.J. Mazurski and R.J. Beninger. Department of Psychology, Queen's University, Kingston, Canada, K7L 3N6.

Environment-specific conditioned locomotor activity has been demonstrated using a number of dopamine agonists. The present study used a classical conditioning paradigm to determine if the stimulant effects of the anticholinergic scopolamine could also be conditioned to specific environmental cues. Two groups (n = 12 and 9) of rats had 12 60-min pairings of a distinctive environment (activity monitors that automatically assess horizontal and vertical activity) with scopolamine (1.0 or 8.0 mg/kg, IP, 30 min prior to each session). Corresponding control (unpaired) groups received saline. After each session all rats received a second injection; the paired groups received saline, and the unpaired groups received the appropriate dose of scopolamine. After every fourth pairing session a test, where all rats received saline 30 min before the session, was conducted to assess conditioning. Both doses of scopolamine produced an unconditioned enhancement of horizontal activity, although conditioned horizontal activity was seen only with 8.0 mg/kg. The low dose increased unconditioned vertical activity during the latter part of the session whereas an initial decrease was observed with the high dose. However, conditioned increases in vertical activity were seen with both doses. The possibility that this conditioned effect was mediated by dopamine was tested in a further experiment. Two groups of rats were treated similarly to the 8.0 mg/kg groups except both received the dopamine antagonist pimozide (0.4 mg/kg IP) 4 h prior to each pairing session. This dose has been reported to block conditioning based on amphetamine or cocaine. As pimozide did not alter the unconditioned or conditioned response, it was concluded that environment-specific conditioning based on scopolamine may not be mediated by dopamine. Possibly, conditioning was a direct result of learned changes within the cholinergic system.

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237.15 AMNESIA PRODUCTION BY SODIUM NITRITE. M.E. Judge, R.P. Wiard* and B.R. Cooper. Department of Pharmacology, Burroughs Wellcome Company, Research Triangle Park, North Carolina 27709.

The effects of sodium nitrite ($NaNO_2$) on the memory of approach/avoidance conditioning were examined to assess the possibility of using $NaNO_2$ induced hypoxia in pharmacological screening for cognitive enhancing compounds. Thirsty mice were trained to inhibit drinking in a small test chamber by using a one-trial approach/avoidance procedure ("lick-suppression test"). Mice were water deprived for 48 hours before the learning trial. Individual mice were placed in a 6 x 6 x 6 in. chamber with water available through a stainless steel drinking tube protruding through one wall. After 5 seconds of drinking, each subsequent contact with the water tube resulted in the delivery of a 1mA shock through the tube. The trial was terminated when contact with the tube was suppressed for 60 seconds. The memory test consisted of an identical session the following day with no shock available. Memory was defined as inhibition of contact with the water tube.

In the first experiment $NaNO_2$ was administered (i.p.) 30 min. before training in a wide range of doses (0.001 to 150 mg/kg). The number of mice discarded for failing to drink within 2 min. was under 10% until the dose of $NaNO_2$ reached 70 mg/kg, then increased through 50% at 120 mg/kg, to 100% at 150 mg/kg. $NaNO_2$ produced amnesia for the training, but only at a high dose (120 mg/kg). Above this dose to few mice completed training for memory to be assessed. The behavior of control groups of mice indicated that the amnesia was genuine, and not due to some non-specific side-effect of the $NaNO_2$ treatment. In the second experiment, 120 mg/kg of $NaNO_2$ was injected at three times: 60 or 30 min. before, or immediately after training. A significant amnesia was produced only with 30 min pretreatment. Studies are in progress to compare $NaNO_2$ and scopolamine induced amnesia , in particular to determine if the $NaNO_2$ induced amnesia is reversible.

237.16 REINFORCING AND MEMORY PROMOTING PROPERTIES OF SUBSTANCE P IN THE REGION OF THE NUCLEUS BASALIS MAGNOCELLULARIS. J.P. Huston, M.S. Holzhauer*, E. Kafetzopoulos* and J.A. Nagel*. Institute of Physiological Psychology, University of Düsseldorf, 4000 Düsseldorf Federal Republic of Germany.

Results from anatomical and cell culture studies suggest that SP provides an input into the nucleus basalis magnocellularis (NBM), an area which may play an important role in learning. To test whether SP in the region of the NBM has mnemonic and reinforcing properties, rats were injected with SP into this region and tested on a passive avoidance task and a place preference task.

Male Wistar rats were unilaterally injected with SP dissolved in a 0.9 % saline vehicle containing .01 M acetic acid. SP or vehicle was applied in a volume of 0.5 µl over 30 sec.

For the step-down task, the rat was placed on a small wooden pedestal in the middle of a plexiglass box having a grid floor. Latency to descend from the pedestal was measured for two baseline trials spaced 24 hrs apart. Immediately after the animal stepped down on the second baseline trial, it received a 1 mA shock for one second. The rat was removed from the box and injected with SP (100 or 10 ng) or the vehicle. Control groups included a 5 hr-delayed injection group, a SP-no footshock group, and a sham-operated group. Retention was tested 24 hours later. Rats injected with 100 ng of SP had significantly longer step-down latencies on the retention test than did vehicle controls, whereas the 5 hr-delay group did not differ from the vehicle group.

For the place preference task, animals were allowed to explore a box consisting of a white and a black chamber for 10 minutes on three consecutive days. About 90 % of the animals showed a preference for the black chamber. On the fourth day, rats received an injection of SP (1 or 100 ng) or vehicle and were than placed into the non-preferred compartment for 10 min. On day 5, the amount of time spent in each compartment was recorded. Rats treated with 1 ng SP changed their place preference as indicated by a significant decrease in time spent in the previously preferred compartment, whereas rats injected with the vehicle or 100 ng of SP retained their previously established place preference.

These results indicate that SP injected into the region of the NBM showed mnemonic and reinforcing properties. SP facilitated the performance of an inhibitory avoidance task in a time- and dose-dependent fashion. SP acted as a positive reinforcer in a conditioned place preference paradigm.

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237.17 EFFECTS OF SCOPOLAMINE AND PRENATAL EXPOSURE TO DIAZEPAM ON RETENTION OF PASSIVE AVOIDANCE. Amalia Márquez-Orozco*, María Cristina Márquez-Orozco* and Roberto A. Prado-Alcalá. (SPON: R. Tapia) Embryol. Dept. and Physiol. Dept., Sch. of Med., Natl. Univ. of México, P.O.B. 70-250, México, D.F., México 04510.

We have reported that prenatal exposure to diazepam does not interfere with learning of active and passive avoidance in 30-day old mice. Since this treatment produces important morphological alterations in structures known to be essential for the retention of passive avoidance, and scopolamine (SCOP) injections produce significant deficits in this process, we decided to test the effects of subthreshold doses of SCOP, injected to mice prenatally-exposed to diazepam. It was predicted that the interaction of treatments would induce a memory deficit. We tested the effects of the i.p. application of a low dose of SCOP (6 mg/kg), which in our conditions of training does not produce memory impairments, or of isotonic saline (SAL) to the offspring of pregnant CD-1 mice that had been treated with 2.7 mg/kg of diazepam (one daily injection from the 6th to the 17th day of gestation); these mice were kept under a 12/12 h light-dark cycle which was initiated at 7:00 a.m., with food and water always available. Right after birth the pups were separated from their mothers, and up to the age of 21 days, half of each litter (that was reduced to n=6) was nursed together with other three newborns by non-treated mice. Behavioral testing was started at 180 days of age. There were four groups of prenatal-diazepam animals: SCOP-treated males, SCOP-treated females, SAL-treated males and SAL-treated females (n = 10/group). All animals were trained to avoid a footshock that was given in the larger compartment of a two-compartment box (one-trial passive avoidance); the i.p. injections were applied 1 minute after training. Retention of the task was tested 6 days later. There were no significant differences in retention between the groups of males nor between the groups of females, although the males performed better than the females, regardless of treatment (P's < 0.05) These results suggest that low doses of SCOP do not interact with the effects of diazepam to produce memory deficits, and that the impairment that was seen in the female mice was due to the effects of prenatal diazepam.

237.18 MEMORY ENHANCEMENT WITH TENILSETAM: ANTAGONISM OF SCOPOLAMINE AND CYCLOHEXIMIDE INDUCED AMNESIA OF PASSIVE AVOIDANCE IN MICE. D. K. Rush and K. Streit*. Cassella AG, CNS Pharmacology, Frankfurt, FRG.

The novel nootropic compound tenilsetam (3-(2-thienyl)-piperazinone, CAS 997) has been shown to improve memory impaired by cholinergic blockade with scopolamine, acute forebrain ischemia resulting from carotid occlusion, and sodium nitrite induced anemic hypoxia in passive avoidance and 1-trial reward tasks in various species (Drug Dev Res, 4:567, 1984). The studies with sodium nitrite, administered post-training to induce an amnesia, suggest that tenilsetam may exert a beneficial influence on memory consolidation. The present experiments were conducted to further characterize the effects of this nootropic compound on memory processes.

Tenilsetam (10, 30, and 100 mg/kg p.o.), administered 60 min prior to training of a step-through dark avoidance response, dose dependently reversed a scopolamine (3 mg/kg i.p., 5 min prior to training) induced amnesia in mice as measured by both latency and duration parameters. Administered immediately after training, tenilsetam (10, 30, and 100 mg/kg i.p.) showed a moderate effect only at the highest dose. This differential effect of pre- and post-training tenilsetam parallels the ability of scopolamine (0.3 and 3 mg/kg i.p.) to induce an amnesia; a much stronger impairment is seen when this anticholinergic is administered prior to rather than after training.

To explore the influence of tenilsetam on memory consolidation processes, the protein synthesis inhibitor cycloheximide (150 mg/kg i.p.) administered either 20 min prior to or immediately following training was found to induce an amnesia, suggesting that the impaired memory resulted from an influence on post-training memory processing. Tenilsetam (10, 30, and 100 mg/kg), administered either 60 min prior to (p.o.) or immediately after training (i.p.), dose dependently antagonized the amnesia induced by pre-training cycloheximide administration. Post-training administration of tenilsetam (10, 30, and 100 mg/kg i.p.) also antagonized an amnesia induced by post-training cycloheximide. These results indicate that, in addition to its ability to counteract the effects of scopolamine, tenilsetam beneficially influences post-training memory processes thought to be dependent on protein synthesis.

Taken together, these findings suggest that tenilsetam may prove clinically useful in treating memory disorders.

237.19 DIFFERENTIAL EFFECTS OF PHYSOSTIGMINE AND 3,4-DIAMINOPYRIDINE ON RETENTION OF A SPATIAL MEMORY TASK FOLLOWING NUCLEUS BASALIS LESIONS IN RATS. B. A. Wirsching, R. J. Beninger*, K. Jhamandas, R. J. Boegman and M. Bialik+. Depts. of Psychology* and Pharmacology and Toxicology+, Queen's Univ., Kingston, Canada, K7L 3N6.

Recent studies have demonstrated that injections of quinolinic acid (QUIN) into the nucleus basalis magnocellularis (nbm) produce significant decreases in cortical choline acetyltransferase (CAT) and impairments of memory (Beninger et al., Neurosci let, 68:317, 1986). Furthermore, pharmacological manipulations of the central cholinergic system have been reported to attenuate the lesion-induced memory deficit. The present study further investigated the effects of QUIN lesions of the nbm on 8-arm radial maze performance and determined the extent to which physostigmine, an anticholinesterase, and 3,4-diaminopyridine (3,4-DAP), a drug that enhances acetylcholine release, can improve the memory impairment. Nineteen experimentally naive food-deprived Sprague-Dawley rats were pretrained on an 8-arm radial maze with 4 arms baited, until choice accuracy stabilized over 4 days to a criterion of $\geq$87% correct. All rats then underwent unilateral infusions of 120 nmoles of QUIN in 1 ul into the nbm. Following recovery, 10 rats received physostigmine (0.0, .01, .05, .10, .20, or .50 mg/kg, ip, 30 mins prior to a session); 9 rats received 3,4 DAP (0.0, .00000001, .000001, .0001, .01, 1.0 mg/kg, ip, 15 mins prior to a session). Drugs were given daily for 4 consecutive days followed by 4 non-drug days. In each drug condition each animal received every dose in a counterbalanced order. Analysis of covariance performed on error scores summed over four days, revealed that physostigmine led to a significant dose-dependent decrease in working memory errors (re-entries into baited arms). Reference memory errors (entries into never baited arms) were not significantly affected. In contrast, 3,4-DAP did not produce a reliable dose-dependent improvement of either type of memory. At completion of behavioural testing rats were decapitated and a section of their parietal-frontal cortex removed and assayed for CAT. Lesioned animals showed a significant decrease (mean$\pm$sem = 41.2%$\pm$2.55) in CAT activity on the injected side compared to the non-injected side. Results confirm that QUIN lesions of the nbm produce memory deficits in the radial maze and suggest that the cholinesterase inhibitor, physostigmine, but not 3,4-DAP attenuates these memory deficits. (Funded by the Ontario Mental Health Foundation.)

HUMAN BEHAVIORAL NEUROBIOLOGY II

238.1 EVENT-RELATED SLOW POTENTIALS: TOPOGRAPHIC DISTRIBUTION DURING PRIMARY MEMORY. C.Berka*,V.P.Clark and E.Halgren, Brain Research Institute, UCLA; VA Medical Center - Southwest Regional Epilepsy Center, West Los Angeles, CA 90024.

In an attempt to probe the neural correlates of Primary Memory (PM), event-related slow potentials were recorded at the scalp in healthy right-handed males performing delayed and immediate matching tasks. Slow negative potentials occurring in the S1-S2 interval (six seconds duration in the delay task) were similar to those reported previously in humans (Butler, S.R., Biol Psychol, 13:157, 1981) and primates (Fuster, J.M., J Neurophysiol, 36:61, 1973). Increases in single-unit activity in prefrontal cortical neurons during the delay between S1 and S2 have also been observed (Fuster, 1973), and may contribute to the generation of the scalp-recorded potentials. In addition, magnetoencephalography suggests at least two possible slow potential generators, both anterior to the central sulcus (Weinberg, H., Il Nuovo Cimento, 2:495, 1983). If the slow potentials reflect the continued firing of neurons encoding S1, then an understanding of the parameters which influence them may provide insight into the role of the frontal cortex in PM. In the present paradigm, maximal load was placed on PM by using complex, nonverbalizable stimuli and three-syllable nonsense words presented briefly (500msec). In both immediate and delay conditions, a two-choice key press response (match/mismatch) was required. Three classes of stimuli were presented: abstract pictures, abstract sounds, and nonsense words. Behavioral performance revealed that the tasks were of equivalent difficulty. DC recordings (at FCz, POz, F3, and F4 all referenced to linked mastoids) evidenced slow negative shifts in the S1-S2 interval for all stimuli in the delay task; consistent negative shifts were not observed in the immediate matching task. The potentials were maximally negative at the anterior midline site for all conditions, but a comparison of the abstract pictures and sounds revealed increased negativity for pictures, particularly at the anterior sites. This may simply imply that visual images require more complex encoding, or could actually reflect differential contributions to frontal cortex from modality-specific sensory regions. The anterior-posterior distribution of the slow potentials recorded during nonsense words resembled that of pictures, but a laterality was observed (F3 more negative than F4) which was not evident in the other conditions. Greater left hemisphere negativity could reflect a general preparation of the left frontal lobe to engage in language processing or could be evidence of a lateralized constellation of neurons involved in PM for verbal material.

Supported by grants from the Mathers Foundation, the Veterans Administration, and the United States Public Health Service (NS 18741).

238.2 NEUROMAGNETIC STUDIES OF VISUAL EVOKED RESPONSES: EFFECTS OF SELECTIVE ATTENTION. C. J. Aine, J. S. George* and E. R. Flynn, Neuromagnetism Lab, M/S M882, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

We have employed neuromagnetometry, a noninvasive technique capable of high spatial resolution, to study the effects of selective attention in a visual evoked response paradigm. Two right handed males and two females participated in the study. In all subjects magnetic recordings were obtained from 14 sensor locations over the left (contralateral) occipital cortex using a 7 channel neuromagnetometer, and from 3 scalp electrode locations. In two subjects, a more extensive mapping was undertaken including 37 magnetic sensor locations and 12 electrode placements. Subjects were shown a random sequence of sinusoidal gratings (1 or 5 cycles per degree, each subtending 2 degrees of visual angle) presented in the central visual field, or 7 degrees into the right visual field. Subjects were required to respond to one of the four stimulus conditions by pressing a fiberoptic-coupled mechanical switch within .5 sec of target simulus onset. At least five components could be reliably identified based on neuromagnetic waveforms and correlations with ERP records: the P1, N1,P2, N2 and P3. As in previous studies, we noted large differences in magnetic field distribution as a function of field of stimulation, and smaller but consistent differences in amplitude and distribution as a function of spatial frequency. The addition of the response task generally enhanced response component amplitude, particularly in peripheral field stimulation. The distribution of attention related differences was similar but not identical to the distribution of the primary components. In most records a significant difference was observed between responses to attended and unattended stimuli at 300 -600 msec poststimulus. Extensive mapping of two subjects suggests that at least two components contribute to the P3 complex. In one subject the early component (~340 msec) has a focal distribution consistent with a parietal-occipital source. Both subjects show a later broad component with a less focal distribution suggesting a deeper source.

238.3 **NEUROMAGNETIC STUDY OF SELECTIVE AUDITORY ATTENTION EFFECTS IN THE HUMAN BRAIN.** Deborah L. Arthur and Edward R. Flynn. Neuromagnetism Laboratory, MS D-434, Los Alamos National Laboratory, Los Alamos, NM,87545.

Controversy persists about whether selective attention enhances the amplitude of the exogenous N1 component or whether it results in the addition of an endogenous process ("Nd")stemming from a different physiological source. We are evaluating these alternatives by the method of neuromagnetism to determine whether the same regions of auditory cortex that are active during the time of the N1m are also responsible for the generation of the subsequent magnetic Nd counterpart (Ndm).

A dichotic listening paradigm was employed in which the standard tone bursts were presented at a constant rate of 800 msec and with overall probabilities of 40% each. The subject's task was to respond to occasional target tones in the attended ear (probability 10%) that were identical in frequency to the standard tones in that ear, but longer (150 msec) in duration. Neuromagnetic data was collected from four right handed subjects using a 7-channel neuromagnetometer. In 2 subjects, simultaneous ERP data was collected from Fz, Cz and Pz. The magnetic field was measured at either 14 or 21 points over temporal regions of both sides of the head. Subsequently, these data were used to construct maps of isofield contours and the underlying dipoles were then fit to equivalent current dipoles using a least squares code. A goodness of fit of the model with the data was calculated to characterize how much of the variance of the measured field pattern could be explained by the dipole model.

Selective attention resulted in a consistent modulation of the response (the Ndm) which began about 150 msec after stimulus onset and lasted throughout most of the remaining epoch. The Ndm component did not typically overlap the N1m component due to the slow rate of stimulation, although in one subject there was also a significant effect of attention on the N1m component. Left hemisphere analyses completed for this subject indicated that the source of N1m activity evoked by an attended tone was indistinguishable from activity evoked by the same tone when it was ignored. The goodness of fit using the N1m source as a model for Ndm data never exceeded 75%, supporting the hypothesis that selective attention invokes additional endogenous activity during the occurrence of the Ndm.

238.4 **TOPOGRAPHIC ELECTROENCEPHALOGRAPH CORRELATES OF THE PERCEPTION OF RHYTHM.** R.G. Niederhoffer,*[1] J.D.E. Gabrieli,[1] and R. Coppola,[2] [1]Department of Psychology, Harvard University, Cambridge, MA 02139 and [2]NIMH, Bethesda, MD 20892

Brain electrical activity during the perception of music has been studied previously by several investigators, but separable activity related to components of music, such as rhythm and pitch, has received less attention. Clinical reports and dichotic-listening studies with normal subjects suggest that although the right hemisphere may be dominant for some aspects of musical perception, the perception of rhythm may be associated with left-hemisphere functions. In order to address this issue, topographic electroencephalography (EEG) was used to measure regional changes in neuroelectric activity associated with four auditory tasks. The subjects were right-handed, young males without familial sinistrality. They performed, with their eyes closed, four 80-second auditory tasks: memorizing musical passages (transformed Bach chorales) and verbal passages (syntactically-reversible embedded-clause sentences) and counting changes in rhythmic patterns and tone frequencies. Recordings made during the tasks were compared to an active 80-second baseline condition (counting randomly spaced clicks) that occurred before and after each task. The recording montage consisted of 16 electrodes, placed at frontal, temporal, parietal, and occipital sites in the standard 10-20 system, but without midline electrodes. Electrodes in each hemisphere were referenced to the ipsilateral ear. Extra-cerebral artifacts were rejected visually, and ten artifact-free 2.56-second epochs were selected for further analysis. Subjects who did not have 10 such records in all 12 conditions (4 tasks, 8 baselines), or whose recognition memory or counting scores indicated that they had not performed the task throughout the recording (i.e. whose scores were more than 1.5 standard deviations below the group mean) were eliminated from further analysis.

Subjects showed regional task-related reversible changes in total alpha-band power. This was most consistent across subjects for the rhythm task, with decreased relative left-hemisphere alpha-band power occurring at posterior sites. To the extent that such a decrease may be interpreted as increased regional activity, these results suggest that different brain regions play separable roles in the normal perception of music and that the posterior left-hemisphere may be critical to the perception of rhythm. Topographic EEG studies may be useful in the development of neurally plausible models of normal human perception and cognition.

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238.5 EVOKED POTENTIAL AND REACTION TIME MEASURES OF SEMANTIC PRIMING IN A PICTURE NAMING TASK. D.S. O'Leary, L. Varholick and M. Seidenberg. Psychology Dept. Univ. Health Sciences/Chicago Med. Sch., N. Chicago, IL 60064

Semantic priming involves facilitation of access to items stored in memory due to prior processing of a conceptually-similar item. We studied semantic priming effects in a picture naming task in 8 normal adult human subjects using both vocal reaction time (RT) and evoked potentials (EPs). Ninety pictures of common objects served as targets. There were three conditions of prime-target pairings: (1) Related Condition: the target was preceded by the presentation of a semantically related picture (e.g. "pear" - "apple") which served as a prime, (2) Unrelated Condition: the prime and target pictures were unrelated (e.g. "hammer" - "apple") and (3) Neutral Condition: a blank slide preceded the target picture. Thirty trials of each condition were randomly intermixed. Duration of both primes and targets was 250 msecs and there was a 1 sec inter-stimulus interval. EEG was recorded from C3 and C4 referenced to tip of nose, and eye EMG was recorded for off-line artifacting and averaging. Subjects named both pictures 2 secs after stimulus offset. Vocal RT's to the pictures were recorded in a second session. Vocal RTs were significantly faster when the preceding picture was semantically related than when it was unrelated. With the exception of one subject, neutral trials were faster than either related or unrelated trials. This replicates previous behavioral findings reflecting a semantic priming facilitation effect. In the EP data, five components could be reliably identified. Repeated measures ANOVAs revealed no differences across conditions or hemispheres in a negative component (N1) peaking at about 130 msecs after picture onset. A positive component with a latency of about 210 msecs (P2) showed a significant difference across conditions, being later in the related condition than in the unrelated or neutral conditions. This pattern was reversed in a following negative component (N2) which had a significantly shorter latency in the related condition than in the unrealted or neutral conditions. Neither P1 nor N2 showed evidence of a hemispheric difference. A late positive wave showed a marginally significant ($p < .08$) interaction between condition and hemisphere. The late positivity peaked significantly earlier over the left hemisphere in the related condition than in the unrelated or neutral conditions. This was not the case for the right hemisphere waveforms. A late negative wave showed a similar pattern. Neither baseline to peak, nor peak to peak amplitude measures showed significant condition or hemisphere effects for these waveforms. These data will be discussed in terms of the value of EP techniques in determining the processing stage at which the semantic priming facilitation effect occurs.

238.6 VISUAL EVENT RELATED POTENTIALS IN VERBAL AND SEMANTIC DISCRIMINATIONS. R. Simson* H.G. Vaughan and W. Ritter*

To examine the differential effects on ERPs of discriminative processes during verbal identification, semantic categorization and target selection, we presented 3-5 letter words at 1.5 sec intervals on a video monitor in 3 discriminative conditions in which an unpredictable change in the stimulus characteristics occured on 20% of the trials. In the semantic condition, an average of 5 words within the same semantic category were sequentially presented and on 20% of the trials the category was unpredictably shifted. In 1 verbal condition, the same word was presented in sequences averaging 5 in length, with an unpredictable shift to another word sequence on 20% of the trials. In the other verbal condition, the same word was repeated and on 20% of the trials 1 of 20 different words was randomly inserted in the sequence. Reaction time (RT) was recorded to each 20% stimulus (target). A simple reaction time (SRT) condition required a rapid response to the same word on all trials.

ERPs were recorded from frontal, central, parietal, occipital and inferotemporal (mastoid) electrodes, referred to the nose. Average ERPs were obtained to the targets (T) and non-targets (NT) in each condition, and to all stimuli in the SRT condition.

Difference waveforms, derived by subtracting the SRT from the NT ERPs in the verbal conditions, revealed negative potentials that began about 200 msec after stimulus onset and terminated about 150 msec later. NT-SRT difference potentials in the semantic task had the same onset but lasted for 300 msec. Comparison of the semantic and verbal NT ERPs revealed a divergence beginning at 280 msec that lasted about 200 msec.

T-NT difference waveforms in the verbal conditions contained a negative wave beginning about 250 msec that was terminated by a large positive wave at 380 msec in the T response. In the semantic condition, T-NT differences were most prominent in a later negative wave, beginning about 300 msec and lasting at least 300 msec. The negative potentials seen in the verbal conditions were equally present in the T and NT ERPs of the semantic condition, and thus did not appear in the difference waveforms. RT to semantic targets was 200 msec longer than to verbal targets. Thus, 3 sequential but overlapping negative potentials appear to be related to processing physical and semantic features and to target selection.

238.7 THE SPATIAL GRADIENT OF VISUAL SELECTIVE ATTENTION: RELATIONSHIPS BETWEEN EVENT-RELATED BRAIN POTENTIALS AND TARGET DETECTIONS. G.R. Mangun* & S.A. Hillyard. Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

The effect of spatial selective attention upon visual processing has been likened in some cases to a small discrete "spotlight", and in others to a more widely distributed "gradient" of facilitated processing that surrounds an attended point in the visual field. Recently, using event-related brain potentials (ERPs) as indices of sensory processing, we reported that the attention-sensitive P135 and N185 waves showed systematic decrements in amplitude as the focus of attention was shifted progressively farther away from the evoking stimulus (Mangun & Hillyard, Human Factors, in press); these findings implied that a rather broad gradient of spatial attention could modify early visual processing. The study reported here obtained behavioral measures of information processing that were correlated with these ERP measures of attentional gradients.

Stimuli were vertically oriented bars flashed one at a time at locations five degrees to the left or right of the vertical meridian or at the midline, all in the upper visual field. Subjects were required to maintain eye fixation upon a central point while focusing their attention upon one of the three visual field locations during an experimental run. They indicated detection of shorter "target" stimuli that occurred 10% of the time at the attended location by making speeded button presses with one hand. In addition, subjects were given a secondary task wherein they responded with the opposite hand if they "happened to notice" any of the shorter target bars at either of the two unattended stimulus locations. ERPs and stimulus detectability measures (d prime) were compared for lateral stimuli when attention was focused upon them, as opposed to when attention was directed to the midline or opposite field stimuli.

The occipitally recorded P135 and N185 waves evoked by lateral stimuli demonstrated systematic amplitude decrements as the focus of attention was shifted to locations increasingly distant from the evoking stimulus location. The longer latency N280 wave did not display such a graded decrement but was instead specifically elicited by the attended stimulus. The behavioral measures of target detection performance (d prime) were also found to decrease systematically as the focus of attention was shifted to locations at increasing distances from the unattended lateral location at which stimuli were incidentally detected.

The parallel changes observed in ERP amplitudes and d prime scores suggest that the decrements seen in the P135 and N185 waves during spatial shifts of attention reflect actual losses in sensory information transmission.

238.8 SIGNAL AVERAGING APPLIED TO POSITRON EMISSION TOMOGRAPHY. M.A. Mintun*, P.T. Fox, and M.E. Raichle (SPON: J. McCasland) Depts of Neurology and Neurosurgery (Neurology) and Radiology and the McDonnell Center for Studies of Higher Brain Function, Washington University Medical School, St. Louis, Missouri 63110

Signal averaging is a powerful, widely employed means of suppressing measurement noise and, thereby, enhancing the detection of state-dependent changes in brain activity. We have applied signal averaging to positron emission tomography (PET) for functional brain mapping.

With PET, task-related regional changes are most effectively detected by direct subtraction of intrasubject pairs of PET CBF images. Image-pair subtraction displays the entire population of regional differences between the parent images while preserving their spatial organization. This population includes noise as well as signal (i.e., task-related regional changes). While robust regional changes can be distinguished from image noise by intensity alone, more subtle activations will be beneath noise levels. Thus the need for improving Signal:Noise ratio.

Task-induced CBF changes are regionally specific, occuring within the brain structures participating in the studied behavior; noise is spatially random. Spatial-domain averaging, then, should increase signal:noise. Spatial-domain averaging was implemented by translating all PET images into a standardized stereotactic coordinate space (Fox et al 1985, J Comput Assist Tomogr 9:141-153) to allow averaging across subjects. Noise suppression due to averaging was tested using same-state subtraction pairs (i.e., control minus control). Noise (computed as the standard deviation of the population of regional differences) fell as the square root of the number of subjects pooled, as tested for averaged-image n's from 1 to 20. Signal strength was tested using vibrotactile finger stimulation, as the primary cortical response is readily detected in all subjects. Signal:noise ratio improved steadily with n from 1.2 (n = 1) to 8.3 (n = 25).

We conclude that inter-subject averaging markedly improves the sensitivity of PET for detecting task-induced regional brain activation.

238.9 A COMPUTATIONAL ANALYSIS OF THE RELATION BETWEEN MEMORY FOR OBJECTS AND THEIR LOCATIONS IN NORMAL AND DEMENTED SUBJECTS. M. Snow*, J.D.E. Gabrieli, C.M. Darnell*, M.M. Kjelgaard*, M.M. Keane*, S. Baylog*, J.H. Growdon and S. Corkin. (SPON: D. Claman). Department of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139

We examined the relationship between memory for an object (what) and for its spatial location (where) in groups with intact or impaired fact-learning capacities: 11 normal subjects, 23 mildly or moderately demented patients with Alzheimer's disease (AD), and 6 patients with Parkinson's disease (PD) who were nondemented. In this experiment (adapted from Smith & Milner, 1981), each subject was presented with 20 toy versions of familiar objects placed on a 30-inch square piece of paper. First the subject was asked to name each object. The examiner then removed the objects and asked the subject to recall them and to replace the objects in their original locations. Three scores were calculated: percentage of objects named correctly, percentage of objects recalled correctly, and a series of scores for recall of spatial location. Relocation accuracy was assessed by calculating the mean displacement per object after transforming each subject's array of locations in the following ways: translating so that the array's center of gravity coincided with the original array; scaling so that the overall dispersion matched that of the original array; rotating to give a minimum-displacement matching with the original array; relabeling the individual locations to give a minimum displacement correspondence with the original array. Analyses of object displacement after those transformations provided measures of location memory independent of several possible distortions; calculating the severity of the four types of displacement errors could be used to characterize computationally the nature of replacement errors. For each subject, analyses were performed for objects recalled or not recalled and objects named or unnamed. The AD patients were impaired in object naming, object recall, and location recall by all measures of location error. The PD patients were unimpaired. For the normal subjects and PD patients, location memory was not significantly different for recalled versus unrecalled objects. For the AD patients, location memory was not significantly different for named versus unnamed objects. Thus, episodic memory for objects in normal subjects and PD patients and semantic memory (names) for objects in AD patients were dissociable from memory for spatial location. These results provide further evidence for the functional separtion of memory for "what" versus "where" and for the idea that different neural systems mediate these two forms of memory.

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238.10 PERCEPTUAL DISORGANIZATION IN PATIENTS WITH PARIETAL-TEMPORAL LESIONS. L. C. Robertson* & M. R. Lamb* (SPON: M. Remler) Depts. of Psychiatry & Neurology, University of California, Davis, & VAMC, 150 Muir Road, Martinez, CA 94553

When normal subjects look at a scene they see objects within other objects. Brain damage often disrupts this type of perceptual organization in different ways depending on the side of lesion. For instance, larger (global) objects are more likely to be misperceived with damage to the right hemisphere and smaller (local) objects are more likely to be misperceived with damage to the left hemisphere. We do not know, however, what specific cognitive mechanism(s) underly the overall performance in these patients. Several mechanisms are involved in normal perceptual organization (e.g., vision, attention, long and short term memory, etc), and damage to any one of these could result in global or local deficits.

In the present work we examined the role of divided attention on perceptual organization in patients with damage to the right or left parietal-temporal junction (PT). There is converging evidence from research using single unit and behavioral measures that subregions of this area are associated with spatial attention. Therefore, it was possible that PT damage also disrupted attention such that attention could not be distributed normally between global and local levels of a stimulus.

Stimuli composed of several local letters arranged to form a global letter were presented to a group of 6 right PT damaged patients (RPT), 10 left PT damaged patients (LPT), and 9 matched controls. Between blocks of trials the probability of a designated target appearing at the global or local level was varied, and reaction times and errors were recorded. Controls allocated more attentional resources to the global level when targets were more frequent at the global level and more attention to the local level when targets were more frequent at the local level. The LPT group was able to allocate attention in a similar manner. However, overall their response times to local targets differed from controls in that reaction times were slower to local targets than to global targets. The RPT group was only able to allocate attention normally when the targets were more frequent at the local level but were unable to do so when the targets were more frequent at the global level. These data will be discussed in terms of attentional and sensory mechanisms as the source of global and local deficits observed in patients with parietal damage.

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238.11 LOCALIZATION OF METABOLIC AND ELECTROPHYSIOLOGIC ACTIVATION DURING VERBAL TASKS. V.I.Nenov, C.Berka, S.Kamath*, E.Halgren, M.Smith, M.Mandelkern*, J.Ropchan* and W.Blahd*, Brain Res Inst UCLA; VA Medical Center -- Southwest Regional Epilepsy Center & Nuclear Medicine Service, Los Angeles, CA 90024.

PET studies allow us to obtain physiologic images of the entire brain. Mapping of local cerebral metabolic rates for glucose (LCMRGlc) with FDG has been used to study the response of the human brain to sensory and cognitive stimulation [Mazziotta JC, etal., Human Neurobio 1983]. Regional cerebral blood flow (rCBF) has been used to study gray matter activation during memorizing and reasoning [Riesberg J, etal. Brain 1973]. Electrophysiological studies have found a series of potentials (termed N4/P3) that are evoked by meaningful complex stimuli. The N4/P3 are generated in the Medial Temporal Lobe (MTL) as well as in other cerebral structures. Furthermore, the N4 to words is larger in the left MTL [Smith ME, etal. EEG Clin Neurophys 1986]. Aphasia is nearly always due to a left brain lesion. However, the isolated right hemisphere has substantial language abilities and rCBF & PET studies have usually indicated that activation in verbal tasks is bilateral and symmetrical or only slightly lateralized. These observations suggest a number of questions: 1) Will the MTL be activated metabolically in a task where it is known to be activated electrophysiologically? 2) What other structures will be activated in this task? 3) To what degree will these activations be lateralized?

Normal right handed males were subjected to three PET studies -- one resting and two stimulations. The lexical decision task was performed during the FDG uptake period when words and nonwords were flashed on a TV monitor and subjects pressed a key when a real word was detected. A number of transaxial PET images covering the whole brain, 11mm apart with 22 mm collimator aperture were obtained. MRI scans in planes corresponding to the PET planes were obtained for each subject prior to the study and were used for structural localization. Structural identification was based on a standardized proportional system [Talairach J, etal. 1967] which uses the anterior comissure - posterior comissure (AC-PC) line as a basis. Data analysis included image subtraction (stimulation minus resting) of corresponding planes; drawing of regions of interest (ROIs) around standard structures; calculation of the percentage change of LCMRGlc; and statistical evaluation of the significance of the observations.

Performing a lexical decision task with nonrepeated words increased LCMRGlc in the macular part of the striate cortex, left MTL (especially the parahippocampal gyrus), anterior cingulate gyrus, and inferior frontal cortex (including orbito-frontal cortex and gyrus rectus). The task related changes in activation were confined to limbic and specific neocortical structures, rather than the moer widespread regions thought to be involved in langauge processing. More sophisticated experiments are required in order to reveal the temporal sequence of activation during memory tasks.

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238.12 COMPARISON OF MANIA VERSUS DEPRESSION FOLLOWING BRAIN INJURY: CAUSAL FACTORS. J.D. Boston*, R.G. Robinson, T.R.Price*. (SPON: G.D. Pearlson) Dept. of Psychiatry and Neuroscience, Johns Hopkins Univ. Sch. of Med., & Univ. of Maryland Sch. of Med., Baltimore, Md. 21205

A consecutive series of patients with either major depression (n=31), mania (n=15) or no affective disturbance as determined by DSMIII symptom criteria (n=28) following brain injury were examined.

Examination of lesion location (based on CT-scan findings or neurological examination) revealed that 10 of 15 patients with mania had exclusive right hemisphere involvement while 4 had bilateral or midline lesions and only 1 had a left hemisphere lesion. In contrast, 19 of 31 depressed patients had left hemisphere lesions, 7 had right hemisphere lesions and 5 had bilateral or brainstem lesions. Among the non-depressed patients, 9 had left hemisphere lesions, 10 had right hemisphere lesions and 9 had bilateral brainstem lesions ($X2=15.2, p<.004$ for lesion location by mood diagnosis). Mania was significantly more frequently associated with right hemisphere injury while depression was significantly more frequently associated with left hemisphere lesions.

Statistical analysis of demographic data indicated no statistically significant differences between groups in mean sex, age and education. Single or double informant family histories revealed that 7 of 15 mania patients (46%) had a family history of affective disorder (4 definite and 3 possible). In the major depressed group 3 (9%) had a family history of affective disturbance (2 definite and 1 possible). One patient (4%) in the no affective disturbance group had a possible family history of affective disturbance ($X2=15.5$, $df=2$, $p<.001$). This unequal distribution of family history of affective disorder remained significant when only definite family history was analyzed ($X=9.52$, $df=2$, $p<.01$). Results of this study indicate that mania following brain injury may be rare because two factors, a genetic loading and a specific right hemisphere lesion, are necessary for its expression. Depression does not appear to require a genetic predisposition and is associated with left frontal injury. Mania and depression associated with brain injury appear to have different etiological mechanisms.

238.13 **SINISTRALITY IN MALE AND FEMALE HOMOSEXUALITY: NEUROBIOLOGICAL IMPLICATIONS.** C.M. McCormick*, S.F. Witelson and E. Kingstone* (SPON: J. Cleghorn). Departments of Psychiatry and Psychology, McMaster University, Hamilton, Ont., Canada, L8N 3Z5.

No reliable evidence of any physical differences between homosexuals and heterosexuals has been found (e.g., circulating sex hormones, body build). However, there is some evidence for biological factors in sexual orientation. For example, the incidence of homosexuality is relatively stable across cultures and through the ages (Whitam, F., Arch. Sex. Beh., 12:207, 1983), and there is a higher concordance for sexual orientation in MZ than DZ twins (Pillard, R. et al., Arch. Sex. Beh., 10:465, 1981).

Given that sexual preference must have some perceptual component and is thus related to brain function, we probed for a possible neurobiological difference between heterosexuals and homosexuals via a neuropsychological route. We report here findings on hand preference, used as an index of hemisphere specialization, in a group of 70 homosexuals recruited through a local homophile organization (males: n=38, median age=30 yr, min/max= 19/60; females: n=32, median age=26 yr, min/max=19/45). The criterion for inclusion was clear homosexual preference on two standardized sexual orientation questionnaires. The hand preference of each subject was classified as either consistent-right-preference (CRP) or non consistent-right-preference (NCRP) based on their responses to a 12-item hand-preference questionnaire (Annett, M., Br. J. Psychol., 61:303, 1970). In large studies of the general population, approximately 65% show CRP and 35% show NCRP, with no difference between males and females (Annett, 1970).

In the present study, the proportion of homosexual females showing NCRP (63%) was significantly greater than that in the general population ($z=3.32$, $p<.001$). The proportion of homosexual males showing NCRP (37%) was not significantly different than that in the general population ($z=0.26$). These results, buttressed with a review of the literature demonstrating (1) a higher incidence of NCRP and (2) increased homosexual behavior in groups of females exposed prenatally to abnormal levels of sex hormones (e.g., congenital adrenal hyperplasia, Nass, R. et al., Annals of Neurol., 18:416, 1985; diethylstilbestrol, Ehrhardt, A. et al., Arch. Sex. Beh., 14:57, 1985) lead us to hypothesize that female homosexuality may have an atypical neurobiological base related to increased prenatal levels of sex hormones. The results also suggest that homosexuality may be different in the sexes. Overall, such results raise the possibility of interrelations among the factors of brain lateralization, sexual orientation, hormone levels, medical disorders (e.g., autoimmune disorders) and cognitive ability (e.g., the higher incidence of left handedness among mathematically gifted individuals). Supported by NIH grant NS 18954 to SFW and by the Dept. of Psychiatry.

238.14 RELATIONSHIP OF POST-STROKE DEPRESSION TO PET SCAN ASYMMETRY IN CORTICAL SEROTONIN RECEPTORS. R.G. Robinson, H.S. Mayberg, D.F. Wong, R.M. Parikh*, P.L. Bolduc*, T.R. Price*, R.F. Dannals*, J.A. Links*, A.A. Wilson*, H.T. Rayvert*, H.N. Wagner,Jr. Dept. of Radiology Psychiatry and Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Stroke patients who had a single lesion of either the right or left hemisphere and no other neurologic or medical disorder were examined using C-11 N Methyl Spiperone positron emission tomography (PET). Patient with left (n=8) or right (n=9) hemisphere strokes were not significantly different in background demographic characteristics, medications taken at the time of PET scanning or time since stroke. In addition, the two groups were comparable in motor, sensory or visual symptoms and anterior-posterior lesion location or lesion volume. Four patients met diagnostic criteria for major depressive disorder but the frequency of major or minor depression and mean depression scores were not significantly different between groups. Spiperone binding was analysed in cortical, temporal and parietal regions on symmetrical brain areas excluding the lesion site. Patients with right hemisphere strokes had significantly greater ipsilateral to contralateral binding of spiperone than did patients with left hemisphere strokes or normals. This increased ipsilateral to contralateral binding ratio in patients with right hemisphere strokes was the result of increased binding in the right hemisphere as opposed to decreased binding in the left hemisphere. In addition, the ipsilateral to contralateral binding ratio in the temporal cortex was significantly negatively correlated with depression rating scales (eg. r=-.93 between negatively Zung Depression Score and PET binding) but not to either intellectual impairment or activities of daily living.

Based on previous study (Robinson et al. Brain, 187, 81 1984), patients with left frontal brain injury are significantly more likely to develop depression than patients with any other lesion location. Findings from the present study suggest that depression is most severe among patients who had the lowest amount of spiperone binding. The failure of the left hemisphere to increase serotonin S2 receptor binding may play a role in the development of depression following stroke and increased binding in the right hemisphere may protect against the development of post stroke depression.

238.15 VISUAL SELECTIVE ATTENTION TO MEANINGFUL TEXT: AN ANALYSIS OF EVENT-RELATED POTENTIALS A.C. Nobre and G. McCarthy VA Medical Center, West Haven, CT and Departments of Neurology and Psychology, Yale University, New Haven, CT 06516

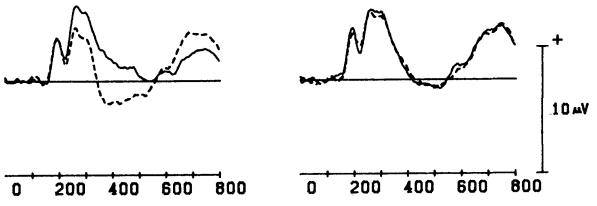
We report a study of focused and divided attention to visually presented stories using event-related potentials (ERPs) recorded from the scalp. The words comprising two stories were randomly intermixed and displayed one word at a time in the center of a computer display. Each word was presented for 50 msec and successive words were presented every 350–500 msec. The words from one story were displayed in red letters while the words from the other story were displayed in green letters. Subjects were asked to read silently either the green or red story (focused attention), or to read both stories (divided attention). Comprehension was tested immediately after each of the 9 runs.

ERPs elicited by each word was acquired from a 12-channel scalp montage referenced to a balanced sterno-vertebral electrode. Additional electrodes were used to record the vertical and horizontal electro-oculogram. The ERPs were sorted by story and attention condition.

ERPs (from Cz) elicited by words from the attended (solid) and unattended (dashed) stories from the focused attention condition are shown at bottom left. These ERPs represent the average of the 8 subjects tested. The ERPs to attended story words diverged from unattended words in a positive direction beginning at about 250 msec and lasting until 600 msec. This difference was related to attentional instruction only and was not affected by the specific color of the attended and unattended stories. The positive difference was largest at midline centro-parietal electrode sites and was larger over the left than right temporal scalp.

ERPs to red (solid) and green (dashed) stories from the divided attention condition are shown at bottom right. No differences were obtained when both stories were attended — ERPs to both colors were of approximate equal amplitude to the attended story of the focused condition. Our data suggest that differential neural processing of attended and unattended stories is evident by at least 250 msec. They further suggest that ERPs may provide a useful probe of attentional resources in word processing tasks.

Supported by the Veterans Administration and by NIMH Grant MH-05286.



238.16 AUTOMATIC SHIFTS OF AUDITORY ATTENTION ARE IMPAIRED BY PARIETAL/TEMPORAL LESIONS D. L. Woods, R. T. Knight, D. Scabini* and C. C. Clayworth* Clinical Electrophysiology Laboratory, Dept. of Neurology, U.C. Davis, VA Medical Center, Martinez, CA, 94553

We examined the effects of various non-target stimuli on reaction times to target tonebursts in a "cocktail party" experiment. Subjects selectively attended to one sequence of tonebursts while ignoring a similar sequence in the opposite ear. Stimuli were presented dichotically through headphones at a rapid rate (2.5-4/sec), with high pitched tonebursts (1300 Hz) in one ear and low pitched tonebursts (700 Hz) in the other. 90% of the stimuli were tonebursts of short duration (25 msec, "standards"), and 3% were novel sounds (computer-generated FM sweeps and digitized environmental sounds). The remaining 7% of the stimuli were "target" tonebursts identical to the standards except for duration (75 msec). The subjects' task was to respond to targets in the attended ear.

Reaction times (RTs) to targets averaged 789 msec. Although novel sounds rarely elicited false alarms, RTs to targets were markedly delayed when the target was immediately preceded by a novel sound (by 302 msec, $p < 0.001$). A greater delay was evident when targets were preceded by novels in the attended ear than when preceded by novels in the non-attended ear (347 vs 253 msec, $p < 0.001$). In comparison with targets that followed attended standards, RTs were delayed when targets followed standards in the non-attended ear (by 27 msec, $p < 0.05$). The results suggest that all auditory stimuli attract attention, but the attention-attracting properties of task-irrelevant stimuli can be inhibited when they are presented frequently.

In comparison with controls, reaction times were prolonged in a group of 16 patients with unilateral parietal-temporal lesions (by 80 msec, $p < 0.001$). RTs were comparable in subgroups of patients with left and right sided lesions, and there was a trend toward slower RTs contralateral to the lesion (lesion side x ear, $p < 0.09$). However, novel stimuli produced a smaller increase in RTs to subsequent targets in patients than in controls (237 vs 302 msec, $p < 0.005$). This suggests that the automatic attention-attracting properties of novel stimuli are reduced by parietal-temporal lesions. Supported by the VA Research Service and NIH Grant 21135 to RTK.

SPINAL CORD AND BRAINSTEM III

239.1 **DOUBLE LABELING OF RED NUCLEUS NEURONS FROM DYE INJECTIONS INTO THE INFERIOR OLIVARY NUCLEUS AND DORSO-LATERAL FUNICULUS OF THE SPINAL CORD IN RAT.** P.R. Kennedy. Biomedical Research Division, Georgia Tech Research Institute, Atlanta, GA 30332.

Despite previous negative findings (Brown et al, J. Comp. Neurol. 176:1, 1977; Carlton et al., Neuroscience Letters, 30:191, 1982; Rutherford et al., J. Comp. Neurol. 229:285, 1984), recent data suggested that a connection exists between the red nucleus (RN) and the inferior olivary nucleus (ION) in the rat (Kennedy, Soc Neuroscience Abstr., 12(1):353, 1986; Kennedy, Neuroscience Letters, 74:262, 1987). This connection was demonstrated using highly concentrated WGA-HRP injections adjacent to the ION that resulted in retrograde transport into neuronal somata where it was visualized as grains of reaction product. The present report extends this observation using Flouro-Gold (FG) dye instead of WGA-HRP. These injections were combined with True (or Fast) Blue dye placements in the Dorso-lateral Funiculus (DLF) of the spinal cord to label the Rubro-spinal Tract (RST) and assess the possibility that the rubro-olivary connection is a collateral of the RST.

In 6 Long Evans rats, the pyramidal tract adjacent to ION was injected with 1.25% FG in volumes of typically 10 to 20 nanoliters. With higher concentrations (2.5, 5 and 10%) of FG in 7 rats, toxic effects on the respiratory center led to gradual onset of fatal apnea within about an hour of injection. After 4 to 7 days survival, perfusion with normal saline and 10% formalin was followed by frozen sectioning at 40 microns, air drying, Xylene immersion and cover slipping with DPX. Visualization with epi-flourescent light at UV wavelengths revealed grains of FG throughout the cytoplasm of RN neurons best seen after the longer survival times. Numerous labeled neurons were seen through the full rostro-caudal extent of RN, similar in number and distribution to the previously reported WGA-HRP labeled neurons (Kennedy, Neuroscience Letters, 74:262, 1987), thus confirming the connection between RN and ION.

Three to 5 days after recovery from the ION injection in three rats, True Blue (TB) or Fast Blue (FB) was soaked in gelfoam plegets and placed against the cut ends of RST axons within the transected DLF of the spinal cord at the C3 to C4 segmental level. Very few neurons were labeled with TB or FB alone: Virtually all were doubly labeled with FG. As expected from known somatotopy, neurons dorsally and rostrally were singly labeled with FG while the doubly labeled neurons were seen ventrally and caudally and extended as far rostral as the fasciculus retroflexus.

This suggests that in the rat, RN neurons have both rubro-spinal and rubro-olivary outputs. It is likely that the rubro-olivary output is a collateral of the rubro-spinal tract.

239.2 CODING OF TARGET AND RESPONSE VARIABLES IN CAT RED NUCLEUS. J.H.Martin, E.Sybirska*, J.Brennan*, and C.Ghez. Ctr. for Neurobiol.& Behav., Columbia Univ. and NYS Psych. Inst., New York, NY 10032

Reversible inactivation of the red nucleus (RN) and the corticospinal tract prolongs reaction time and impairs trajectory control (Neurosci. Abst. 12,1986). The present study examines the role of the RN in the initiation and execution of rapid limb responses by recording the activity of single neurons during performance of a series of skilled tracking tasks in cats.

Animals were trained to make adjustments in isometric forearm force or position to match shifts in a target. A feeder moving to the right or the left displayed force or position errors. Shifts of opposite direction evoked forelimb extension and flexion, as well as right and left neck responses toward the moving display. To dissociate the coding of target stimulus and response variables in task-related neuronal activity, forelimb responses of a given direction were evoked by stimuli in either of the two directions by inverting display polarity. To dissociate the coding of forearm and neck responses, animals were trained to make adjustments in neck torque applied to the head fixation frame in isolation.

Task-related neurons were recorded from forelimb regions of RN (threshold microstimulation effects in contralateral forelimb muscles at currents <15 uA) in two cats. Neurons had receptive fields on the contralateral forelimb and some also showed convergence of afferent input from other body sites. RN neurons whose activity was modulated in advance of a change in forelimb force (lead cells, N=57) were the focus of this study, however, cells whose activity lagged the response were also observed. Lead cells were modulated during spontaneous reaching movements. The activity of some lead cells was better synchronized with the onset of the forearm response, whereas others were better synchronized with the stimulus. Three classes of lead cells were identified. The first (33%, n=14) showed reciprocal changes in activity correlated with a single direction of forearm force production. These cells were better timed to the response than to the stimulus and could participate in specifying response direction. The activity of the second class (18%, n=8) showed a consistent relation to changes in neck torque, either in isolation or associated with forearm responses and this activity was better timed to the stimulus. These cells may serve to coordinate synergic coupling between distal and proximal muscles during task performance. The third class (49%, n=21) had non-reciprocal discharge patterns which varied in the different task conditions. Their activity could be timed to the stimulus in one condition but to the response in another, or modulated before responses of one direction while lagging in another. The function of these more complex lead cells remains unclear.

Motor cortex lead cells specifically related to the direction of forearm force production are timed to the stimulus (Exp Brain Res 57, 1985). The more consistent lead time of RN neurons related to forearm force suggests that the red nucleus may have a more direct role in response initiation than motor cortex. (Supported by NS 19205)

239.3 RED NUCLEUS IN THE NON-HUMAN PRIMATE: THE FINE STRUCTURE OF PROJECTIONS FROM THE DEEP CEREBELLAR NUCLEI DEMONSTRATED BY THE AXON TRANSPORT OF WHEAT GERM AGGLUTININ-HORSERADISH PEROXIDASE.

D.D. Ralston, A.M. Milroy* and H.J. Ralston, III. The Department of Anatomy, University of California, San Francisco, California 94143.

The red nucleus of the non-human primate, located in the midbrain, is divided into a rostral 2/3 composed of small (10-15u) and medium (20-30u) sized cells, and a caudal 1/3 composed of small (10-15u), medium (20-30u) and large (40-90u) sized cells. These two anatomical components are set apart, not only for the morphological differences at the light and electron microscopic level, ie. cell size and distribution, but also for the division of labor between the two regions of the nucleus, as well as the afferent and efferent projections to each region.

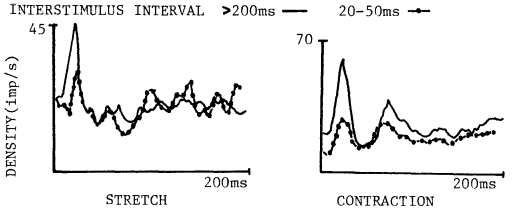
The fine structure of the synaptic populations within the magnocellularis and the parvocellularis portions of the nucleus have been described and categorized into several groups in a previous study (Ralston, et al; Neurosci: Abstr. 12: 418.2, 1986). The present study attempts to define the morphological nature of the synapses which mediate excitatory input from the deep cerebellar nuclei to both the parvocellularis and magnocellularis regions of the nucleus. Given the data derived from physiological experiments which have demonstrated that the input from the cerebellum is a powerful one and exerts a major influence on the rubral neurons, it is anticipated that the cerebellar input would be composed of the large round pale population of terminals, because of their synaptic input to the soma and proximal dendrites.

Macaque fascicularis were used in this study to determine the nature of the afferents. The animals were immobilized with ketamine hydrochloride (10mg./kg.) I.M. and anaesthetized with I.V. pentobarbital (28 mg./kg.). All surgical procedures were carried out using sterile technique. Wheat germ agglutinin-horseradish peroxidase was injected into selected regions of the deep cerebellar nuclei using stereotaxic coordinates. After 3-4 days survival time the monkey was reanaesthetized and perfused intracardially with an aldehyde solution. Serial vibratome sections of midbrain were processed for LM using TMB as a chromogen (Mesulam) and for EM using the ammonium molybdate technique of Olucha, 1985, and the slow osmication procedure of Henry, 1985. Results demonstrate that the terminals of the deep cerebellar nuclei constitute a portion of the population of the pale round vesicle terminals ending on cell bodies and proximal dendrites. The majority of the labeled structures are composed of myelinated axons suggesting that some of the labeled terminals may be collaterals of myelinated fibers en route to the thalamus, as these axons are known to course through the red nucleus. There were no other labeled terminal profiles observed within the nucleus. (Supported by NS-23347 from N.I.H.)

239.4 THE EFFECT OF TEMPORAL PATTERNS OF NATURAL STIMULATION ON DSCT ACTIVITY IN CAT.

C.E.Osborn, R.E.Poppele and Liming Shen*. Lab. of Neurophysiol.,Dept. of Physiol.,U. of Minnesota, Minneapolis, MN

DSCT units are preferentially activated by stimulation of muscle nerves when the interval between stimuli is either $<$ 20ms or $>$ 60ms (Osborn and Poppele, Neurosci. Abs. 12:249, 1986). We have extended these investigations to include the more natural stimuli of muscle stretch and contraction. Spike activity of 211 DSCT units was recorded during randomly applied stretches or contractions of Gastrocnemius-soleus (GS). Single stretches ($<$1mm) reached a peak in 40 ms; single contractions were either maximal twitches elicited by direct stimulation of cut ventral roots, or were produced through the crossed-extensor reflex. Regardless of the type of stimulus used, the probability that a stimulus produced an excitatory response depended on the time since the preceding stimulus (see figure below). Interstimulus intervals of 20-50 ms (dotted line) were much less effective in producing a spike than either longer (solid line) or shorter intervals (not shown). The effect of interstimulus interval was therefore similar to that observed during direct stimulation of afferent fibers. However, the underlying mechanisms may not be the same. Our preliminary experiments on muscle spindles in GS suggest that long stimulus intervals produce a more synchronous and therefore more potent afferent fiber discharge to the DSCT than do intermediate stimulus intervals. Using electrical stimulation of nerves, however, each afferent fiber volley is synchronous and of equal size, and the sensitivity of DSCT units to stimulus intervals emerges from properties intrinsic to the cells and the interneuronal circuitry. Thus, the ability of DSCT to encode temporal patterns in natural stimuli may be a cascaded effect of stimulus history on afferent fiber discharge and the behavior of the intrinsic spinal cord circuitry. Supported by NSF:BNS 85-18714 and NIH:NS 21143.



239.5 **ELECTROPHYSIOLOGICAL PROPERTIES OF GUINEA-PIG FACIAL MOTONEURONS:A COMBINED STUDY IN THE ACUTE PREPARATION AND THE IN VITRO WHOLE BRAIN.**

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Cranial motoneurons innervating somatic musculature have not yet been studied as far as their ionic conductances are concerned . In order to do so we considered facial motoneurons , in an isolated whole brain,to be an adequate preparation . A prerequisite for that work , was the comparison of their membrane properties in vitro and in vivo (acute preparation) .

Electrical stimulation of either the facial nerve trunk(in vitro) or its branches (in vivo) evoked similar triphasic field potentials , although conduction velocity was slower in vitro . Intracellular recordings indicated that antidromically elicited action potentials were comparable in both preparations as far as amplitude , rise time and duration were concerned.When present , depolarizing and hyperpolarizing after-potentials were of the same order of magnitude . As already observed in other in vitro preparations,membrane input resistance was consistantly higher in vitro than in vivo . Membrane time constants were similar . In view of these results it was concluded that facial motoneurons in the isolated whole brain were suitable for pharmacological experiments.

Using ionic channel blockers(TTX,TEA,4-AP,Cs++, Co++ and Cd++) it was found that FN neurons exhibited the usual fast Na and K conductances. Our experiments suggest the additional presence of an anomalous rectification , a calcium dependant potassium conductance and more interestingly, high threshold calcium spikes of presumed dendritic origin. Moreover in presence of TEA we were able to reveal the existence of fast rising spiklets , which were presumably dendritic sodium spikes.

In conclusion the facial motoneurons appeared to be equiped with a set of conductances which make them capable of actively processing the incoming flow of afferent informations . Understanding the roles of these conductances will require the study of facial motoneurons in the alert preparation. (Supported by Swiss NSF no 3.288-0.85)

239.6 **ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF RETICULAR NEURONS IN NUCLEUS GIGANTOCELLULARIS : A STUDY IN BRAINSTEM SLICES AND ISOLATED WHOLE BRAIN OF GUINEA-PIG.**

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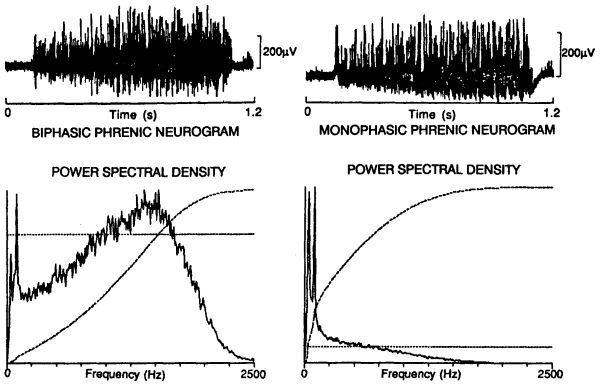
Ponto-medullary reticular neurons belong to networks subserving very different functions. Whether such heterogeneity is reflected at the level of the ionic conductances of these neurons is yet unknown. As a first step we have recorded intracellularly from a restricted pool of cells located above the inferior olive and known to contain a large number of reticulo-spinal neurons. Membrane properties and pharmacology allowed to divide the neurons in three classes.

Neurons of the first class were characterized by a thin (M±SEM:.29 ± .02 ms,n=15) action potential (AP) followed by an early fast and a delayed slower after-hyperpolarization (AHP). Moreover these cells displayed strong anomalous rectifications (AR) and rebound responses. Application of TTX in conjunction with potassium channel blockers (TEA and 4-AP) revealed both high threshold calcium spikes (Ca-HTS) as well as large and prolonged calcium plateau potentials. Neurons of the second class had wider AP (.46 ± .03 ms,n=31) followed by a single AHP. In presence of ionic channel blockers they displayed only Ca-HTS. Although more or less prevalent from one cell to another, rebound responses, AR and a delayed return to baseline following hyperpolarizing pulses were found in these neurons. This latter response (possibly due to an A-current) was large and prolonged in a minority of cells (up to 600 ms). Neurons of the third class were characterized by a broad AP having a pronounced shoulder on its repolarizing phase. In presence of TTX, the major part of the AP could be ascribed to a calcium spike probably initiated at somatic level. The shape of the AP and the slow oscillatory behaviour at rest were reminiscent of raphe neurons.

In conclusion, it remains to be determined whether one or more of these cell groups represent reticulospinal neurons. At present this can only be tested in vivo with antidromic stimulation from the spinal cord. (Supported by Swiss NSF no 3.288-0.85)

239.7 **MONOPHASIC NEUROGRAM REQUIRED FOR DETECTION OF HIGH-FREQUENCY SYNCHRONY (*HFS*) IN INSPIRATORY ACTIVITY.** *Charles A. Richardson.* Cardiovascular Research Institute, Departments of Anesthesia and Physiology, University of California, San Francisco, CA 94143-0542

Although high-frequency synchrony (*HFS*) in the phrenic neurogram of cats is a common phenomenon first reported in 1912 and observed by many investigators since, some researchers have reported low or variable incidence of *HFS* and discounted its importance in central respiratory pattern generation. To investigate the possibility that nerve recording technique might play a role in the low incidence of detectable *HFS* in some experiments, I recorded the biphasic and monophasic neurogram simultaneously in the right phrenic nerve in four decerebrate, paralyzed, artificially ventilated cats. About 2-3 cm of the right phrenic nerve was desheathed. A proximal bipolar electrode yielded the biphasic signal; a distal bipolar electrode with the nerve crushed between its pair of wires yielded the monophasic signal. In the example below, each power spectral density plot is normalized to its maximum spectral line, that of the monophasic signal being 8.9 times greater than that of the biphasic signal. In every case, I found that action potentials summed and **interfered** with each other at the biphasic electrode resulting in a broad band of noise with only 7% of the power below 200Hz. At the monophasic electrode, action potentials summed and **reinforced** at the frequency of *HFS* resulting in sharp spectral peaks with 52% of the power below 200Hz. The average fraction of total power in a frequency band 20Hz wide, centered at the frequency of *HFS* was only 2.5% in the biphasic signals, compared to 14.1% in the monophasic signals. Clearly, any signal processing technique designed to detect *HFS* in the phrenic neurogram is at a marked disadvantage if applied to the biphasic neurogram instead of the monophasic neurogram. (Supported by NIH HL-26176)



239.8 Specific responses to a passive movement in the cerebellar and olivary projecting neurons in the main cuneatus nucleus (MCN) in cats.

Domich.L,F.J.Rubia* and R.Alvarez-Caceres.* Centro Ramón y Cajal. Ctra.del Colmenar Km 9.Madrid.28034.Spain.

It is well accepted that two afferent systems to the Purkinje cell,the mossy and climbing fibers have an interdependent behaviour with a parallel or reciprocal activity and that both systems transmit an accurate information about the dynamic and statics parameters of the movement.One would expect that this precise and complex information is already present at a precerebellar level.

In cats lightly anesthetized with Nembutal,we have analized the responses of the cells of the main cuneatus nucleus provoked by a passive movement of the paw at wrist level.A ramp at 40°/s and the displacement angle between +20° and -20° according to the horizontal plane was used as stimulus.

Two populations of cells were recorded,those of cerebellar projection located in the rostrolateral part of the MCN and the cells projecting to the inferior olive situated on the basal-caudal part of the MCN.Specific responses to dynamic and static parameters are observed within both populations.

In agreement with our expectations,similar responses to those observed at the cerebellar level have been detected.Three types of responses are present:type A a phasic discharge at the onset and the offset of the ramp,type B a phasic -tonic discharge appear at the onset and during the steady high position and type C similar to type B but inversed phase.The proportion of each type of response (A:47%,B:34% and C:19%)is very closed to the values reported at cerebellar level at least for the cuneocerebellar cells,because for the cuneolivary projecting neurons a striking difference is observed.The majority of these cells exibit a type A response,86% over 47% of the cerebellar projecting cells.

The results show that at this precerebellar level there exist almost the same specific responses to the parameters of the passive movement suggesting that the information is processed at this site. The significant quantitative differences between the cells of cuneocerebellar projection which represent one of the mossy fibers sources and the cells projecting to the inferior olive and indirectly related to the climbing fibers would suggest a different role about the specific information coming out from both neuronal populations.

239.9 CHARACTERISTICS OF MEDULLARY NEURONS THAT PROJECT TO THE FACIAL OR TRIGEMINAL MOTOR NUCLEI IN THE RAT.

L. Evey, J. Travers,*and R. Norgren. Dept. Behavioral Science, College of Medicine, Pennsylvania State Univ., Hershey, PA 17033, and Dept. of Oral Biology, School of Dentistry,*Ohio State Univ., Columbus, OH 43210

Anatomical evidence indicates that interneurons that synapse in either the facial (VII) or trigeminal motor (mV) nuclei are scattered in the medullary reticular formation (mRF). Axons arising from the nucleus of the solitary tract (NST) pass through this area of mRF providing a potential basis for sensorimotor integration of ingestion and rejection (Grill, & Norgren, Br. Res., 143:281-297, 1978). Rats were prepared with two chronically implanted bipolar stimulating electrodes, one positioned in VII and the other in contralateral mV. After a week of recovery, animals were reanesthetized with a combination of barbiturate and chloralose or urethane and secured in an atraumatic head mount with the nose tilted down. Microelectrode penetrations were made from 1.5 mm rostral to 1.0 mm caudal to obex and from 2.0 mm lateral toward the midline. Search stimuli (0.1 ms, 150 uA, 1 Hz) were applied concurrently to mV and VII. Selected recording sites and stimulus electrode locations were marked with microlesions. Due to a lack of spontaneous activity, the criterion for antidromic invasion was that elicited action potentials must follow each of five stimulus pulses at 500 Hz. Antidromically driven units were tested for responses to jaw stretch, light touch, and pressure (pinch with forceps) applied to oral-facial regions. Taste responses were checked by washing midrange concentrations of NaCl, sucrose, quinine, and HCl over the anterior tongue. A total of 32 antidromically driven neurons, representing approximately 5 % of all units isolated in the mRF, were recorded from 8 rats during 116 electrode penetrations. Antidromic thresholds ranged from 20 to 150 uA. There were 23 neurons driven from VII. Of these, 11 were activated from the contralateral nucleus (mean latency = 1.0 ms, Sd = .46) and 12 from the ipsilateral nucleus (mean latency = 0.7 ms, Sd = .28). Eight neurons were driven from mV, all from the contralateral nucleus (mean latency = 0.8 ms, Sd = .38). Spontaneous activity was never present and, with one exception, no responses occurred to somatosensory or taste stimulation. One neuron antidromically driven from mV responded to light touch of the gingiva near the ipsilateral lower incisor. Based upon background neural activity, antidromically driven cells were located near neurons that responded to pinching of the tongue, lips, or ears, or to neurons that were synchronized with respiratory rhythms. Gustatory responses were not observed in any cells tested. Neurons were distributed ventrolateral to the hypoglossal nucleus in the mRF between the roots of the hypoglossal nerve medially, and the efferent vagal fascicles, laterally. These findings are consistent with previous experiments using neuroanatomical tracers. Supported by NIH NS07686, NS20397, NS24889, and NIMH Research Scientist Award, MH00653, to R.N.

239.10 DISTRIBUTION OF HIGH AND LOW FREQUENCY OSCILLATIONS (HFO, LFO) AMONG PHRENIC (PHR) MOTONEURONS AND DORSAL MEDULLARY INSPIRATORY (I) NEURONS OF NEWBORN PIGS.

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To obtain information about the development, distribution and origin of central I activity in newborn pigs, we have compared: a) auto power spectra (APS) of PHR discharges in pigs at two different ages (4-6 d and 10-15 d); b) APS of discharges in different PHR nerve roots in 4-6 d pigs; c) APS of PHR nerve and dorsal medullary I neuron discharges in 5-15 d pigs. Pigs were anesthetized with Saffan, paralyzed with decamethonium bromide and artificially ventilated with 100% O_2. End-tidal CO_2, rectal temperature and abdominal aortic blood pressure were within normal limits. Signals were processed as follows: a) PHR signals were successively high pass filtered (10 Hz), low pass filtered (200 Hz) and acquired at 512 Hz; b) for neuronal activity, each action potential in a spike train was converted to a 100 microsec pulse, low pass filtered (200 Hz) and acquired along with PHR signal. APS were estimated with a Fast Fourier Transform routine synchronized to the onset of I; final APS were obtained by averaging of spectral estimates in each I phase and included at least 30 central I phases. Examination of PHR spectra in younger pigs showed that major peaks occurred with equal likelihood in 90-150 Hz band (HFO) and in 20-50 Hz band (LFO). However, PHR spectra of older pigs had major peaks only in HFO band, which is similar to PHR spectra of cats (this confirms the results of others). Comparison of APS in PHR roots showed that peak power was in HFO band in some roots and in LFO band in other roots. Squared coherence estimates between PHR roots were usually >0.5. For I neurons, there was good correspondence between discharge pattern and location of major spectral peaks; neurons with augmenting patterns had peaks in HFO band, those with decrementing patterns had peaks in LFO band. Squared coherence estimates between neurons and PHR were usually <0.2. In conclusion, these data suggest that the rate of maturation in PHR motoneuron pools is uneven, some have mature HFO in their APS, other APS have immature LFO. This difference is also observed in APS of I neurons, which are probably part of the neural network for I pattern generation. (ALS and DFD are Parker B. Francis Fellows. This work was supported by a grant from Long Island Jewish Medical Center, LIJMC #3-818.)

239.11 THE FINAL COMMON PATHWAY IN VOCALIZATION IN THE CAT
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The neuronal framework of vocalization in mammals has been intensively investigated, but their precize pathways have not been elucidated. Vocalization is a coordinated pattern of motor activities of 1: abdominal, 2: laryngeal, 3: pharyngeal, 4: peri-oral, 5: mouth opening and 6: tongue musculature. These muscles are respectively innervated by 1: motoneurons located in the ventral horn of the mid- and low thoracic and upper lumbar spinal cord, 2: the caudal half of the nucleus ambiguus, 3: the dorsal group of the nucleus ambiguus, 4: the ventrolateral facial subnucleus, 5: the ventro-medial motor trigeminal nucleus and 6: the hypoglossal nucleus. Vocalization can be elicited by stimulation in different parts of the limbic system, but most easily in the periaqueductal grey (PAG). However, direct projections to vocalization involved motoneurons have not been demonstrated, which suggests that interneurons play an essential role. A specific interneuronal cell group projecting to all the vocalization involved motoneuronal cell groups has not been found. In this report we will demonstrate that the nucleus retro-ambiguus (NRA) in the lateral part of the caudal medulla projects to the vocalization motoneurons and that the NRA receives strong afferent projections from the PAG.
In 6 cases 0,5µL containing 50µCi ^{3}H-leucine was injected in the caudal brain stem and in 15 cases in the PAG. In the 6 caudal brain stem injected cases labeled fibers crossed the midline just caudal to the obex and descended in the ventral funiculus of the contralateral half of the spinal cord. Labeled fibers were distributed to the abdominal muscle motoneuronal cell group and in the more rostrally located injections also to the phrenic nucleus. These projections were bilateral but with a contralateral preponderance. HRP-injections at the T1 level of the spinal cord indicated that these contralaterally descending fibers were derived from neurons in the NRA.
Ascending fibers were distributed to the nucleus ambiguus, the ventrolateral facial subnucleus and the ventromedial motor trigeminal nucleus. All these projections were mainly contralateral.
In the cases with ^{3}H-leucine injections in the PAG, labeled fibers descended laterally through the caudal mesencephalic and pontine reticular formation and medially through the medullary reticular formation. Many fibers terminated diffusely in these same areas including the nucleus raphe magnus and in some cases the nucleus raphe pallidus. However, especially in those cases in which the ventrolateral part of the caudal PAG was involved in the injection site, a very specific distribution of labeled fibers was observed in the NRA. No labeled fibers were found in the motoneuronal cell groups, with the exception of the dorsal vagal nucleus. These results suggest that the NRA plays a vital role in the neuronal framework of vocalization.
Supported by a grant of NASA/Ames Research Center CA to G.H..

239.12 **EFFECTS OF PAG STIMULATION ON LARYNGEAL EMG AND VOCALIZATION IN THE AWAKE MONKEY.**
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Previous studies have demonstrated that single PAG neurons change their firing rates prior to vocalization in monkeys trained to vocalize. Vocalization-related neurons were located in the dorsolateral and ventrolateral PAG. Clear functional relations were not demonstrated for many neurons, which prompted the present study.

Three macaque monkeys were reinforced for sitting quietly in a restraining chair. Monkeys were surgically prepared for recording from laryngeal muscles with chronically implanted EMG electrodes, and a recording chamber was attached to the skull. A tungsten microelectrode was used for microstimulation of the PAG. The PAG was explored by moving the electrode vertically in steps of 0.5 mm. Each day the electrode was moved horizontally in steps of 0.5 mm by means of an X-Y stage on the recording chamber. Twenty trains of stimuli (20 ms train, 0.1 ms biphasic pulses, 200 pps, 20-150 µA) were delivered at each location and rectified EMGs were averaged for the 20 trains. Trains of pulses (200 pps) lasting 500 ms also were delivered to elicit vocalization. Vocal and EMG signals were recorded on magnetic tape for later analysis.

Short-train stimulation typically excited thyroarytenoid (TA) and cricothyroid (CT) muscles and suppressed posterior cricoarytenoid (PCA) muscles. The latencies from onset of the train to beginning of the responses was 12-20 ms, and durations of responses were 20-30 ms. Responses could be elicited from widespread stimulation in the PAG, but most were obtained from ear bar zero anterior 3 mm. Most responses were obtained by stimulation of the dorsal anterior PAG.

Long-train stimulation only elicited vocalization from areas in which laryngeal muscles were also excited by stimulation. Clear calls such as "coos" were elicited predominantly from the dorsal anterior PAG, whereas, rough sounding calls such as "barks" were elicited more posteriorly. During each type of vocalization the elicited muscle patterns were similar to the short-train stimulation patterns. During the barks, the CT and TA muscles gave bursts of activity that corresponded to bursts of sound.

These data indicate a rough organization of the PAG. The dorsal anterior PAG seems more related to the laryngeal system than other areas. Analysis of the patterns of EMG responses suggests that the PAG may be organized into small groups of cells, which when stimulated, produce coordinated muscle responses. That different types of vocalizations are elicited from different locations, suggests the PAG may also be organized on the basis of qualitative aspects of vocalization. The vocalizations we elicited by stimulation in different regions may be related to a variety of other behaviors, e.g. aggression, that are also elicited by PAG stimulation.

239.13 BRAINSTEM GENERATION OF ACTION ORDER AFTER DESCENDING DECEREBRATION
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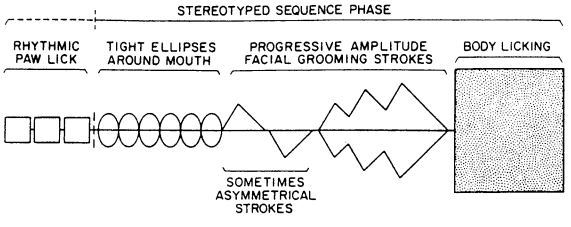
Descending levels of decerebration were employed in rats to examine the capacity of the caudal brainstem to order actions into complex sequences. We have described 'action syntax' rules that order acts into predictable sequences (Berridge, Fentress, & Parr, Behav. Brain. Res., 23:59, 1987). The focus of this study was upon a chaining rule that generates highly stereotyped and recognizable sequences from combinations of up to 15 coordinated actions. We reported last year that this rule can be generated without somatosensory feedback. We also reported that chain completion is partially disrupted by lesions of the neostriatum & globus pallidus.

The ability of various chronic decerebrate preparations to produce sequentially ordered grooming chains in response to fur wetting was examined after complete spatula transection either rostral to the midbrain (MESENCEPHALIC Decerebrate), or at the midbrain/pons junction (METENCEPHALIC Decerebrate), or at the pons/medulla junction (MYELENCEPHALIC Decerebrate). Behavioral testing began 10 days after decerebration.

Complete and structurally coordinated chains were produced by mesencephalic decerebrates, although not as frequently as by intact rats. Complete but prolonged and partially degraded chains were occasionally produced by metencephalic decerebrates. No perfect chains were produced by myelencephalic decerebrates. However, extremely degraded chain 'skeletons' were sometimes generated by myelencephalic rats.

These results suggest that a) Rostral brain (e.g., striatal) lesions that impair sequential chaining may do so by disrupting syntax implementation rather than generation per se, b) the basic generation of this chaining rule can be carried out by brainstem circuitry, and c) this sequencing circuit may not be localized at a particular level within the brainstem, but rather is distributed as a degenerate and partially redundant network that is only reduced incrementally by descending transections.



240.1 FLUORESCENCE ACTIVATED CELL SORTER ANALYSIS OF INCREASED DIHYDROFOLATE REDUCTASE ENZYME LEVELS INDUCED BY METHOTREXATE IN PRIMARY CULTURES DERIVED FROM MOUSE BRAIN. E.E. Serrano* and R.T. Schimke*. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305. (SPON: C. Gundersen)

Methotrexate (MTX) is a 4-amino analog of folic acid and a commonly used antineoplastic drug which acts by competitive inhibition of the enzyme dihydrofolate reductase (DHFR). Stepwise selection of cultured mouse, hamster and human cell lines with MTX has been shown to result in the emergence of resistant populations of cells. MTX resistance can be imparted by a variety of mechanisms such as altered MTX transport, reduced DHFR affinity for MTX or increased levels of DHFR. The resistance conferred by overproduction of DHFR is a consequence of amplification of the DHFR gene and has been well documented in a variety somatic cell lines (Schimke, Cell 37: 705-713 1984).

In order to examine the effects of MTX treatment on cells whose origin approximated the in vivo condition more closely, we chose to develop a primary culture system using the brains from 16-18 day old Swiss Webster mouse embryos. Following enzymatic and mechanical dissociation, cells were plated on T25 flasks at a concentration of 1.5 X 10^6 cells/flask in control medium and allowed to grow for a week. The first passage eliminated neurons and left a glial culture comprised primarily of astrocytes which could be stained with GFAP. Healthy dividing cultures could be easily maintained up to five months. At the first passage cells were divided into control and MTX groups and subjected to a two or three step selection procedure where cells were split and placed in higher concentrations of MTX at each passage. Cultures received nanomolar concentrations of MTX over a three to four month period.

The Fluorescence Activated Cell Sorter (FACS) at the Shared Cell Sorter Facility at Stanford was used to analyze the characteristics of the cell population following MTX treatment. DHFR levels were assayed with fluoresceinated MTX (F-MTX). Cultures were examined on the FACS with regard to: a)autofluorescence b)viability (propidium iodide) c)size (forward scatter) d)cell division (Hoechst) e)DHFR (F-MTX).

Cultures exposed to stepwise selection of 30/60/90 or 60/120 nM MTX showed a significant two to three fold increase in fluorescence (and hence DHFR levels) relative to controls. When DHFR from the 30/60/90 culture was probed with ^{32}P labelled DHFR cDNA and mouse alpha fetoprotein cDNA using the slot hybridization technique, there was a twofold increase in gene copy number.

Thus, while the magnitude of the response in the primary cultures was smaller, our findings were consistent with the results obtained with somatic cell lines.

(Supported by a Ford Foundation Postdoctoral Fellowship to E.E. Serrano)

240.2 STRUCTURAL ANALYSIS AND ISOLATION OF THE HUMAN S-ANTIGEN GENE. C.M. Craft, and T. Shinohara*. Dept. of Psychiatry, University of Texas Health Science Center at Dallas, Dallas, TX 75235, LDN, NICHD, and LMDB, NEI, NIH, Bethesda, MD 20892.

S-Antigen (48K protein) is a tissue-specific, soluble protein in the retina and pineal gland. In visual transduction, S-antigen binds to photolyzed, phosphorylated rhodopsin in retinal rods. This binding prevents rhodopsin from interacting with transducin and turns off the photon induced signal. The functional role of pineal S-antigen in neural transduction is not known. In disease, this highly antigenic protein induces an inflammatory response in retinas and pineal glands (experimental autoimmune uveitus). To better understand the physiological function of S-antigen in normal and disease states, we first analyzed the molecular structure of this protein.

We have previously characterized bovine retinal S-antigen cDNAs, the primary and secondary structure, immunogenicity, and its mRNA (Craft et al., 1985, Society for Neurosci., 339.2; Wistow et al., 1986, FEBS, 196:23-28). We now present the gene analysis of bovine and human genomic DNA. Total genomic DNA was digested with restriction enzymes, electrophoresed, blotted and hybridized with bovine S-antigen cDNA probes (BSC-440 and BSC-880). The results suggest a single gene per haploid genome and indicate that the S-antigen of retina and pineal gland is the same gene product. However, from other experiments we found S-antigen from bovine retina and pineal gland show different isoelectic points and glycosylation patterns, which suggest alternate post-translational modification.

We constructed a lambda EMBL human genomic library of DNA enriched 50-fold for human S-antigen gene fragments isolated by large scale electrophoresis. We isolated 8 clones containing a 9.4 kilobase fragment from this library. Cross-hybridizations of Southern blots with a bovine S-antigen cDNA (BSC-880) probe verified the 9.4 kilobase fragment was analogous to the human S-antigen genomic fragment. This fragment was subcloned into plasmid vectors for restriction analysis and M13 for dideoxy sequencing. Deduced polypeptide sequence of the human S-antigen from DNA sequence revealed extensive homology with the bovine sequence. Although the 5' end of the S-antigen gene is absent in this fragment, a 3' exon of this human gene with a 90% homology to the bovine cDNA sequence and a 100% homology to a bovine tryptic peptide sequence was verified. Splice junctions were highly conserved and long stretches of introns surrounded this exon. Currently, we are isolating the 5' end of the gene from a human cosmid library and determining the human chromosome localization of S-antigen with CHO/human chromosome hybrids.

240.3 ISOLATION AND CHARACTERIZATION OF THE HUMAN TYROSINE HYDROXYLASE GENE. K.L. O'Malley,* L. Vinnedge, B.M. Martin, J.R. Kelsoe,* A.L. Winfield and E.I. Ginns. Molecular Neurogenetics Unit, NIMH, and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

We are studying the structural organization and regulation of the human tyrosine hydroxylase (TH) gene in order to investigate whether mutations in this gene occur in bipolar affective illness or other neuropsychiatric disorders. A human brain genomic DNA library constructed in EMBL3 was screened using a full length rat tyrosine hydroxylase cDNA as a probe (Brown et al., Biochem., in press). Out of one million recombinant phage we identified one clone which hybridized to probes derived from the 5' and 3' ends of the rat cDNA. Using restriction endonuclease mapping, Southern blotting and DNA sequencing, we have determined the organization of the human tyrosine hydroxylase gene. Like its rodent counterpart, the human gene is single-copy and spans approximately 8 kb. Comparison of the rat and human gene 5' flanking regions suggests that these sequences may be conserved because of their importance for transcriptional regulation. A cAMP enhancer element is located upstream of the transcriptional start sites in both the rat and human genes. However, unlike the rat gene, the human gene undergoes alternative splicing to generate several exon 1 sequences (see abstract E.I. Ginns et al.). Evidence will be presented that the proteins encoded using these alternative splice sites can be expressed.

It has been previously shown that tyrosine hydroxylase and phenylalanine hydroxylase (PH) evolved from a common ancestral gene. The human PH gene spans over 90 kb with introns as large as 23 kb (DiLella et al., Biochem. 25: 749, 1986). Our characterization of both rat and human tyrosine hydroxylase genes demonstrates that this size difference between TH and PH is primarily a result of changes in intervening sequences. Major changes in noncoding regions have occurred since the divergence of these genes.

240.4 Cloning and Expression of Multiple cDNA's For Human Tyrosine Hydroxylase. E.I. Ginns*, J.R. Kelsoe, B.M. Martin*, S.L. Winfield*, M.E. LaMarca*, M.D. Luu*, S.M. Paul* and K.L. O'Malley [2], (Spon: F.K. Goodwin) Molecular Neurogenetics Unit, Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892, [2]Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO.

In order to study the possible involvement of tyrosine hydroxylase (EC 1.14.16.2) in the pathogenesis of bipolar affective illness and other neuropsychiatric disorders, cDNA clones for human tyrosine hydroxylase have been isolated from a lambda GT10 library constructed from a neuroblastoma. Four clones were isolated by hybridization using a unique 5' fragment from the human tyrosine hydroxylase gene (see abstract O'Malley et al.). These cDNA clones contained the full coding region as well as both 5' and 3' untranslated sequences. In contrast to the previously reported rodent tyrosine hydroxylase cDNAs, the human cDNAs were heterogeneous in their 5' coding sequences. These differences correspond to alternative splice sites observed by us in the human tyrosine hydroxylase gene (see abstract O'Malley et al.).

In order to explore the significance of these multiple forms of tyrosine hydroxylase, baculovirus expression vectors (Summers and Smith, 1986) containing tyrosine hydroxylase cDNAs having the different human 5' sequences are being used to produce enzymatically active protein. The availability of large quantities of the different forms of purified tyrosine hydroxylase will permit comparative kinetic and structural analyses, as well as production of specific antiserum for immunocytochemical and pulse-chase studies. These approaches should clarify the physiologic significance of the various forms of tyrosine hydroxylase, including possible differences in their subcellular distribution (bound versus soluble), cofactor activation, and phosphorylation.

240.5 SEQUENCE COMPARISON OF BOVINE ADRENAL PHENYLETHANOLAMINE N-METHYLTRANSFERASE AND ITS GENOMIC DNA CLONE E.P. Weisberg*, D.K. Batter, W.E. Brown*#, and B.B. Kaplan (SPON: James Byrd, III, M.D.) Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15213, and #Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, PA 15213

A cDNA clone for bovine adrenal PNMT was used to screen a Charon 28 bovine genomic library. One clone, designated λP1, was identified which putatively contained the entire PNMT gene. The structure and complete nucleotide sequence of this genomic clone was subsequently delineated by restriction endonuclease mapping and dideoxy sequence analysis (Batter, et al., submitted for publication). In order to unambiguously establish the identity of this clone, the nucleotide sequence was compared to partial amino acid sequence data derived from the purified protein.

PNMT was purified to apparent homogeneity by a modification of the procedure of Pohorecky and Baliga (Arch. Biochem. Biophys., 156:703-711, 1973). A bovine adrenomedullary tissue homogenate was fractionated by ammonium sulfate precipitation, gel filtration and ion exchange chromatography. The specific activity of the purified protein was 610 nmole/mg-hr. PNMT has an apparent molecular mass of 31,000, as judged by SDS-PAGE. Results of an amino acid analysis of the purified protein is consistent with the amino acid composition deduced from the genomic sequence.

The purified protein was subjected to specific chemical cleavage by both cyanogen bromide and formic acid. These procedures cleaved the protein at a Met residue near the C-terminus, and an acid labile Asp-Pro bond near the N-terminus. N-terminal amino acid sequence analysis of the CNBr cleavage product identified 13 amino acid residues. Nine additional residues were obtained from the formic acid cleavage product. Our results also suggest that the N-terminus of the protein is blocked since it is not labile to the sequencing reagents.

The purified protein was also subjected to enzymatic digestion by trypsin. The products of complete digestion were fractionated by reverse-phase HPLC, and four well-defined fractions subjected to sequence analysis. Amino acid sequence analysis identified 3, 10, 11, and 15 amino acids in the four tryptic peptides.

In total, six peptides were sequenced, accounting for approximately 20% of the entire PNMT protein. The amino acid sequence of all peptides isolated was identified within the protein sequence deduced from the nucleotide sequence of the gene. This work provides an independent and unambiguous verification that the genomic clone, λP1, contains the entire coding region for adrenomedullary PNMT. (This work was supported by USPHS grant NS19608, MH29670 and MH18273).

240.6 HUMANS AND RATS CONTAIN A SINGLE GENE ENCODING BRAIN GLUTAMIC ACID DECARBOXYLASE. M.G. Erlander, N.J.K. Tillakaratne*, R.M. Goldstein*, S. Gonzalez*, D.L. Kaufman*, Y. Kobayashi* and A.J. Tobin. Department of Biology, Molecular Biology Institute, and Brain Research Institute, University of California, Los Angeles, CA 90024.

The fusion protein produced by bacteria infected with lambda-GAD catalyzes the stoichiometric conversion of glutamate into CO_2 and GABA (Kaufman et al, Science, 232, 1138-1140). We have compared the K_m for glutamate and the K_i for two GAD inhibitors (aminooxyacetic acid and 2-mercaptopropionic acid) of this fusion protein with that of partially purified brain extracts. These data support the identification of this cDNA as that encoding the major species of brain GAD.

Lambda-GAD, constructed in lambda gt-11, contains a 2.3 kb feline cDNA, whose sequence we have recently reported (Kobayashi, et al., J. Neurosci., in press). We have isolated several dozen human GAD cDNAs by plaque hybridization to a cDNA library from human fetal brain (obtained from R. Neve). One of these cDNAs, 3.3 kb long, contains extensive homology with 2 kb of the 2.3 kb feline cDNA.

We have used feline cDNA to isolate eight overlapping lambda and cosmid clones from human genomic libraries (obtained from T. Maniatis and H. Lehrach). These clones extend over 75 kb of human DNA. All the restriction fragments revealed by Southern blotting of human DNA are contained within this contiguous region. This result indicates that the human genome contains only a single gene homologous to our cloned GAD cDNA.

We have similarly analyzed 20 clones from a rat genomic library obtained from J. Bonner. These clones, and the corresponding rat Southern blots, span only 30 kb of rat DNA. The structure of the rat GAD gene thus appears to differ from that in the human genome.

This work was supported by a grant to AJT from NINCDS (#NS 22256) and a program project grant to Dr. A.V. Delgado-Escueta (#NS 21908). DLK has been supported by a USPHS Training Grant in Cell and Molecular Biology (#GM 07185), MGE by the ARCS Foundation, SG by the UCLA Summer Research Program for Minority Undergraduate Students, and NJKT by the American Association for University Women, Phi Beta Kappa, and the Ursula Mandel Fellowship.

240.7 **TISSUE-SPECIFIC EXPRESSION OF THE RAT GALANIN GENE: ISOLATION AND CHARACTERIZATION OF CLONED cDNAs.** Lee M. Kaplan*, Eliot R. Spindel, Kurt J. Isselbacher*, & William W. Chin*. Laboratory of Molecular Genetics, Brigham & Women's Hospital, Boston, MA 02115, and Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA 02114.

Galanin is a 29-amino acid peptide localized in central and peripheral neurons of several vertebrate species. In the CNS, galanin-like immunoreactivity is found in cholinergic neurons of several nuclei which have been shown to undergo specific degeneration in Alzheimer's disease. Galanin is also widely distributed in the myenteric and submucous plexuses of the GI tract, where it has been shown to have a variety of direct and neurally-mediated effects on smooth muscle contractility.

In order to permit study of the regulation of galanin gene expression in an easily manipulated animal model, we cloned cDNAs encoding rat galanin (rGal). Mixed synthetic oligonucleotide probes were used to isolate several rGal clones from a cDNA library prepared from rat hypothalamic tissue. Sequence analysis of three such clones revealed rGal to be synthesized initially as a 124-amino acid precursor. The deduced amino acid sequence of the precursor includes a signal peptide, galanin, and a 60-amino acid carboxy-terminal peptide (galanin mRNA-associated peptide; rGal-MAP). The galanin portion is flanked on each side by Lys-Arg tryptic cleavage sites. The predicted amino acid sequence of rGal is 90% homologous with porcine galanin, with all 3 amino acid differences in the C-terminal heptapeptide. However, the amidated C-terminal amino acid of rGal is threonine rather than alanine. These data confirm the phylogenetic conservation of the N-terminal region of galanin predicted from immunological studies. Although rGal-MAP shows only 58% overall homology with its porcine counterpart, its amino acid sequence includes a large region of near identity (>90% homology). The high interspecies conservation suggests the existence of and a biological role for this galanin mRNA-associated peptide.

Northern blot analysis using a ^{32}P-labeled anti-sense cRNA probe reveals a single band of hybridizing mRNA which is approximately 900 nucleotides long. Rat galanin mRNA is localized predominantly in the CNS and GI tract. Highest CNS concentrations are found in the hypothalamus, with lower levels in the cerebral cortex and brainstem. In the GI tract, highest levels are in the duodenum with progressively lower concentrations in the small intestine, stomach, and colon. In contrast to its relative abundance in porcine adrenal medulla, galanin mRNA is barely detectable in rat adrenal tissue. The cloning of rat galanin will facilitate further examination of tissue-specific and developmental expression of the galanin gene.

240.8 REGULATION OF MYELIN BASIC PROTEIN PRODUCTION IN MICE HETEROTIC FOR MYELINATION. Robin Miskimins* and Robert K. Yu. Dept. of Neurology, Yale University School of Medicine, New Haven, CT 06510, USA

We have recently shown that there is some form of transcriptional or post-transcriptional control operating in the regulation of myelin basic protein (MBP) production in the CNS (Miskimins et al., Proc. Natl. Acad. Sci. USA, 83:1532, 1986) using mice heterotic for myelin production during the most active phase of synthesis. These mice are the F_1 generation of a cross between C57Bl/6J and DBA/2J. We were interested in determining how this control might be exerted, whether the effects we have seen are also present in the PNS, and whether the reciprocal cross offspring (DBA/2J X C57Bl/6J) also show heterosis for MBP production. We have quantitated several markers for myelin in the sciatic nerve of 16 day old mice. No heterosis for myelin content in the PNS has been observed. In addition we have performed Northern blot analysis of MBP RNA from the reciprocal cross mice. We have seen that these mice also show increased levels of MBP mRNA production at 16 days of age. Our approach to understanding how transcription of the MBP gene might be regulated has been to use low ionic strength polyacrylamide gels to identify the regions of the 5' end of the MBP gene that specifically interact with DNA binding proteins. We have mapped several fragments of DNA in the 5' end of the MBP gene that interact with these proteins. One includes the first exon, part of the first intron and about 200 bases of 5' flanking sequence. We are also using these gels to isolate the fragments that bind the proteins and footprint the binding sites. Our data indicate that there are several protein binding sites in the 5' end of the MBP gene, some of which may be for the specific regulation of transcriptional activation of the gene during myelinogenesis. In addition we have obtained a glioma cell line (courtesy of Dr. S. Pfeiffer) in which we have observed low levels of MBP mRNA production. We are hopeful these cells will prove useful in identifying regulatory proteins involved in transcriptional control of myelin genes. (Supported by USPHS grant NS-23102)

240.9 MOLECULAR BASIS FOR THE DIFFERENTIAL REGULATION OF NEURON-SPECIFIC AND NON-NEURONAL ENOLASE GENE EXPRESSION IN THE BRAIN. S.M. Forss-Petter* and J.G. Sutcliffe, Research Institute of Scripps Clinic, La Jolla, CA 92037.

To illuminate the features of lineage-specific and developmentally regulated gene expression in the rat brain, we are analyzing the regulatory signals required for transcription of the enolase isozyme genes. We have previously cloned the rat neuron-specific enolase (NSE) mRNA, determined its complete nucleotide sequence (2222 bases excluding polyA), and analyzed its pattern of expression (Forss-Petter et al., J.Neurosci.Res.16:141, 1986). In the adult, NSE is one of the most abundant (0.5%) neuron-specific mRNAs. The first NSE transcripts can be detected in the 14-day rat embryo. A major increase in expression takes place during the second postnatal week, correlating with the appearance of NSE protein but accumulating faster to adult level.

Primer extension and S1 nuclease protection show that transcription of NSE initiates at 8 or more sites spanning 60 nucleotides, whereas transcription of non-neuronal enolase (NNE) initiates at a single site. Southern blot analyses indicate a single NSE gene and at least ten copies of the NNE gene (some of which are probably pseudogenes) in the rat genome. NSE cDNA probes were used to isolate genomic clones of NSE and NNE (which cross-hybridize at low stringency) from a lambda phage library, and oligonucleotides were used to confirm the assignments and indicate fragments proximal to the promoter regions. Analysis of the nucleotide sequence of the NSE promoter region reveals no canonical TATA/CAAT motifs but a very GC-rich (potential S1-binding) sequence, similar to other genes using multiple sites for transcription initiation. We have analyzed three different genes for NNE, two of which contain no TATA or CAAT boxes within 250 nucleotides upstream from the cap site. We cannot yet tell whether one single gene or many genes with identical regulatory signals are actually transcribed. Multiple copies of the rat ID sequence are present within and surrounding the NSE gene, whereas none could be detected in any of the three NNE genes analyzed.

NSE mRNA is expressed in rat C6 (glioma) and PC12 (pheochromocytoma) cells and in mouse neuroblastoma lines; NNE mRNA is abundant in every line we tested. In the adrenergic mouse neuroblastoma line NIE-115, DMSO induction stimulates differentiation to a neuronal phenotype and also NSE mRNA expression (roughly ten-fold). The extent of the cis-acting signals required for proper transcription initiation, investigated in transfection assay with NSE/NNE promoter-driven expression of a reporter gene will be discussed.

240.10 **NEURON SPECIFIC OLFACTORY MARKER PROTEIN: GENE STRUCTURE STUDIES.** **K. E. Rogers and F. L. Margolis.** Department of Neurosciences, Roche Institute of Molecular Biology, Nutley, New Jersey, 07110.

Olfactory marker protein (OMP) is a 19kd cytoplasmic protein which is expressed by mature olfactory receptor neurons. Although the biochemical properties of this developmentally regulated macromolecule have been extensively characterized, its function remains unknown. We have previously cloned and characterized rat OMP-cDNA and subsequently used that cDNA as a probe to investigate OMP-mRNA regulation. In the study presented here, we used random-primed labeled OMP-cDNA restriction fragments as probes to identify and isolate genomic DNA encoding rat OMP from a Charon 4A lambda phage library. After screening 9 x 10^5 pfu, three recombinant phages were isolated (λ-OMP1, λ-OMP2, λ-OMP3). Each isolate was shown to contain approximately 16.5 kb of rat genomic DNA. A restriction map was constructed for λ-OMP1, and exon-containing fragments were identified by Southern blot hybridization to OMP-cDNA. Various regions from the cDNA, containing either non-coding or coding information, were used as probes to select genomic fragments which contained information primarily upstream to the coding region. These fragments were sub-cloned into M13mp10 vectors and sequenced by the Sanger dideoxy method. Sequence analysis of a 900 base-pair Pst I fragment demonstrated that the fragment included 198 nucleotides of coding information as well as 700 additional nucleotides upstream from the ATG start site. However, the Pst I fragment did not contain intron sequences within the coding region. A TATA-like sequence was noted 53 nucleotides 5' to the ATG start codon, but no apparent CAAT box was detected in the appropriate region. Additional characterization of λ-OMP1 is now underway. Furthermore, we are now engaged in determining whether the Pst 1 fragment contains actual OMP promoter activity or other features which confer tissue-restricted expression.

240.11 GENETIC TRANSFORMATION WITH DNA OF MEC-3, A GENE REQUIRED FOR DEVELOPMENT OF A SINGLE NEURONAL TYPE IN C. ELEGANS Jeffrey C. Way* and Martin Chalfie, Dept. of Biological Sciences, Columbia University, New York, NY 10027

The nematode C. elegans has six mechanosensory neurons for the sensation of gentle touch. Many mutations which abolish touch-sensitivity have been isolated (mec mutations); most of these are in genes which appear to affect the functioning of the touch receptors, and which abolish at most one of the special features of these cells.

In mec-3 mutants of C. elegans, the six touch receptor neurons possess none of their distinctive features, such as 15-protofilament microtubules and associated extracellular material. The cell division pattern which produces these cells is normal and no other cell type appears to be altered. Thus, unlike other mec genes, mec-3 appears to be involved in the specification of the touch receptors, and not their function.

Previously, (Way and Chalfie, Neurosci. Abst. 52:10 [1986]) we showed that in mec-3 mutants, the altered touch receptor still retains neuronal characteristics. We also described cloning the gene by transposon tagging.

We have rescued a mec-3 mutant by microinjection of cloned DNA, using the procedure of A. Fire (EMBO J. 5, 2673 [1986]). In each transformant, the DNA behaves genetically as a free duplication. This duplication can be lost during mitosis to generate mosaic animals, which may be touch sensitive in the head or tail only, or which may have no transformed progeny. As determined by Southern blotting, there are between 50 and 250 copies of the transforming DNA per haploid genome. By injection of deleted DNAs, we have delimited the mec-3 gene to at most 8,000 bases.

In dominant mec-4(d) mutants, the touch receptors degenerate, presumably because a toxic mec-4(d) product is expressed in the touch cells. In mec-3;mec-4(d) double mutants, the cells do not degenerate, but appear as they do in mec-3 mutants. This suggests that mec-3 is necessary for the expression of the normal mec-4 gene. Intriguingly, in one particular mec-3;mec-4(d) strain carrying the mec-3(+) transforming DNA, additional cells degenerate. The precise mechanism for this phenomenon is unknown.

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240.12 EXPRESSION OF THE NERVE GROWTH FACTOR GENE: CELL-SPECIFIC REGULATION. P.J.Alvarez, S.A.Quarless*, E.C. Azmitia and G.Heinrich. Lab for Mol. Endo., Howard Hughes Med. Inst., Dept. Med., Mass.Gen.Hosp., Boston, MA 02114

Nerve Growth Factor (NGF) is produced by targets of specific neurons in the CNS and PNS. In these targets NGF production is correlated with the density of innervation and differs markedly among targets, suggesting that regionally specialized cells express the NGF gene in quantitatively and qualitatively different ways. The specific cells that secrete NGF in the CNS and the molecular mechanisms that restrict NGF gene expressions to and regulate it within these cells are still unknown. We are using several approaches to address these questions.

In order to identify the cis elements in and around the NGF gene that determine cell-specificity we created a fusion gene from the mouse NGF gene promoter region and a reporter gene encoding human growth hormone (hGH). This construct was transfected into the L929, C6, HL60, PC12, and NIE115 cell lines and assayed for hGH with a two-site radioimmunoassay (RIA) as a measure of transcription of the fusion gene. Expression of the fusion gene was then correlated with the cell's intrinsic ability to transcribe the NGF gene into NGF mRNA by Northern blot and S1 protection hybridization assays. We are now using deletion mutants of the fusion gene to further characterize the cis-acting elements that are responsible for cell-specificity and will transfect fusion genes containing the cell-specifier(s) into primary hippocampal neurons and glia to determine whether these cells are permissive for NGF gene expression.

Our second approach is to identify by primary culture the cells that express the NGF gene, and its mode of regulation. Brains of newborn rats were dissociated with trypsin, and cultured in DMEM/20% fetal calf serum. After 5 weeks and at least one passage the cultures contained predominantly fibroblasts by immunostaining and immunoprecipitation analysis. The cultures produced hybridizable NGF mRNA. 1 mM Na-butyrate in the medium for 24h increased NGF mRNA 2.5-fold, and decreased beta-actin mRNA 10-fold. These data demonstrate that fibroblasts derived from brain synthesize NGF mRNA in primary culture in a regulatable fashion.

240.13 RAT GROWTH HORMONE (rGH) GENE 5' FLANKING SEQUENCES TARGET THE EXPRESSION OF A MARKER GENE TO THE PITUITARY OF TRANSGENIC MICE. S.A. Lira*, E.B. Crenshaw III*, L.W. Swanson and M.G. Rosenfeld*. (SPON: R. Emeson). Howard Hughes Medical Institute, La Jolla, CA, 92093.

Growth hormone (GH) is a polypeptide hormone produced by the somatotrophs of the anterior pituitary. In the rat it is coded by a single gene which is under transcriptional control of growth hormone releasing factor, glucocorticoids and thyroid hormone. Rat pituitary cells in culture produce at least 10^8 more GH than liver cells in the same conditions. To address questions concerning the tissue-specific expression of the rGH gene we performed gene transfer experiments in pituitary and non-pituitary cell lines using the chloramphenicol acetyltransferase (CAT) gene as a reporter (Nature 322:557, 1986).These experiments provided the identification of a 5' flanking region (from -47 to -235bp) that conferred pituitary cell-line expression when fused to a heterologous gene. This region was shown to act in a position and orientation independent fashion and on a heterologous promoter, resembling an enhancer element. We decided to examine further the nature of the signals present on the 5' flanking region by fusing three different segments with the human growth hormone gene (hGH) and injecting them into fertilized mouse oocytes. The fragments encompassed respectively 1.7kb, 320bp and 180bp of the rGH gene upstream region. Fragments were excised from the vectors, purified and injected according standard techniques. At 3 weeks of age a segment of the mouse tails was excised, DNA extracted and blotted on nitrocellulose filter. Blots were hybridized with a PvuII probe from the hGH gene . Positive animals were selected and mated to generate transgenic lines. We generated 5 founders for the 320bp-hGH series,9 founders for the 180bp-hGH series and 13 founders for the 1.7kb-hGH series. When analyzed around 45 days of age none of the animals showed signs of gigantism when compared to their littermates.
Immunohistochemical analysis of the pituitary gland of at least two different integration events for each series revealed the expression of hGH in the anterior lobe of the transgenic animals but not in their control littermates. Double labeling studies using antibodies against LH, ACTH, TSH, PRL and mGH revealed the expression of the transgene in the somatotrophs of all three series and in a sub-population of TSH producing cells. No expression of the hGH was detected in gonadotrophs, corticotrophs, or lactotrophs in the 320-GH or 180-GH animals analyzed, but co-labeling with hGH was sometimes detected in the lactotrophs of the 1.7-GH animals. Work is presently being conducted to determine if the transgene is being expressed in sites other than the pituitary.
The findings described above are suggestive of the presence of genetic information located on the 180bp upstream of the rGH gene that allows for expression of a marker gene in the somatotrophs.
Sergio Lira is partially supported by the Brazilian Research Council (CNPq).

240.14 RAT CALCITONIN (rCal) GENE 5' FLANKING SEQUENCES TARGET THE EXPRESSION OF A MARKER GENE TO THYROID C-CELLS IN TRANSGENIC MICE. S.E. Leff, L. Stolarsky*, S.A. Lira*, E.B. Crenshaw III*, L.W. Swanson and M.G. Rosenfeld*. Howard Hughes Medical Institute, La Jolla, CA 92093.

We have been studying the cell-type specific expression of the rCal gene, a gene that produces primarily calcitonin mRNA in thyroid C-cells and calcitonin gene-related peptide (CGRP) mRNA in some peripheral sensory neurons also derived from the neural crest. In addition, CGRP and its mRNA are found in several central nervous system nuclei. Transfections of fusion genes comprised of the 5' flanking DNA and promoter of rCal, and the reporter gene chloramphenicol acetyl transferase (CAT) identify a segment of 5' flanking DNA between -810 to -1350 bp from the promoter that specifically enhances the expression of genes directed from the rCal promoter in medullary thyroid carcinoma derived cell lines. However, the promoter segment alone from -155 to +30 bp is competent to direct expression of reporter genes at moderate to high levels in a number of heterologous cell lines in this transient transfection paradigm (L.Stolarsky et al, in preparation). To test whether 5'flanking DNA from the rCal gene that includes this segment contains developmental information to direct expression of a marker gene, human growth hormone (hGH), to thyroid C-cells and the appropriate neuronal cell types, we introduced fusion genes comprised of hGH or a modified "midi" hGH gene under the control of the rCal promoter and 5' flanking DNA to -1350 bp into newly fertilized mouse embryos.

Northern blot hybridization analysis detected high levels of hGH mRNA in thyroids, moderate levels in lung, and low to moderate levels in the brainstem. In situ hybridization analyses using ^{35}S-hGH antisense riboprobes identified high levels of hGH mRNA in thyroid C-cells, and low levels of hybridization in the trigeminal ganglion. These data indicate that rCal 5' flanking DNA can direct its cell-specific expression to thyroid C-cells. Expression of this fusion gene in the nervous system is under investigation.

240.15 COMPLEMENTARY DNA CLONING OF A NERVOUS SYSTEM SPECIFIC INTERMEDIATE FILAMENT FROM *XENOPUS LAEVIS* WITH HOMOLOGY TO MAMMALIAN NEUROFILAMENT. L. Charnas, K. Richter*, T. Sargent*, and I. Dawid*. Hum. Gen. Branch and Lab. of Mol. Gen., NICHD, NIH, Bethesda, MD 20892

Neurofilaments are a neuron specific class (Type IV) of the intermediate filament gene family. They are expressed after neurite outgrowth begins and are a useful marker of neuronal differentiation. We were interested in developing nucleic acid probes to be used as markers of early neuronal differentiation. We screened 5 X 10^4 plaques from a *X. laevis* brain cDNA λgt11 library with NF68, a cDNA probe for mouse NF-L (Lewis, S. and Cowan, N., J. Cell Bio. 1985, 100:843-850). Four distinct clones were isolated and partially sequenced. The derived amino acid sequence of each clone was homologous to regions within the rod domain of mouse neurofilament.

The complete nucleotide sequence of a 1.9 kB clone, XNK3, demonstrated a 1.07 kB coding region and an 0.86 kB 3' noncoding region. The derived amino acid sequence coded for 357 amino acids, 286 amino acids within the rod domain and 71 amino acids in the carboxy terminus. The rod domain showed some homology to nonepithelial intermediate filaments but had the highest homology to mammalian neurofilament, with identity in the final 29 amino acids of the 2B portion of the rod domain. The carboxy terminus began with the sequence -**TSVG**-, distinctive for NF-L proteins, and in the middle, had a single -**KEEKE**- sequence, characteristic of mammalian NF-M's. The remainder of the C-domain was rich in glycine and serine and had limited homology to other known intermediate filament

Northern blot analysis probing with XNK3 demonstrated a 2.4 kB band present in brain, but absent in liver, ovary, skin, and muscle. This corresponds to a maximum size of 58kDa for the protein encoded by XNK3. We speculate that XNK3 is a novel, low molecular weight neurofilament in *X. laevis*. Developmental expression profiles and cell localization experiments with *in situ* hybridization analysis are in progress.

241.1 IMMUNOHISTOCHEMICAL LOCALIZATION OF GABA, CHOLINE ACETYLTRANSFERASE, GLUTAMATE AND ASPARTATE IN THE VISUAL SYSTEMS OF GOLDFISH AND MICE. G.H. Kageyama and R.L. Meyer. Dev. Biol. Center, University of California, Irvine, CA 92717.

The immunohistochemical localization of GABA, choline acetyltransferase (ChAT), glutamate (GLU) and aspartate (ASP) was examined in the visual systems of normal and monocularly enculceated (or optic nerve crushed) goldfish and in normal mice.

GABA (Immuno Nuclear). In the goldfish optic tectum GABA was found in Type III cells in stratum opticum (SO), sparsely distributed neurons in the stratus griseum centrale (SGC) and neurons in stratum periventriculare (SPV). Large GABA+ puncta were localized sparsely distributed in stratum marginale (SM) and SGC, and densely distributed within sublamina b of SO, throughout the stratum fibrosum et griseum superficialis (SFGS), sublamina a of the SGC and neuropil of the stratum album centrale (SAC). In the primary optic innervation layer (SFGS) neither optic nerve crush or enucleation resulted in any notable changes in GABA labeling. At the E.M. level GABA was localized within predominantly large non-optic vesicle-containing profiles. In the retina of goldfish and mice GABA was found as previously reported within certain classes of amacrine cells and their processes stratified within the inner plexiform layer (IPL). GABA was also found within small neurons located sparsely distributed within the mouse LGN and superior colliculus.

ChAT (Immuno Nuclear). In goldfish ChAT was localized within a class of small type XIV tectal periventricular neuron and retinal amacrine cells and processes as previously reported. ChAT labeling was not notably affected by either optic nerve crush or long-term enucleation. At the E.M. level ChAT was localized within non-optic vesicle-containing profiles within the SFGS.

GLU (Bob Wenthold, NIH). In goldfish optic tectum GLU was localized within the Type I pyramidal neurons in the SFGS and their dendrites in SM, as well as neurons sparsely distributed throughout the SGC, large neurons (presumed Type XIII) in the SAC and in the smaller Type XIV periventricular neurons in the SPV. Fibers in the SO and fibers and terminals in the SFGS were also labeled and disappeared 3 days after optic nerve crush without notably affecting the labeling of Type I neurons. In the retina of both goldfish and mice GLU labeled some retinal ganglion cells, cone pedicles, cells in the inner nuclear layer and processes in the inner plexiform layer. In the mouse large cells in the LGN and large and small cells in superior colliculus were labeled. GLU labeling was blocked by 0.05M L-glutamate, but not aspartate.

ASP (Chemicon and Bob Wenthold, NIH). In both goldfish and mice ASP was localized in the same cells and processes as described for GLU I and GLU II but differed in staining intensity. ASP labeling was blocked by 0.05M L-aspartate but not glutamate.

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241.2 IDENTIFICATION OF N-ACETYL-ASPARTYLGLUTAMATE-LIKE IMMUNOREACTIVITY IN THE ANTERIOR HYPOTHALAMUS OF THE RAT. J.R. Moffett, M.A.A. Namboodiri and J.H., Neale. Dept. of Biology, Georgetown Univ., Wash. D.C. 20057.

Several lines of evidence developed in recent years suggest that the acetylated dipeptide, N-acetyl-aspartylglutamate (NAAG), might serve in some capacity as a neuroactive agent. Support for this idea has come from studies utilizing anti-NAAG sera, which demonstrate specific NAAG-immunoreactivity (IR) in numerous cell types in the CNS, including retinal ganglion cells, and the projection zones of the primary and accessory optic tracts in rat, cat and primate. In order to investigate the role of this dipeptide in the retino-hypothalamic axis, the distribution of NAAG IR was mapped in the retinohypothalamic pathways and anterior hypothalamic areas of the rat following optic nerve transections.

At the anterior margin of the third ventricle, the anterior periventricular nucleus exhibited NAAG-IR which was both intracellular, and diffuse within the neuropil. Additionally, many NAAG positive neurons were visible throughout the preoptic area. More caudally, densely staining NAAG positive neurons were observed in the hypothalamic periventricular nucleus, and in a dorsal crescent of cells in the suprachiasmatic nucleus. Both of these nuclei contained a high degree of diffuse staining in the neuropil as well. Laterally, immunoreactive cell groups and diffuse staining neuropil were visible throughout the extent of the lateral hypothalamus, encompassing several nuclear groups. NAAG positive axonal cross sections were seen scattered throughout the optic chiasm proper, with the greatest density of such fibers occurring in the dorsal half of the chiasm. Notably, diffuse NAAG-IR was present in several presumptive ganglion cell projection zones, including, the suprachiasmatic nuclei, supraoptic nuclei, and the lateral hypothalamus. Unilateral optic nerve transection in albino rats resulted in loss of reactivity in corresponding optic nerve axonal crossections, and reductions in the diffuse neuropil reactivity in the ventral suprachiasmatic nucleus, the supraoptic nucleus, and lateral hypothalamus.

These data expand the number of visual pathways in which neurons utilizing NAAG are likely to participate, and suggest that this dipeptide may be involved in some form of direct retinal regulation of hypothalamic function. (Supported by NIDA grant DA 02297).

241.3 CHOLINERGIC MODULATION OF GLUTAMATE IN THE THALAMIC RETICULAR NUCLEUS. G.A. Marks, Andrew Hoelscher* and H.P. Roffwarg. Dept. of Psychiatry, Univ. of Texas Health Science Ctr. at Dallas, Dallas, TX 75235.

Evidence supports a central role for cells of the thalamic reticular nucleus (TRN) in the control of the transfer of sensory information through the thalamus. The TRN exerts a GABAergic inhibitory influence upon specific thalamic relay nuclei. It, in turn, receives input from collaterals of relay cells in the respective nuclei, putatively glutaminergic-facilitating influences from cortex and ascending influences from the brain-stem. Work in several laboratories, utilizing the anesthetized cat preparation, indicates that at least one brainstem input to TRN is cholinergic and that cholinergic agonists, mediated through muscarinic receptors, inhibit TRN cells. Recently, Kayama et. al., working with the anesthetized rat, concluded that the brainstem cholinergic influence on TRN neurons is excitatory. To investigate this controversy, we have microiontophoretically applied cholinergic agonists to TRN cells in anesthetized rats. We found responses that are too complex to characterize as solely inhibitory or excitatory.

Male Long-Evans hooded rats were anesthetized under chloral hydrate (IP). Four-barrel micropipets were used to apply drugs iontophoretically. Three barrels contained active drug solutions and a fourth contained 3M Nacl for current balance and current testing. An insulated etched-tungsten electrode was cemented to the micropipette (tip to tip $< 20\mu m$) and used to record the activity of single cells extracellularly.

The TRN cells studied were responsive to either visual or somatosensory stimulation and were physiologically identified by a spike-burst response to single shocks of the OT (visual) and ML (somatosensory). With the vast majority of cells, carbachol (carb) ejection elicited a quick-onset inhibition followed by facilitation. Low currents of carb, which in itself had little effect on activity, caused marked increases in the facilitatory response to glutamate (glu). This effect took several minutes to develop and several minutes to recover. In rarely encountered cells capable of very regular tonic discharge, carb only elicited inhibitory responses, and no interaction with glu was observed.

Our conclusion, based on these preliminary data, is that the influence of cholinergic agonists on the vast majority of TRN cells is twofold: (1) a short-acting, mode-dependent inhibition and (2) a long-lasting neuromodulatory effect, potentiating the putatively glutaminergic cortical influence. Responses may be mediated by two different muscarinic receptor subtypes.

241.4 MODULATORY ACTION OF THE RETICULAR TRANSMITTERS NOREPINEPHRINE AND 5-HYDROXYTRYPTAMINE (SEROTONIN) IN THE CAT'S VISUAL THALAMUS. H.-Ch. Pape* and U.Th. Eysel Institute of Physiology, University of Essen, D-4300 Essen 1, West Germany.

Acetylcholine (ACh), norepinephrine (NE) and 5-hydroxytryptamine (5HT; serotonin) are regarded as transmitters of reticular influences in the geniculate nuclei (recent review by Sherman, S.M. and Koch, C. Exp.Brain Res.,63: 1, 1986). While a range of specific ACh effects on visual thalamic function is well documented, NE and 5HT have so far been reported to act more globally in terms of depression or facilitation of geniculate activity. We applied NE and 5HT locally by microiontophoresis during single cell recording in the perigeniculate (PGN) and dorsal lateral geniculate (dLGN) nuclei of the cat. Effects were compared with those of ACh and the excitatory transmitter L-glutamate. In the dLGN, visually and glutamate evoked activities were depressed by NE in 91% of the cells (n=34), and in 89% (n=18) by 5HT. Facilitation was not observed. Possible influences of the monoamines on inhibitory processes in the dLGN were investigated during continuous 5HT and NE ejection, with simultaneous application of glutamate compensating monoamine induced depression. In roughly one-third of the cells, long-range lateral inhibition was reduced by NE (n=21) or 5HT (n=10). Effects ranged from weak disinhibitory modulation to complete disinhibition. Binocular and center/surround inhibition were not affected by NE nor 5HT. For comparison, ACh elicited increased firing rates in 79% of the cells (n=29), disinhibited long-range inhibition in 77% (n=13) and enhanced center/surround antagonism. In the perigeniculate nucleus, NE, 5HT and ACh depressed neuronal activity, with ACh being most effective.

In conclusion, differential reticular influences on dLGN activity (depressant action of NE and 5HT, facilitation with ACh) and modulation of inhibitory mechanisms in the geniculate nuclei (disinhibition of global inhibition by all reticular transmitters, enhanced specific inhibition only with ACh) might reflect gating of geniculate transmission in relation to various states of sleep and arousal.

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241.5 DISTRIBUTION AND MORPHOLOGY OF CHOLINERGIC AXONS IN THE LATERAL GENICULATE NUCLEUS AND OTHER THALAMIC NUCLEI IN THE CAT.
D. Fitzpatrick and D. Raczkowski. Department of Anatomy, Duke University Medical Center, Durham, NC 27710.

We examined the distribution and morphology of putative cholinergic axons in the cat's thalamus using a monoclonal antibody to choline acetyltransferase (ChAT). Fine ChAT-immunoreactive (ChAT+) fibers with small varicosities are visible throughout the thalamus, but there are conspicuous regional differences in their density. ChAT+ fibers are particularly dense in the dorsal lateral geniculate nucleus (LGNd) and this density stands in sharp contrast to the sparse distribution of ChAT+ fibers in other principal sensory (GMv and VP) and motor (VA and VL) nuclei. Within laminae A, A1, C and the MIN of the LGNd, ChAT+ varicosities are grouped together into clusters. In contrast, ChAT+ axon terminals in laminae C1-C3, appear less dense and have a uniform distribution. In the A laminae, ChAT+ profiles contain pleomorphic vesicles and make synaptic contacts with the dendritic processes of projection and local circuit neurons as previously reported by deLima et al. (1985).

ChAT+ varicose fibers are also numerous within the pulvinar nucleus (Pul), the ventral lateral geniculate nucleus (LGNv), the intermediate nucleus of the lateral group, the lateral medial - suprageniculate complex (Graybiel and Berson, 1980) and the paracentral and central-lateral components of the intralaminar nuclei. With the exception of the LGNv, these nuclei are not direct targets of the retina. Nevertheless, they all receive projections from the visual midbrain and have been implicated in visual sensory or motor functions. In general, the distribution of ChAT+ fibers is similar to the pattern of acetylcholinesterase (AChE) staining in the thalamus, but there are exceptions: the tecto-recipient portion of the lateral posterior nucleus and the centre median-parafascicular complex, both rich in AChE activity, are only sparsely innervated by ChAT+ fibers.

Finally, ChAT+ fibers outline the cell bodies and proximal dendrites of neurons throughout the reticular nucleus (Ret) of the ventral thalamus, including the perigeniculate nucleus. Since most nuclei of the dorsal thalamus receive projections from the Ret, this may be the main route by which cholinergic neurons in the midbrain and pontine reticular formation exert a global influence over thalamic function. However, the differential distribution of ChAT+ fibers within the dorsal thalamus suggests that the cholinergic system has an additional, more specialized role in influencing cellular activity in thalamic nuclei linked to the visual system. Supported in part by NSF BNS 84-11964, NSF BNS 85-19709, NIMH MH04849, and The Sloan Foundation.

241.6 THE CELLULAR MECHANISMS OF THALAMIC PGO WAVES. B. Hu*., M. Deschênes and M. Steriade. Lab. Neurophysiology, Sch. of Med., Laval University, Quebec, Canada, G1K 7P4.

This study investigated the effect of peribrachial (PB) stimulation on neurons of the lateral geniculate nucleus (LGN) in the cat. Intracellular and field potential events were recorded simultaneously in normal or reserpinized cats under a variety of anesthetic conditions. In all animals, the visual cortical areas were removed and, in most preparations, both retina were also lesioned. In LGN relay neurons, PB stimulation triggered an all-or-none IPSP superimposed on a slow depolarization. The IPSP occurred at a latency of ~80 msec, lasted for ~50 msec, and was reversed by intracellular Cl injection. This IPSP was observed in all LGN neurons. The slow depolarization started at a latency of ~20 msec and lasted for 200-300 msec. Its amplitude was very variable, being barely detectable in some neurons. This depolarization grew with hyperpolarization of the cell and was accompanied by an increased conductance. The all-or-none IPSP appearing on the plateau of the slow depolarization had an intrageniculate origin since perigeniculate neurons were always inhibited by PB stimulation. Results obtained after reserpine treatment were identical to those just described, showing that these responses did not originate from stimulation of NE or 5-HT fibers. In addition, in reserpinized cats, spontaneous PGO waves were recorded in the LGN. These PGO could also be triggered by auditory or somatic stimuli that usually evoked orienting reactions in behaving animals. The intracellular events associated with these field potentials were identical to those triggered by PB stimulation. It is then concluded that LGN-PGO waves are the thalamic correlates of orienting reactions and that the PB nucleus is the final common path transmitting these corollary discharges toward the diencephalon. Supported by MRC grants MT-5877 and MT-3689.

241.7 EFFECT OF RETINAL IMPULSE BLOCKAGE ON THE ACTIVITY AND AMOUNT OF CYTOCHROME OXIDASE IN THE ADULT CAT LATERAL GENICULATE NUCLEUS: a HISTOCHEMICAL AND IMMUNOCYTOCHEMICAL STUDY. T. M. Cheng* and M. Wong-Riley (SPON. D. A. Riley). Dept. of Anat. & Cellular Biology, Med. Coll. of Wis., Milwaukee, WI 53226.

At the light and EM levels, histochemically-localized cytochrome oxidase (C.O.) activity has been shown to vary between different neuronal types in relationship to functional neuronal activity (Brain Res.,171:11,'79;J.Comp.Neurol.,222:1,'84). In addition, we have shown that the relative amount of C.O. immunolabeled with our specific antisera at the light and EM levels vary between neuronal profiles in the normal adult cerebellum, in direct correlation with the relative level of histochemical C.O. activity (Soc.Neurosci. Abstr.,12:695,'86). The present study investigated the influence of monocular tetrodotoxin (TTX)-induced blockage of retinal action potentials on both the amount and activity of C.O. in neurons of the lateral geniculate nucleus (LGN), and also served as an extension of previous light microscopic observations of the effect of TTX on C.O. activity in the cat visual system (Brain Res., 261:185,'83). Quantitative analysis of cellular immunolabeling at the light microscopic level and histochemical C.O. activity at the light and EM levels revealed functionally-related enzymatic changes in both the amount and the activity of C.O.. A relative decrease in histochemically-detected C.O. activity correlated with a relative decrease in immunocytochemically-labeled C.O.. In addition, not only were neurons in the normal CNS not all alike, but not all neurons responded alike to the blockage of afferent impulse activity. For example, in the cat LGN, the large cells normally are physiologically more active (Physiol.Rev.,62:738,'82), have a higher level of histochemical C.O. activity (J.Comp. Neurol.,242:338,'85), have a higher amount of C.O., and have more mitochondria per unit of cytoplasmic area. These metabolically more active large neurons were also more affected by the inhibition of afferent input than the smaller, less active neurons. At the EM level, the overall decrease in cellular activity and amount of C.O. observed at the light microscopic level appeared to be mainly due to changes in mitochondrial distribution. These alterations were consequences of blocking retinogeniculate impulses which normally maintain the "driving force" or presynaptic input to the LGN neurons. These neurons may, in turn, reduce their output to the striate cortex, thereby decreasing their oxidative demand for energy. The large cells being metabolically more active normally, were more affected than the smaller, less active ones. In summary, the maintenance of the functional and enzymatic integrity of post-synaptic geniculate neurons in the adult cat, as demonstrated by decreases in both the amount and activity of C.O., appears to be dependent upon presynaptic input. (Supp. by NIH NS18122 and EY05439).

241.8 ULTRASTRUCTURAL STUDIES OF THE PRIMATE LATERAL GENICULATE NUCLEUS: RETINAL, SUPERIOR COLLICULAR AND PARABIGEMINAL INPUTS TO THE SMALL-CELLED LAYERS. S. Feig*, D. Van Lieshout* and J.K. Harting* (SPON: A. Berman). Dept. of Anatomy, Univ. of Wis., Madison, WI 53706.

We have used electron microscopic anterograde autoradiography to analyze the synaptic relationships of retinal (RT), superior collicular (SC) and parabigeminal (PB) axon terminals within the small-cell, koniocellular layers of the Galago lateral geniculate nucleus (LGN). These koniocellular layers are quite large in Galago and are an important component of the small-cell retino-cortical channel. Thus, they receive predominantly W-cell input from the retina and project to the supragranular layers of area 17. They also receive substantial projections from the superficial layers of the superior colliculus and from the parabigeminal nucleus [Harting et al., Brain Res. 366(1986) 358-363].

At the ultrastructural level, koniocellular RT's have a very distinctive appearance. They are very closely clustered and are small in comparison to RT's in the parvocellular and magnocellular layers. Koniocellular RT's are almost equally presynaptic to pleomorphic vesicle-containing elements (f2) and small-diameter dendritic shafts and spines. Many of the postsynaptic targets of RT's in the koniocellular layers are targets of other RT and f2 terminals. However, we have never observed an f1-type terminal (another profile with pleomorphic vesicles) presynaptic to one of these retinal targets, even though f1-type endings are present in the koniocellular layers.

A preliminary analysis of the interlaminar zones reveals many of the ultrastructural features described above for the koniocellular layers. Available data regarding SC and PB inputs indicate that these terminals contain round vesicles and dark mitochondria and make asymetrical contacts.

RETINAL TERMINAL	%RT CONTACTING POSTSYNAPTIC TARGET			χTERMINAL
	DENDRITIC SHAFT	DENDRITIC SPINE	F2	DIAM.(μm)
magno	96%	12%	44%	2.37±.48
parvo	84%	36%	21%	1.64±.48
konio	57%	49%	49%	.93±.32

Supported by NIH Grant #EY01277.

241.9 LATERAL PULVINAR RESPONSES RELATED TO REWARD AND STIMULUS SIGNIFICANCE. D. Burman and L.A. Benevento. Dept. of Anatomy, Univ. of Illinois at Chicago, Chicago, IL 60680.

Single-cell recording was undertaken in the lateral pulvinar (PL) of a behaving monkey (Macaca fascicularis). Prior to recording, the monkey had been trained on a series of visuomotor tasks. In a form discrimination task, the monkey was required to discriminate between a circular and a square spot in order to anticipate the direction and timing of a saccade required later in the task. The monkey had to anticipate the direction and timing of the saccade in order to consistently perform the task correctly, due to limitations on the time allowed for completion of the saccade. In the original discrimination task, the discriminandum (S1) appeared two seconds after the appearance of the initial fixation spot (i.e., S1 appeared at t=2), then remained onscreen until completion of the task. Another spot (S2) appeared at t=4, and the monkey had to decide whether to saccade to S1 at t=4 or to S2 at t=6 based upon whether S1 was circular or a square. As reported earlier (Burman and Benevento, Soc. Neurosci. Abstr. 11: 234, 1985), some PL cells responded preferentially to the S1 circular spot. These cells also responded to other cues that signified the monkey should prepare to saccade within two seconds (such as the S2 circle and the reward); the responses to these cues may be termed time-dependent. Other PL cells have been found which had direction-dependent responses. The response of these cells to S1 depended on the direction of the saccade which would follow, as signified by the combination of the location and form of S1. In addition, PL responses during performance of a modified form discrimination task have been analyzed. In the modified task, S1 appeared at t=2 for only 30 ms, reappeared at t=4, and remained onscreen once the monkey was cued to saccade promptly. A tonic response to S1 could not be a purely visual response, since it did not depend on the presence of a visual stimulus or its offset; such a response would be related to the significance of S1. Some PL cells did indeed have a tonic response to S1 in the modified task, and many of these cells also responded during the reward period. The reward also served as a cue for a saccade, since the monkey consistently moved its eyes shortly after the reward period ended; this saccade was typically in the direction opposite that required by the task. (Thus, if the monkey moved its eyes to the right during the task, it would move its eyes back to the left after the reward.) Although the reward-associated responses of many PL cells could be related to the direction or timing of the saccade which would follow, a few cells responded more vigorously to the reward than to any visual stimulus or to any saccade. A hypothesis of PL function is expounded, which suggests that PL is involved in the analysis of stimulus significance and increasing the efficiency of cortical mechanisms involved in the selection of saccade targets.

241.10 VISUAL RESPONSES OF NEURONS IN THE PULVINAR OF THE MACAQUE. John W. McClurkin, Caroline Kertzman, and David Lee Robinson. Lab. of Sensorimotor Res., National Eye Inst., Bethesda, MD 20892, USA.

Most neurons in the two retinotopically organized areas of the pulvinar are visually responsive. However, many have weak and/or inconsistent responses to identical presentations of a stimulus. We have observed that brief and rapidly flashing stimuli are very effective in locating and delineating visual receptive fields. Here we have studied the responses of pulvinar neurons to stroboscopic stimuli presented at frequencies from 1 to 8 pulses per second and compared the results with those found for the same cells to oscillating stimuli. We also tested the responses of cells in the superficial layers of the superior colliculus and parvocellular layers of the lateral geniculate nucleus to stroboscopic and oscillating stimuli.

All neurons were studied in awake, trained rhesus monkeys while they fixated a spot of light throughout the periods of stimulation of the visual receptive fields. We found a subset of pulvinar cells which responded weakly, if at all, to a stationary stimulus presented in the visual receptive field for over one second. When a similar stimulus was flashed briefly (10 microsec) at rates of one or two per second, there was still only a weak response. When the same stimulus was flashed at rates of from 4 to 6 per second, there was a dramatic and consistent response entrainment from the cell. This response consisted of a brief burst of spikes following each flash of the stimulus. We never observed a tonic or prolonged elevation in activity associated with our stroboscopic stimuli. The responses of these cells declined markedly to presentation rates of over 6 pulses per second. Thus the rate at which the strobe entrained the cell was very finely tuned. Furthermore, there was an augmentation of the response to the optimum presentation rate. The first and second stimuli in any individual train of pulses evoked much weaker discharges than later ones. When the same cell was tested with a comparable stimulus which oscillated back-and-forth across the visual receptive field, there was no similar frequency selectivity or discharge augmentation. When these same tests were conducted on visually responsive cells in the superior colliculus and lateral geniculate nucleus, we did not observe the selectivity for stimulation frequency. Furthermore, we did not find neurons which showed response augmentation with repetitive stimulation. In fact, most cells had a response decrement.

It is clear from these studies that there is a population of cells in the pulvinar which is very responsive to repetitive stimulation at about four per second. Although the frequency selectivity probably depends on synaptic processes in the pulvinar, its unique presence in this area may reflect the function of this part of the visual system.

241.11 THE MACAQUE PULVINAR: INCREASES IN VISUAL SENSITIVITY FOLLOWING SACCADIC EYE MOVEMENTS. David Lee Robinson, Caroline Kertzman, and John W. McClurkin. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892, USA.

Previously we have shown that the activity of many cells in the pulvinar is altered in association with saccadic eye movements. This can be either an excitatory or inhibitory pattern. Furthermore, the activity change also follows saccades to remembered locations in the dark, thus ruling out visual signals as its source. In this study, we sought to determine whether the sensitivity of pulvinar cells to visual stimuli changes following saccadic eye movements.

Monkeys were trained to fixate a spot of light and to make saccadic eye movements to peripheral targets. Eye position was monitored by computer with a scleral search coil. Stimuli were positioned with a pair of mirror galvanometers controlled by the computer and could be stabilized on any part of the retina. Stimulus onset was triggered from the start of the eye movement in saccade trials or at least 500 msec after the monkey achieved fixation on the standard fixation trials. The responses of cells to a receptive field stimulus during fixation were compared to their responses to comparable stimuli presented at varying delays after saccadic eye movements. We found an increase in visual sensitivity following eye movements in about one third of the cells sampled in the two retinotopically organized areas of the pulvinar. There was considerable variability in the time course of the increase in sensitivity. For some cells, visual responsivity was at a maximum immediately after the eye movement and declined to fixation levels over 500 milliseconds. For others, maximum sensitivity was not reached until 250 to 500 msec after the saccade. Finally, in some cells, there was a complex interaction between the post-saccadic changes in spontaneous activity and the increase in visual sensitivity.

New information is processed by the visual system following saccadic eye movements. The function of increased visual sensitivity in the pulvinar, therefore, may be to alert the cortex to the presence of new stimuli.

241.12 CORTICAL PROJECTIONS TO THE CAUDAL SUBDIVISION OF THE LATERAL POSTERIOR-PULVINAR THALAMIC COMPLEX IN THE CAT. M.L. Rodrigo-Angulo and F. Reinoso-Suárez. Dpto. Morfología. Fac. Medicina. Universidad Autónoma de Madrid. 28029 Madrid. Spain.

In the cat thalamus, the most caudal part of the lateral posterior-pulvinar thalamic complex (LP-Pu) has been defined by Graybiel and Berson (Neurosci.,5:1175-1238, 1980) as the LP-Pu extrageniculate visual subdivision. It is formed by the following nuclei:caudal part of the pulvinar (Pu), lateralis posterior lateralis (LPL), lateralis posterior medialis (LPM) and lateralis medialis-suprageniculate complex (LM-Sg). Based on cytoarchitectonical and histochemical data we have delimited these nuclei, have separated LM from Sg and excluded the latter. The aim of this study was to elucidate the organization of the cortical afferent connections to each one of those nuclei using the retrograde HRP transport method. By means of glass micropipettes, stereotaxically guided injections of an aqueous solution of HRP (20%) were made in the various nuclei in 10 adult cats. Our results demonstrate that caudal LP-Pu receives abundant cortical projections which amount of an average of 79.05% with respect to the total number of afferents from the telencephalon, diencephalon and brainstem. In the case of LPL, the percentage of cortical afferents reached 93.0% of the total. However, no one or just a few of these projections come from the contralateral hemisphere. We did not find any specific projection from given cortical areas to cytoarchitectonically defined nuclei, although preferences could be observed. The Pu receives projections mainly from visual cortical areas 17,18,19,20, and 21. The lateral suprasylvian visual areas (LS), the visual splenial area (AVS) and the retrosplenial area (Rs) also project to the Pu. Visual areas also represent the main source of projections to LPL. Area 17 projects most heavily upon LPL, which also receives projections from areas 18,19,20,21,and the posteromedial (PmLS), posterolateral (PlLS), dorsal (DLS) and ventral (VLS) subdivisions of the LS. Sparse projections were also detected from area 36, Rs, and posterior suprasylvian area (Ps). The LPM receives a rather uniform projection from cortical areas 17,18, 19,20,21,DLS,PlLS,Ps, and Rs. Different sectors of LM exhibit specific projection patterns. The dorsolateral sector receives its main projection from the insular granular and agranular areas, the visual area of the anterior ectosylvian sulcus, caudal part of the sylvian sulcus, cortical areas 5 and 7, AVS, and cingulate area (Cg). Less abundant projections arise from 18,19,20,21,4,6, anterior limbic (LA) and prelimbic (Pl) areas, and from prefrontal cortex (Pf). The ventromedial sector of LM receives projections from the insular agranular cortex, PF, LA, Cg, Pl and area 5, while projections from visual cortical areas were absent. Overall, we observed a shift of cortical labeled neurons, from caudal to rostral areas, corresponding to a sequence, from lateral to medial, on the caudal LP-Pu. Supported by Grant CAYCT 512/84.

241.13 RETINOHYPOTHALAMIC PATHWAY REVISITED IN MAMMALS: AN UNUSUAL IPSILATERAL ASYMMETRY IN PRIMATES. H.M. Cooper*, G. Mick*, M. Magnin* (SPON: H. Kennedy), INSERM-Unité 94, 69500 Bron, France.

In mammals the proportion of decussating and non-decussating retinal axons within the optic chiasm varies according to the degree of frontal orientation of the optical axes of the eyes. In animals with laterally placed eyes, the majority of retinal axons cross in the chiasm, whereas in animals with frontally directed eyes such as primates, the number of crossed and uncrossed fibers is approximately equal. However, the bilateral distribution of retinal efferents terminating within a given primary visual nucleus is not strictly related to the degree of decussation and, in the case of the primate retinohypothalamic projection we find that the density of retinal terminals is greater ipsilaterally.

We studied retinal projections in both strepsirhine (Microcebus murinus, Galago alleni) and haplorhine (Callithrix jacchus, Macaca fascicularis, Hylobates concolor) primates as well as in non-primate species from 7 other orders of mammals using anterograde transport of tritiated amino acids or HRP injected into the vitreous of one eye. The density and spatial distribution of label was quantified using computerized image density analysis. The pattern of distribution of anterograde label in various visual nuclei agreed with classical descriptions of retinal projections in each species, indicating that the tracers were transported normally from all areas of the retina. However, in primates the amount of label (silver grains or HRP granules) throughout the suprachiasmatic nucleus (SCN) was greater ipsilateral to the injected eye. This was particularly striking in the gibbon and in the macaque where the ipsilateral density of label was 6 to 9 times greater than contralateral label. By contrast retinal input to the SCN in rodents, carnivores and marsupials was predominantly contralateral. In megachiropteran bats, edentates and pholidotes label was equally distributed on both sides of the SCN.

The results demonstrate that, in contrast to other primary visual nuclei, the retinohypothalamic projection in primates is the only visual sub-system with a distinct ipsilateral bias. Whether this reflects a higher proportion of ganglion cells in the ipsilateral retina or a difference in the densities of ganglion cell terminal arborizations in the SCN requires further investigation.

241.14 NONRETINAL AFFERENTS TO THE MEDIAL TERMINAL ACCESSORY OPTIC NUCLEUS IN THE RABBIT. R.A. Giolli, R.H.I. Blanks and Y. Torigoe. Dept. of Anatomy and Neurobiology, Coll. Med., Univ. Calif. Irvine, CA 92717.

The distribution and density of nonretinal afferents to the rabbit medial terminal accessory optic nucleus (MTN) have been determined after injections of horseradish peroxidase (HRP) into the MTN in seven rabbits, and verification of certain of these projections has been made in rabbit and rat utilizing anterograde transport of tritiated leucine or leucine/proline from appropriate injections into cerebral cortical areas and brain stem nuclei.

Horseradish peroxidase labeled neurons have been identified: (A) In four visual and/or oculomotor nuclei in which available autoradiographic brain series have confirmed the presence of afferent projections to the MTN: (1) The nucleus of the optic tract/dorsal terminal accessory optic nucleus, (2) the interstitial nucleus of the superior fasciculus (posterior fibers), (3) the periaqueductal gray (including its supraoculomotor portion), and (4) the pars medialis, deep mesencephalic nucleus. (B) In the ventral lateral geniculate nucleus, in which a projection to the MTN has been confirmed autoradiographically in the rat by others. (C) In brain stem nuclei and cortical areas in which available autoradiographic brain series fail to confirm the presence of MTN afferents: (1) The nucleus reticularis pontis, pars oralis and pars caudalis, (2) intermediate intersitial nucleus of the medial longitudinal fasciculus, (3) nucleus raphe pontis, and (4) five cortical areas (area retrosplenialis granularis dorsalis, striate area, parietal area 3, subicular cortex and regio praecentralis granularis). (D) In other nuclei, in which autoradiographic brain series are unavailable for study, but others have failed to confirm the presence of these afferents to the MTN suggesting a false-positive interpretation with neuronal labeling probably resulting from spread of HRP into ventral tegmental nuclei adjacent to the MTN. Thus in the medulla and pons, labeled neurons are found in the medial, lateral and superior vestibular nuclei, medullary reticular formation including nucleus reticularis gigantocellularis, lateral reticular nucleus, raphe magnus, spinal nucleus of V, nucleus gracilis/cuneatus, dorsal and ventral division of the parabrachial nucleus, central pontine gray, nucleus K of Meessen and Olszewski, and dorsal nucleus of the lateral lemniscus. In the midbrain labeled cells are present in the intersitital nucleus of Cajal and strata griseum intermedium and profundus of the superior colliculus. In the diencephalon, labeled neurons are found in the posterior and medial pretectal nuclei, zona incerta, and ventral lateral geniculate nucleus. Labeled somata also are observed in the caudate-putamen, fundus striati, lateral preoptic area and lateral hypothalamic nucleus of the telencephalon. These nonretinal afferents to the MTN probably function in the fine tuning of visual-oculomotor information. (Supported by NIH grant EY03642 and NSF grant BNS 8612919).

241.15 VISUAL CORTICAL PROJECTIONS TO THE CAUDATE NUCLEUS IN MACAQUE MONKEY. W. Fries* and A.-M. Lehmann* (Spon. U. Buettner). Dept. of Neurology and Institute for Medical Psychology, Ludwig-Maximilians-University,Munich (FRG).

The cerebral cortex has been recognized as a major source of input to the caudate nucleus. The precise mode of corticostriatal connectivity is still an issue of debate; concepts of both rostrocaudal as well as mediolateral arrangements have been proposed (Kemp and Powell, Brain 93, 1970; Selemon and Goldman- Rakic, J. Neurosci. 5, 1985). We have studied the termination of efferent fibers from striate and prestriate visual areas in the caudate nucleus using anterograde axonal transport of HRP or WGA-HRP after cortical injections and subsequent TMB-histochemistry. Terminal fields of visual corticostriatal fibers were located in the rostrocaudal part of the caudate, confirming previous reports. However, depending on the injection site, major differences in their topographical arrangement were found with respect to their rostrocaudal extent. Striate cortex injections into central visual field representation resulted in none or little labelling, whereas injections into far periphery yielded terminal fields of moderate rostrocaudal extent suggesting a gradient of strength in the projection from central to peripheral similar to that of corticopontine projections (Fries, Neurosci. Abstr. 7, 1981). When area V4 was injected, the terminal fields were restricted to a small region of caudal caudate. Yet, injections into neighbouring area V5 yielded substantial labelling extending from dorsal to ventral caudate. Thus, neighbouring areas of visual cortex do not project to neighbouring parts of caudate, as one would expect following the topographical concept, but overlap with respect to their rostrocaudal extent. In contrast, the visual area in the posterior bank of intraparietal sulcus (POa) and the frontal eye fields were found to project throughout almost the entire rostrocaudal extent of the caudate. Hence, both topographical as well as nontopographical principles govern the mode of termination of efferents from visual and visually dominated cortical areas. These findings are relevant in view of recent findings of visually and eye movement triggered responses of caudate neurons in the awake monkey (Hikosaka and Sakamoto, Exp. Brain Res. 63, 1986).

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241.16 SIZE-DIFFERENCE THRESHOLDS AFTER LESIONS OF VISUAL THALAMIC NUCLEI IN PIGEONS. C. Kertzman and W. Hodos. Dept. of Psychology, Univ. of Maryland, College Park, MD 20742.

Nucleus rotundus (Rt) and nucleus dorsolateralis posterior (DLP) are the thalamic components of two parallel ascending pathways within the tectofugal division of the pigeon visual system. An earlier study (Hodos, Weiss, & Bessette, 1986) had shown that lesions of the telencephalic components of these pathways produced postoperative elevations in size-difference thresholds only if the lesion included both structures. What was not revealed by their study was whether the integrity of the thalamic components is necessary for pigeons to discriminate small differences in the size of stimuli or whether the birds could still make the discrimination with only one of the two nuclei intact. Moreover, no prior behavioral evidence existed to indicate that DLP plays a role in visual information processing.

Fourteen pigeons were tested preoperatively using a variant of the method of constant stimuli to determine the smallest difference between the size of two annuli that the subjects could discern. The comparison stimuli ranged from 3.5 mm to 15 mm in diameter. After surgery, in which lesions were placed bilaterally in Rt, DLP, or both structures, subjects' size-difference thresholds were again determined. Combined lesions of Rt and DLP resulted in impaired psychophysical performance, which was characterized by initial elevations in threshold followed by a gradual improvement in performance. Some birds eventually returned to their preoperative level. In contrast, subjects with lesions in Rt or DLP alone showed an immediate return to their preoperative sensitivity levels.

These results indicate that both nuclei can process information about the size of visual stimuli. Moreover, the processing that occurs within either nucleus is sufficient for the pigeon to discriminate size differences. These data provide the first behavioral evidence that DLP participates in visual information processing and suggest that DLP may be specialized for the visuomotor control of feeding.

241.17 VISUAL RESPONSE CHARACTERISTICS OF CELLS IN NUCLEUS ISTHMI OF PIGEONS. Wang, Yong-Chang* and Frost, B.J., Departments of Physiology and Psychology, Queen's University, Kingston, Ontario K7L 3N6.

In the parvocellular division of the avian nucleus isthmi (Ipc) neurons receive an input from the optic tectum, and in turn project back to that structure (Hunt, Streit, Kunzle and Cuenod, 1977). We have employed standard extracellular recording techniques and computer generated stimuli to study the visual response properties and retinotopic organization of Ipc in urethane anesthetised pigeons.

From sequential penetrations in stereotaxic coordinates it was determined that the rostral regions of Ipc receive projections from the nasal areas of the visual field, while the temporal visual field is mapped onto the caudal pole. Also, the upper visual field is mapped onto the more dorsal aspects of the nucleus and the lower visual fields onto the more ventral aspects of the structure. The receptive fields of Ipc units are quite large ranging from 15 degrees in diameter to over 90 degrees, with more frontally located RFs exhibiting an oval shape where the long axis is oriented approximately vertical. In spite of their large size all RFs contained a "hotspot" where they responded vigorously.

All Ipc cells were driven optimally by moving black or white stimuli; direction of contrast being unimportant. Directional tuning curves were quite broad, and like tectal neurons, characterised by a distinct null for nasal to temporal directions of motion in the visual field. Within the receptive fields there were often small subregions which responded to the off-set, or onset and off-set of light spots, although these responses were relatively weak. Size tuning curves revealed that most units preferred stimuli about 2° diameter. Ipc neurons did not respond to whole-field motion or diffuse changes in illumination. Ipc units also respond well to kinematograms and some responded only to the leading edge of kinematographic bars moved in the direction of their long axes. Most Ipc cells appear to have inhibitory regions surounding their ERFs where additional moving stimuli totally inhibit responses. In some instances these zones do not extend completely around the ERFs.

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241.18 EFFERENT CONNECTIONS OF THE LENTIFORM NUCLEUS OF THE MESENCEPHALON IN CHICKEN. Stefan R. Bodnarenko and Olivia C. McKenna. Biology Dept., City College, N.Y., N.Y. 10031 and Biopsychology Program, Hunter College, N.Y., N.Y. 10021.

The avian lentiform nucleus of the mesencephalon (LM) and the mammalian nucleus of the optic tract (NOT) are two pretectal retinorecipient areas known to receive large field retinal slip signals and to play a role in the generation of horizontal optokinetic eye movements. The notion that the LM and NOT are homologous structures is based on their similar embryological derivation and their position at the mesodiencephalic border. Further evidence for their homology is based on the similarity of their afferents which originate from the retina, visual telencephalon, and accessory optic system (AOS). In this study we have explored the efferent projections of the LM in 6 week old chickens using anterograde tract tracing techniques to determine whether the homology of the LM to the NOT extends to their efferent projections. After iontophoretic injections of HRP or tritiated amino acids into the LM, three groups of axons were seen leaving the injection site traveling caudally, but each in separate directions. One group of axons which exited from the lateral LM, directly entered the adjacent optic tectum and coursed through the inner layers to terminate in lamina 6. Another group of axons which exited from the dorsal LM, moved medially and coursed around the dorsal and ventral surfaces of the lateral spiriform nucleus, to reach the dorsal pretectal nuclei---the pretectal nucleus, principal precommissural nucleus, and pretectal area. Some fibers from this group crossed to contralateral dorsomesencephalic areas via the posterior commisure. Other fibers from this group terminated in the nucleus of Darkschewitsch. The third group of axons, which exited from the ventral LM, projected caudally and terminated in the three subdivisions of the nucleus of the basal optic root (nBOR) of the AOS. Caudal to the nBOR, fibers were followed ipsilaterally to their terminations in the ventral portion of the nucleus of the deep mesencephalon, the medial pontine nucleus and the inferior olive. In addition, axons were seen entering the cerebellum bilaterally in the brachium conjunctivum.

The targets of the avian LM are remarkably similar to those of the mammalian NOT. Both provide projections to the cerebellum indirectly via the medial pontine and inferior olivary nuclei; the LM also contributes a direct projection. Both the LM and NOT project to the nucleus of Darkschewitsch providing an indirect pathway to the vestibular nuclei. In addition, both project to other visual areas---pretectal nuclei, optic tectum and AOS. The similarity of these efferent targets further strengthens the idea that the LM is homologous to the NOT. (Supported by NIH EY 03613).

BIOLOGICAL RHYTHMS V

242.1 PRENATAL ENTRAINMENT BY MELATONIN: THE PREDICTED PHASE RELATIONSHIP BETWEEN HAMSTER MOTHER AND FETUS. F.C. Davis. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

Evidence of the last 10 years indicates that the rodent suprachiasmatic nucleus (SCN) is entrained by maternal rhythmicity before birth. Although the mechanism for fetal entrainment is not known, there is evidence that a rhythm in melatonin can mediate prenatal entrainment in Syrian hamsters; daily injections of melatonin during gestation can set the phase of activity rhythms measured after birth (Davis and Mannion, 1986). This result has now been repeated with a dose of melatonin smaller than those used in initial experiments (10 vs 50 or 100 ug/day), and results now show that the phase established by melatonin injections is the same phase that would be predicted to be established by the mother's endogenous melatonin rhythm if it caused entrainment. As in earlier studies, pregnant Syrian hamsters (Mesocricetus auratus) were maintained in constant dim light and received SCN lesions on day 7 of gestation. On days 8 to 15 they were given subcutaneous injections of sesame oil every 12 hours (1100 and 2300 hours). One group received melatonin at 1100 hrs and the other at 2300 hrs. Pups remained in dim LL after weaning (PN 20) and were placed in individual running-wheel cages to measure the phase of their activity rhythms (activity onset as phase reference). With 10 ug/day, the average phase of 6 litters (31 pups) exposed at 2300 was 18.41 hrs, and 2 litters (9 pups) exposed at 11.00 was 5.79 hrs, for an average difference of 11.38 hrs. The average phase of all litters exposed at 2300 (10, all doses) was 18.84 hrs and that of all litters exposed at 1100 (9) was 6.77 hrs. The average postweaning freerunning periods were the same for both groups of pups, 24.20 hrs. The average freerunning period and the average phases at weaning were used to determine the hypothetical phases of the pups on the last day of melatonin injections (day 15 of gestation). The phases were 14.84 for the 2300 group and 2.77 for the 11.00 group so that activity onset preceeds the melatonin injection by 8.16 hrs and 8.23 hrs, respectively. This is the approximate phase relationship between activity onset and the peak of the endogenous pineal melatonin rhythm in Syrian hamsters (Elliott and Tamarkin). Because the activity onsets of mothers and pups are approximately in phase at weaning (Davis and Gorski, 1985), this phase relationship may also exist for the mother and fetus. If so, the relationship between the activity onset phase of the fetal pacemaker and the mother's melatonin rhythm is predicted to be approximately 8 hrs. Evidence that the phase relationship between exogenous melatonin and the fetal pacemaker is also 8 hrs, supports the hypothesis that melatonin is a physiologically important signal for fetal entrainment. Supported by NIH grant HD-18686 to F.C.D.

242.2 PRECOCIOUS SPINY MICE AS A MODEL TO ASSESS THE POTENTIAL FOR RETINA-MEDIATED LIGHT PERCEPTION IN UTERO. S.L. Jacques*, D.R. Weaver, and S.M. Reppert. (SPON: E.J. Hunnicutt, Jr.). Department of Dermatology and Children's Service, Massachusetts General Hospital & Harvard Medical School, Boston, MA 02114.

The mammalian fetus is generally considered to be isolated from environmental lighting. The light-dark cycle during the prenatal period does influence physiology and behavior of the developing animal, however. This occurs by indirect mechanisms, in which the dam senses the lighting cycle and communicates relevant aspects of the cycle (e.g., phase, day length) to the fetus. Direct perception of environmental lighting by the fetus in utero has not been extensively investigated.

In a recent paper, we studied the penetration of light into the uterine lumen in pregnant albino rats and guinea pigs with a surgically implanted optical fiber (Photochem Photobiol 1987 45: 637). Transmission was greatest for wavelengths > 600 nm because of lower attenuation by tissue absorbance. Interestingly, there was also a band of increased transmission at around 500 nm, with approximately 2% of incident light reaching the lumen of the uterus. This wavelength corresponds to the wavelength of maximum sensitivity to light in rodents, and the peak in the action spectrum of rhodopsin. It thus appears that light of biologically relevant wavelengths does reach the fetus within the uterus, and it may be sufficiently intense to be perceived.

Perception of light depends not only on light being present, but also on the presence of sufficiently developed receptive tissues. We are studying the development of the circadian timing system and its responsiveness to light in a precocious rodent, the spiny mouse (Acomys cahirinus). The retinohypothalamic tract, the primary pathway for entrainment of circadian rhythms, is clearly present within the suprachiasmatic nuclei (SCN) of spiny mouse pups on the day of birth. Pups were injected with horseradish peroxidase (30%, 4 ul per eye, bilaterally, under anesthesia) within 24 h of birth; survival time was 24 h. Thus the anatomical substrate for detection of light by the circadian timing system is present at birth, and may be present earlier.

To determine whether the retinohypothalamic tract is functional on the day of birth, we used 2-deoxyglucose to study the metabolic activity of the SCN. In both adult and fetal spiny mice, metabolic activity of the SCN is higher during subjective day than at night. On the day of birth, exposure to light at night increased the metabolic activity of the pup SCN compared to siblings not exposed to light. This indicates that environmental lighting is perceived by the circadian system of the pup on the day of birth. The stage is thus set to examine whether light reaching the fetal spiny mouse is perceived, and whether prenatal light perception is physiologically important. Supported by AM25395 and HD14427.

242.3 VOLUNTARY EXERCISE (EX) PREVENTS THE ANESTRUS INDUCED BY EXOGENOUS MELATONIN (MEL) IN PINEALECTOMIZED (PX) FEMALE GOLDEN HAMSTERS. D.R. Pieper, C.A. Lobocki*, D. Samuels* and K.T. Borer. Providence Hospital, Department of Physiology, Southfield, MI 48037 and The University of Michigan, Department of Kinesiology, Ann Arbor, MI 48109.

Female golden hamsters become anestrus after about 10-15 weeks in a short photoperiod. This anestrous state is a result of altered MEL secretion from the pineal, and a similar cessation of estrous cyclicity results from twice daily MEL injections into PX hamsters on long photoperiod. A recent study has indicated that access to a running wheel reverses the anestrus associated with short photoperiod in golden hamsters. The present study tested whether EX would also prevent the anestrus induced by exogenous MEL injection in PX hamsters on long photoperiod.

Forty 50 day old golden hamsters were PX and allowed to recover for 2 weeks. Twenty of the animals were then placed in running wheel cages (EX) while the other 20 were left in suspended cages without wheels (sedentary, SED). One week later, one half of each group began receiving 15 ug MEL s.c. at 10 a.m. and 4 p.m. daily, while the other half was injected with vehicle (VEH). Estrous cyclicity was followed for 7 weeks, and all animals were then sequentially bled through atrial catheters for determination of serum LH levels.

Sixty-70% of the SED MEL group became anestrus after 4 wks of MEL injections and remained acyclic for the remainder of the experiment. On the other hand, at least 90% of the hamsters in all of the other 3 groups (including the EX MEL group) continued having normal estrous cycles throughout the experiment. The SED MEL hamsters that became acyclic had daily surges in serum LH peaking at 8-11 hrs after lights on, but all other animals in all groups had LH surges only on the afternoon of proestrus.

These results indicate that the effect of exercise on short photoperiod induced anestrus are not due to an influence of exercise on the secretion of MEL from the pineal. Rather, EX somehow influences the site of action of MEL. Supported by NSF Grant #DCB 8509689 to DRP and #DCB 8502902 to KTB.

242.4 EVIDENCE FOR A SEX DIFFERENCE IN THE FUNCTIONAL ANATOMY OF HYPOTHALAMIC CIRCUITS INVOLVED IN SEASONAL REPRODUCTION IN HAMSTERS. L.L. Badura*, C.L. Sisk, and A.A. Nunez, Psychology Dept./ Neuroscience Prog., Michigan State University, E. Lansing, MI, 48824.

In hamsters of both sexes, exposure to a nonstimulatory photoperiod (<12.5 hr light/24 hr day) results in gonadal regression and reproductive quiescence (Science, 158: 925-928, 1967; Biol. Reprod., 12: 223-231, 1974; Science, 191: 197-199, 1976). The suppression of reproductive functions in nonstimulatory photoperiods involves the pineal product melatonin (Biol. Reprod., 20: 32-50, 1979). The current model of the pathway by which photic information reaches the pineal gland includes the suprachiasmatic (SCN) and paraventricular (PVN) nuclei of the hypothalamus (Science, 227: 714-720, 1985). In male hamsters, horizontal knife cuts that either interrupt connections between the SCN and the PVN or projections travelling dorsal from the PVN prevent testicular regression in short days (Brain Res. Bull., 15: 149-153, 1985; Neurosci. Lett., 61: 261-266, 1985; Brain Res., 370: 102-107, 1986). In the present study, we employed similar knife cuts to investigate the role of hypothalamic pathways in the photoperiodic control of female reproductive physiology.

Cycling female hamsters were initially housed in a long-day (16L:8D) photoperiod. One group of females received a horizontal knife cut aimed either ventral or dorsal to the PVN; another group received sham surgery. After a one week recovery period, half of the animals in each group were transferred to a short-day (6L:18D) photoperiod. Vaginal discharge was monitored daily until all sham-operated animals in 6L:18D showed discharges characteristic of anestrus. The animals were then laparotomized, the uteri bilaterally exposed, and photographed with a Polaroid land camera for determination of uterine width.

Animals with knife cuts located ventral to or through the ventral half of the PVN continued to show regular 4-day estrous cycles in both long- and short-days. Thus, similar to findings for the male, these cuts prevented photoperiod-induced gonadal regression, suggesting that projections from the SCN to the PVN are a component of the neural mechanism modulating pineal responses to photoperiod and thus seasonal reproductive cycles. In contrast, animals with cuts dorsal to or through the most dorsal portions of the PVN and sham-operated animals in 6L:18D ceased to show estrous cycles after 8-10 weeks of exposure to short days, and had uteri significantly smaller than all other groups. Animals with cuts dorsal to the PVN in 16L:8D continued to show estrous cycles and maintained stimulated uteri, indicating the projections interrupted by these cuts are not themselves necessary for the expression of estrous cyclicity. These findings differ from those obtained with males in which knife cuts dorsal to the PVN prevented testicular regression in short-days. Therefore, a sex difference may exist in the functional anatomy of the hypothalamic circuits involved in the control of hamster seasonal reproduction.

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242.5 COMPARISON OF THE EFFECTIVENESS OF SINGLE VS. PAIRED WEEKLY LIGHT PULSES FOR MAINTAINING TESTES IN SYRIAN HAMSTERS. (MESOCRICETUS AURATUS) C.E. McCormack Dept. of Physiology. The Chicago Med. Sch., N. Chicago, IL 60064

It has been proposed that in Syrian hamsters, the photoinducible zone (PIZ) for maintenance of gonadal function coincides with the time interval during which light exposure causes phase-shifts in the circadian rhythm of locomotion (J. Elliott in "Biological Clocks in Seasonal Reproductive Cycles," ed. by B.K. & D.E. Follett, 1981) i.e., the PIZ is within the PRC (phase-response-curve). The following experiment was performed to quantitate the capacity of light pulses given during the PRC to maintain gonadal function. Male, 70 day-old hamsters (LVG-Charles River), housed in individual isolated activity wheels, were exposed for 2 wk to daily 14h photoperiods (400 lux, cool white fluorescent), and then were placed in continuous darkness (DD) and given weekly light pulses until autopsy 9 wk later. Six experimental groups were given either 1 min (1') or 15' light pulses (400 lux) each week at a circadian times (ct) which elicited phase-delays ($-d\emptyset$) (1300-1600 ct), phase-advances ($+d\emptyset$) (1700-2000 ct), or no change in phase (0600 ct) of the locomotor rhythm. A 7th experimental group was given a weekly 0.5' pulse at the onset of spontaneous locomotion (1200 ct) and another 0.5' pulse 10h later (ct 2200). One control group remained in DD, and one in continuous light (LL). Given in the table are mean paired weights of testes (TW)(g/100g body wt±SEM), the % of hamsters with TW > 2.0g, and the mean $d\emptyset$ of the locomotor rhythm in hours. Of the 6 groups given single weekly light pulses, those given 15' pulses at times which produced a $-d\emptyset$ (15' at 1300-1600) or a $+d\emptyset$ (15' at 1700-2000) had larger TW than DD controls or hamsters showing no $d\emptyset$ (0600)($P < .01$). Regardless of when given, 1' pulses produced small $d\emptyset$ and failed to maintain testes. However, 1' of light per week did maintain testes if given as paired 0.5' pulses at times which entrained the locomotor rhythm (1200+2200 ct)($P < .01$ vs DD). These results indicate that while a PIZ does exist within the PRC, the hamster is more sensitive to paired light pulses given at opposite ends of the PRC than to single pulses during the PRC. (Supported by 1-R01-HD-13131 and BRSG-S07-RR05366-24)

Duration (time)	n	TW	%w TW > 2.0g	$d\emptyset$
1' (1300-1600)	9	.92±.31	11	-0.15±.06
15' (1300-1600)	9	1.93±.36	67	-0.32±.08
1' (1700-2000)	9	.73±.32	11	+0.18±.07
15' (1700-2000)	17	1.68±.28	59	+1.19±.13
1' (0600)	8	.27±.05	0	0
15' (0600)	26	.35±.04	0	0
0.5' (1200+2200)	8	1.85±.37	75	0
LL	6	2.68±.09	100	-
DD	6	.47±.20	0	-

242.6 **THE EFFECTS OF FEEDBACK LIGHTING ON THE PHOTOPERIODIC RESPONSE IN THE MATURE DJUNGARIAN HAMSTER.** F.M. Sulzman*[1], H.N. Krum*[2] and J.S. Ferraro[2] (SPON: R. Zec). [2]Dept. of Biol. Sci., SUNY-Binghamton, Binghamton, NY 13901; [1]Div. of Life Sci., NASA, Washington, DC 20546.

Mature male Djungarian hamsters (*Phodopus sungorus*) were housed in cages where the animals had free access to food, water and an activity wheel. These cages were placed in individual light-tight sound-attenuated chambers. The animals were exposed to one of the five following lighting conditions for 43 days: a light-dark cycle of 16 hours of light followed by 8 hours of dark (LD16:8), constant light (LL, 45-75 lux), constant dark (DD, 0 lux), feedback lighting (LD_{FB}, a condition which illuminates the cage in response to locomotor activity) or a feedback lighting neighbor control ($LD_{FB\ NC}$, the animal receives the same light pattern as a paired animal in LD_{FB}, but has no control over it). Locomotor activity was continuously monitored. At the end of the experimental exposure, animals were autopsied. Testicular and sex accessory glands weights were determined. The reproductive and circadian period results are shown in Figure 1 (mean±SEM); weights are in mg/10g total body weight and period lengths are presented in hours.

Figure 1

	Lighting Condition				
	DD	$LD_{FB\ NC}$	LD_{FB}	LL	LD16:8
Paired Testes Wt.	17.3±1.3[a]	115.9±42.9[b]	186.9±14.7[c]	199.4±16.4[c]	191.2±7.8[c]
Sex Acc. Gland Wt.	8.7±1.7[a]	29.5±9.1[b]	29.6±7.6[b]	43.6±6.1[b]	34.1±2.7[b]
N	8	7	8	8	14
Free-Run Period	24.0±0.1[a]	N/A	24.6±0.1[b]	24.7±0.1[b]	N/A
N	15	N/A	12	12	N/A

Means with similar superscripts are not significantly different. Unlike previous results observed in mature Syrian hamsters or immature Djungarian hamsters, LD_{FB} was photostimulatory and thus maintained reproductive function. Furthermore, it is apparent that LD_{FB} not only mimics LL in its ability to maintain reproductive function in the mature Djungarian hamster, but LD_{FB} is also capable of mimicking the period lengthening effects of LL, similar to previous studies in other species. Part of the explanation of why LD_{FB} is more stimulating in the mature than the immature Djungarian hamster, may be that Djungarian hamsters become less responsive to nonstimulatory photoperiods with age (K. Hoffmann, BOR, 32 Suppl. 1:57, 1985). However, this explanation does not account for the differences between mature Djungarian and mature Syrian hamsters. Another difference arises from the fact that $LD_{FB\ NC}$ is photostimulatory in the mature Syrian hamster while it is only submaximally stimulating in mature Djungarian hamsters. It is quite obvious that further work is necessary to determine how these organisms interpret light signals. Supported by NIH grant 1 R01 NS23128-01 (JSF).

242.7 PHOTOPERIODIC HISTORY CONTROLS THE NEUROENDOCRINE INTERPRETATION OF THE PINEAL MELATONIN SIGNAL IN THE MALE SYRIAN HAMSTER.
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Although it is well established that the pineal gland mediates photoperiodic control of neuroendocrine activity through the actions of its hormone melatonin (MEL), the factors which influence interpretation of this nocturnal signal are poorly understood. This study tests the hypothesis that photoperiodic history may control the central response to pineal MEL. Male Syrian hamsters were held on long days (LD, 16L:8D) for 4 weeks and then half of them were transferred to short days (SD, 8L:16D). Seven weeks later, half of the animals in both LD and SD were transferred to 12L:12D (groups LM and SM, respectively) while the rest remained on LD or SD (groups LL and SS). After an additional 7 weeks, animals were sacrificed at intervals through the night, and trunk blood and pineal glands retained.

In all groups, pineal MEL content was low (<100 pg) for the first 4 hr of darkness and then rose rapidly (>250 pg). The duration of the nocturnal peak was directly proportional to the length of the dark phase (LL 4.5 h, LM 8 h, SM 8 h and SS 11 h). There was no significant difference in the MEL rhythms of groups SM and LM. Exposure to SD for 14 weeks caused a marked decline in serum LH (LL 1.49 ± 0.25, SS 0.58 ± 0.09 ng/ml). However, the effect of 12L:12D upon serum LH levels was dependent upon photoperiodic history. Animals entering 12L:12D from LD (LM) showed a decline (0.32 ± 0.09) whereas in animals previously held on SD, 12L:12D stimulated LH release (SM, 2.63 ± 0.50 ng/ml). Serum prolactin (PRL) was also sensitive to photoperiod. After 14 weeks of exposure, animals in LD had higher levels than those in SD (LL 10.68 ± 1.39, SS 0.72 ± 0.05 ng/ml). However, photoperiodic history did not have a significant effect upon serum PRL levels of animals exposed to 12L:12D. In both groups, levels were not significantly different from SD controls (LM 0.98 ± 0.16, SM 1.69 ± 0.24 ng/ml).

This study demonstrates: (a) The duration of the pineal MEL signial is a high-fidelity representation of the length of the dark phase in any photoschedule. (b) At an intermediate daylength, the interpretation of the MEL signal by neuroendocrine systems regulating LH secretion is controlled by photoperiodic history. (c) This effect of photoperiodic history is not apparent in the control of PRL secretion, implying that MEL may be read in several ways and possibly at several sites within the brain.

242.8 PHOTOPERIODIC INFLUENCES ON SEXUAL BEHAVIOR IN MALE SYRIAN HAMSTERS
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Photoperiod history affects the neuroendocrine response to altered daylength. Male Syrian hamsters interpret an LD 12:12 photoperiod as inhibitory or stimulatory, depending on whether they have been previously exposed to long or short days. This differential response is reflected in the secretion of both androgens and gonadotrophins, and in the activity of brain aromatase (see Hastings et al; Hutchison et al; Neurosci. Abstr. 1987). Here the effects of photoperiod history (PPH) on masculine sexual behavior were determined.

Male hamsters remained gonadally intact (I) or were castrated and given subphysiological doses of testosterone (CT) chronically; they were placed in long (LD 16:8) or short (LD 8:16) days for 16 weeks. Sociosexual behaviors were assessed during wks 7 and 15. On wk 8, half the males in long and short days were transferred to medium days (LD 12:12) generating LL, LM, SS,and SM groups; I and CT conditions were equally represented in each of the 4 PP's.

PP treatment affected sociosexual performance on both wk 7 and wk 15 tests, but only among I males; CT hamsters did not differ significantly on any behavioral measure in either test. Among I males, PP did not affect the initiation of copulation; mount latencies were not significantly different between groups. However, on both wk 7 and 15 tests, intromission and ejaculation latencies of LL males were shorter than those of any other PP group ($p < .05$). The proportions intromitting and ejaculating were 9/10, 3/10, 5/9, 7/10 and 8/10, 1/10, 0/9, 2/10 for LL, LM, SS and SM groups, respectively. Anogenital investigation rates were lower among LM and SS, compared to LL males ($p < .05$); LL and SM groups were not significantly different. These findings indicate that short days impaired sociosexual responsiveness, and that LM and SM males behaved differently on some measures even though they experienced the same PP for 8 wks.

PP affected endogenous levels of T among I males. Mean values at sacrifice on wk 16 were 1.01, 0.12, 0.44 and 0.69 ng/ml for LL, LM, SS and SM groups, respectively. T levels were significantly higher among LL than among LM or SS males ($p < .05$). Interestingly, levels of T among the CT hamsters (0.30, 0.41, 0.53 and 0.46 ng/ml) were not significantly different from intact SS levels, yet behavioral performance among the latter group was remarkably impaired. This suggests that T may be much less effective behaviorally among gonadally regressed males, compared to castrates receiving equivalent levels of T. Factors that might account for this difference (T availability patterns; specific testis secretions) are now being investigated.

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242.9 DJUNGARIAN HAMSTERS UNDERGO CHANGES IN REPRODUCTIVE FUNCTION WHEN EXPOSED TO CHANGES IN DAYLENGTH AT PHOTOPERIOD EXTREMES.
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For long-day breeders such as the Djungarian hamster (Phodopus sungorus), long photoperiods (>14L/day) are characterized as stimulatory, while short days (<14L/day) are considered to inhibit reproductive function. More recent evidence suggests that reproductive activity in this species is also a function of an animal's photoperiodic history. However, it is unknown whether changes in photoperiod can induce reproductive changes when the change occurs at extreme photoperiods. We addressed this issue by examining the reproductive response of animals maintained in either (1) extremely long photoperiods and then shifted to a shorter, but "stimulatory," photoperiod, or (2) extremely short photoperiods and then exposed to a longer, but "inhibitory," photoperiod. To determine the extent and temporal sequence of reproductive responses, ultrasonography was employed to determine serial changes in testis size in individual animals.

60-day-old male Djungarian hamsters were assigned to two groups: Group I (n=5) was maintained in 6L:18D for 8 weeks and then changed to 10L:14D for 8 weeks. Group II (n=5) was maintained in 20L:4D and then transferred to 16L:8D for 16 weeks. Using ultrasonography, serial measurements of testis size of individual animals were performed every two weeks by the same observer using a Diasonics Real-Time Scanner with a 10 MHz transducer. Testis size was calculated from ultrasonographic length and width measurements using the equation $\pi/6 \times L \times W^2$. Ultrasonographic estimation of testis size had a coefficient of variation of 8% and closely matched actual testis size (n=22, Y=1.01X + 0.2, R=.98, p<.01).

Both groups responded to changes in photoperiod. Within two weeks of transfer from 6L:18D to 10L:14D, testes of males in Group I increased in size, but complete recrudescence did not occur: individual testes measured 16±0.8 mg (M±SEM) after 8 weeks in 6L:18D, and 106±13 mg after 8 weeks in 10L:14D. Animals in Group II transferred from 20L:4D to 16L:8D experienced a 50% decrease in testis size, with testes decreasing from 254±15 mg in 20L:4D to 132±10 mg after 8 weeks in 16L:8D. Testicular recrudescence subsequently occurred with testes increasing to 411±70 mg after an additional 8 weeks in 16L:8D.

These findings suggest that the reproductive axis of Djungarian hamsters can respond to changes in photoperiod at extreme daylengths that have previously been considered to be either purely stimulatory or inhibitory. Supported by NIH grant HD14427.

242.10 LONG DAY LENGTHS INCREASE BRAIN WEIGHT IN THE SIBERIAN HAMSTER, PHODOPUS SUNGORUS.
N. Spears*, J. Dark & I. Zucker. (SPON: M. Breedlove). Dept Psych., Univ of California, Berkeley, CA 94720.

Male meadow voles reared in long day lengths have heavier brains with greater total DNA content than males reared in short day lengths (Dark et al, Brain Res., 1987). Meadow voles and Siberian hamsters both undergo similar seasonal changes in their anatomy and physiology, many of which can be simulated in the laboratory by varying photoperiod. Here, we investigated whether Siberian hamsters also showed photoperiod-induced inhibition of brain development.

Female hamsters were mated and maintained in long day lengths (16L:8D). Offspring were weaned at 18 days of age, and randomly assigned to a long or short (8L:16D) photoperiod. Autopsies were performed at 60 days of age (42 days after weaning), and testes and uteri weighed. The brains were dissected, weighed and frozen for later determination of DNA content.

Male hamsters reared in long day lengths had greater body weights, testicular weights and brain weights than those reared in short day lengths ($P<0.05$ in each case). Females reared in long day lengths had greater body weights and uterus weights than those reared in short day lengths ($P<0.05$ in each case). Photoperiod did not affect brain weight of females. In long days, males had greater body and brain weights than did females ($P<0.05$ for each comparison). In the short day lengths, there was no sexual dimorphism in body weight, but males had greater brain weights than females ($P<0.05$). The findings will be discussed in terms of changes in cell number, as revealed by DNA analysis.

Male,but not female, Siberian hamsters undergo photoperiod-induced brain changes similar to those described for male meadow voles. However, Siberian hamsters differ from meadow voles in that short day lengths do not eliminate the sexual dimorphism in brain size.

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242.11 ANDROGENS AFFECT BRAIN WEIGHT AND DNA CONTENT IN THE MEADOW VOLE. C.S. Whaling* J. Dark and I. Zucker. Group in Endocrinology and Psychology Department, Univ. of Calif., Berkeley, CA 94720.

Microtus pennsylvanicus exhibit seasonal variation in total brain weight and total brain DNA content; male voles maintained during development in short day lengths (10 h light/day) have smaller brains with less DNA than counterparts kept in long days (14 h light/day) at 70 days of age (Dark et al., Brain Res., 1987). Because testicular activity is also inhibited in short day lengths, we tested the hypothesis that androgens mediate these differences in brain weight and cell number. At 19 days of age male voles were either gonadectomized or sham-operated and intact 19 day old females were implanted subcutaneously with Silastic capsules (Dow Corning #602-305, length of 4 mm) filled with testosterone propionate (Tp) or left empty. At 70 days of age brains were removed and weighed and blood was collected for assay of T. In each experiment voles with reduced plasma T concentrations had significantly heavier brains.

		Plasma T (ng/ml)	Brain Weight(mg)
Male voles:	Controls (N=20)	3.5±1.2	680.0±7.8
	Castrates (N=21)	0.3±.04	708.7±5.9*
Female voles:	Controls (N=20)	0.5±.05	672.3±9.3
	Tp (N=20)	6.8±0.9	647.4±7.0*

*indicates $p<0.05$, two-tailed t-test

Body weight did not differ significantly between treatment groups. Increased brain weight in long day male voles cannot be attributed to increased androgen secretion, which acts to restrain rather than stimulate brain growth.

Supported by NIH Grant HD 02982.

242.12 LONG PHOTOPERIODS REVERSE PRIOR SHORT DAY LENGTH-INDUCED RETARDED BRAIN DEVELOPMENT IN MEADOW VOLES. J. Dark, C.S. Whaling* and I. Zucker. Dept. of Psychology and Group in Endocrinology, Univ. of California, Berkeley, CA 94720.

Meadow voles (Microtus pennsylvanicus) reared in short day lengths are smaller and show retarded reproductive development, compared with controls kept in long day lengths. In addition, their brains are lower in weight with fewer cells (Dark et al, Brain Res., 1987). The effects of short day lengths on body size and reproductive apparatus can be reversed by switching voles to long-days. The purpose of this investigation was to determine whether transferring voles from short to long photoperiods would reverse the previously retarded brain development of male meadow voles.

On the day of birth, dams and their litters were assigned to either remain in the maternal photoperiod (LD 14:10) or were moved to a short photoperiod (LD 10:14). At 70 days of age, half of the voles in each photoperiod were transferred to the opposing photoperiod, thus forming 4 groups: LL-voles in long-days throughout testing, LS-voles initially in long then switched to short-days, SS-voles remaining in short-days throughout testing, and SL-voles switched from short to long-days. All animals were autopsied at 140 days of age: brains were dissected, weighed and frozen for determination of DNA content.

Final photoperiod significantly affected brain mass of male meadow voles: animals kept in long day lengths during the final 70 days of testing possessed significantly heavier brains than those in short day lengths. Voles maintained in short days and then switched to long photoperiods(SL) had heavier brains than animals remaining in short day lengths (SS). Initial photoperiod did not significantly affect brain mass.

Short photoperiod-induced retardation of brain development can be successfully reversed by exposing voles to long day lengths, even at a chronological age when brain growth is ordinarily assumed to be complete.

Supported by NIH Grant HD 02982.

242.13 INTERACTIONS BETWEEN CIRCADIAN BODY TEMPERATURE AND WHEEL RUNNING RHYTHMS OVER THE ESTROUS CYCLE IN ALBINO RATS. S.Kent*, D. Freedman* and E.Satinoff (SPON: J.Malpeli). Dept. of Psychology, University of Illinois, Champaign, IL 61820.

We examined the relationship between body temperature (Tb) and activity in cycling female rats of the Sherman strain. The rats were housed in activity wheels in a room kept at 23 ± 1°C. Tb's were recorded with telemetry and activity counts cumulated and recorded every 10m. Analyses from 5 rats with regular 4-day cycles are reported here. Data were collected for 7-8 cycles in a 12:12 LD photoperiod, and then for 8-9 cycles in 10:14 LD.

In LD 12:12, mean daily Tb was 37.8 ± 0.1°C (± sem) and did not vary more than 0.1°C over the cycle. Highest hourly mean Tb was 39.2 ± 0.2°C and lowest hourly mean Tb was 36.9 ± 0.1°C. Highest mean Tb occurred on the day of proestrus (P). Peak Tb was 0.2-.3°C higher and trough Tb from 0-.1°C lower on P than on any other day in the cycle. In 4/5 rats the acrophase of the Tb rhythm on P was advanced 57 ± 13m compared to the other 3 days. In the fifth rat there was no change. Activity increased a mean of 48 ± 10% on the night of P and an advanced phase (41m) was seen in 4/5 rats. The rat that showed no change in activity phase was different from the one that showed no change in Tb phase.

In LD 10:14, mean daily Tb declined 0.3°C and mean activity declined 24%. The highest hourly mean Tb was 39.0 ± 0.1°C and the lowest was 36.6 ± 0.1°C. Tb and activity phases advanced on P in 4/5 and 2/5 rats, respectively. Mean Tb and mean peaks and troughs were similar to those seen in LD 12:12.

The wheels of 4 rats were then shut for 28 days. Mean Tb declined 0.4°C but differences over the estrous cycle remained, with peak Tb staying 0.2°C higher on the night of P. The most striking change was the decline in Tb amplitude, to a mean peak of 38.0 ± 0.1°C and a mean trough of 36.1 ± 0.1°C. Changes within the estrous cycle remained constant.

These results indicate that 1. Tb and activity increased on the night of P. 2. Tb and activity rhythms phase-advanced on the night of P, but not necessarily together. 3. When the rats had access to the wheel, trough Tb's during the day were significantly higher than when the wheels were shut, even though over 95% of the activity occurred at night.

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242.14 PHOTOPERIOD INFLUENCES ANDROGEN-INDUCED AROMATASE ACTIVITY IN THE BRAIN OF THE MALE SYRIAN HAMSTER. J. Hutchison, R.E. Hutchison*, T.H. Steimer*, M.H. Hastings*, J. Herbert*, J.B. Powers, L. Steel*, and A.P. Walker*. M.R.C. Unit on the Development and Integration of Behaviour and Department of Anatomy, University of Cambridge, U.K., Division of Biology of Growth & Reproduction, University Hospital, Geneva, Switzerland, Department of Psychology, Vanderbilt University, Nashville, U.S.A.

Estrogens, formed locally in the brain by aromatization of androgens, are important in the activation of male behavior. In the male hamster, estradiol-17β (E_2) acts with testosterone to influence mating behavior. Exposure to short photoperiod reduces male behavioral responsiveness to androgen (Powers et al, Soc. Neurosci. Abstr., 1987). Our study examines whether (a) short photoperiod affects aromatization of testosterone (T) in the brain, (b) aromatase activity is related to photoperiodic effects on plasma levels of T, LH, prolactin, (PRO) and pineal melatonin (MEL). The latter provides a representation of the length of the dark phase (Hastings et al, Neurosci. Abstr., 1987).

Estrogen formation in individual brain samples was assayed in vitro using the stereospecific production of ^3H_2O from (1β-^{3}H)T as a measurement of aromatase activity. Male hamsters, either castrated or intact, were maintained on long days (LD, 16L:8D) for 4 weeks and then half were transferred to short days (SD, 8L:16D) for 16 weeks. The other half remained on long days. At the time of castration, a group of SD and LD castrates received subcutaneous silastic implants of T.

Formation of estrogen from T was higher in the preoptic area (POA) than other areas. Despite negligible plasma T levels in LD castrates, POA aromatase activity was in the same range as intact LD males. Aromatase activity did not differ significantly between SD and LD intact or castrated males, indicating that photoperiod does not directly influence basal levels of enzyme activity. There were no correlations between T, LH, PRO, MEL and aromatase activity. However, POA aromatase activity was increased by T in SD castrates. There was no comparable increase in LD castrates. This photoperiodic effect appears to be limited to the aromatization pathway, since production of androgenic metabolites was similar in SD and LD males.

We conclude that (a) aromatization of T is localised in POA, (b) preoptic aromatase activity is not related directly to plasma T in LD males, (c) activity of the enzyme is increased by T in short day conditions. Sensitivity of the aromatase to the inductive effects of T appears to be increased by short photoperiod.

242.15 GONADAL, THYROID AND PINEAL NYCTOHEMERAL RHYTHMS IN THE HYPERPROLACTINEMIC MALE RAT. M.K. Vaughan, D.C. Herbert, G.M. Vaughan*, F.J. Weaker*, A.I. Esquifino*, J.A.F. Tresguerres & R.J. Reiter, Dept. of Cellular & Structural Biology, Univ. Texas Health Science Center, San Antonio, TX 78284 & Institute of Surgical Research, Ft. Sam Houston, TX 78234.

Young, male Sprague-Dawley rats were separated into 2 groups: controls and transplanted. Males in the transplanted group received a pituitary under a kidney capsule from an adult female donor. All transplanted and control rats were kept in a 14:10 LD room (lights on 0600 h) for 3 weeks at which time groups of 8-9 rats were decapitated at 1200, 1600, 2200, 0200, 0400 or 0800 h. Pineal glands, pituitaries (pit) from the eutopic position and sera were collected. Grafted animals had elevated serum prolactin (PRL) concentrations that exhibited a rhythm with the highest values in the light phase. Serum PRL in control animals did not exhibit a significant 24-h rhythm. Eutopic pit PRL content, manifesting a biphasic (12-h) rhythm with crests during the day and night in controls, exhibited a similar pattern in grafted rats though an overall reduction in pit PRL content was seen in the grafted animals. Neither the normal biphasic serum testosterone rhythm nor the normal 24-h rhythm (nocturnal surge) of pineal N-acetyltransferase activity and melatonin content was altered in the hyperprolactinemic rats. Serum thyroxine (T_4) and triiodothyronine (T_3) and their free indices (FT_4I, FT_3I) were highest during the day in control and grafted rats. The overall serum T_4 and FT_4I levels were lower in grafted rats though overall T_3 and FT_3I levels did not differ between grafted and controls. Grafted animals showed an additional nocturnal peak in T_4, FT_4I, T_3, and FT_3I. T_3 uptake (T_3U) values were similar between controls and grafted rats, in both cases exhibiting a fall during the night. Changes in serum thyronines were not explainable by changes in serum binding as assessed by the T_3U, and thus may represent changes in thyroidal secretion of T_4. The rhythms in serum PRL suggest the presence of rhythmic circulating factor(s) capable of influencing ectopic lactotrophs and the reduced eutopic pit PRL content suggests a role for PRL in influencing eutopic lactotrophs in the hyperprolactinemic male rat model. Though circulating testosterone and pineal melatonin synthesis were not altered in this model, thyroid function appeared to be altered (Supported by NSF grant PCM 8118487 and North American-Spanish Joint Committee Grant #CCA 8309108).

242.16 NEUROTRANSMITTER CHANGES ASSOCIATED WITH SEASONAL REPRODUCTIVE ACTIVITY IN THE EUROPEAN STARLING. G.F. Ball, D.L. Allen, and V.N. Luine, Rockefeller University, New York, N.Y. 10021.

European starlings (Sturnus vulgaris), like many songbird species, show dramatic seasonal changes in their response to long photoperiods that stimulate vernal gonadal recrudescence. These changes in responsivity are thought to be mediated in part by variations in hypothalamic neurochemistry. For example, Dawson et al. (1985 J. Endo. 105:71-77) have demonstrated that hypothalamic levels of gonadotropin-releasing hormone (GnRH) are low in starlings refractory to the effects of long days but that GnRH levels increase sharply when the birds have experienced a sufficient number of short days to regain photosensitivity. In order to identify other neurochemical systems that may be involved in the mediation of this differential response to photoperiod, we examined differences in neurotransmitter activity in captive male starlings in different seasonal states. These states consist of birds that are photorefractory, photosensitive, or photosensitive and photostimulated (i.e. with fully developed gonads).

Epinephrine (E), norepinephrine, dopamine, 5-hydroxyindole acetic acid (5-HIAA) and serotonin (5HT) levels were measured in discrete brain areas. Catecholamine turnover rates were estimated following injection of the tyrosine-hydroxylase inhibitor, α-methyl-para-tyrosine. Areas selected for microdissection include regions implicated in the control of reproduction such as the pre-optic area (POA), which contains GnRH cell bodies that regulate pituitary secretion, as well as a number of nuclei that are a part of the sexually dimorphic song control complex, such as Area X.

In the POA and Area X, no differences in monoamine levels were noted between photosensitive and photostimulated birds, so the data were combined. Striking differences were found in serotonin activity in the POA. Levels of 5-HIAA and the ratio of 5-HIAA to 5-HT were higher in refractory birds. This suggests that serotonin may inhibit gonadal recrudescence. In Area X, a highly sexually dimorphic nucleus that has been implicated in the control of song, levels of epinephrine were increased in photorefractory birds. Thus, seasonal variation in brain neurochemistry appears to occur in both hypothalamic and extra-hypothalamic areas implicated in the regulation of hormonal and behavioral changes associated with reproduction. (Supported by HD-12011, Revson Foundation Fellowship, BRSG-S07RR07065, and NIH Training Grant).

NEUROETHOLOGY III

243.1 A REIDENTIFIABLE MOLLUSCAN NEURON RESPONSIVE TO MAGNETIC STIMULI. K. J. Lohmann and A. O. D. Willows. Dept. of Zoology NJ-15, University of Washington, Seattle, WA 98195 and Friday Harbor Laboratories, 620 University Road, Friday Harbor, WA 98250.

Behavioral experiments have indicated that the marine mollusc Tritonia diomedea can derive directional information from the magnetic field of the earth (Lohmann, K. J., and Willows, A. O. D. Science, 235: 331, 1987). We have initiated electrophysiological experiments designed to isolate neurons involved in the detection of the geomagnetic field.

Using a whole animal preparation (Willows, A. O. D., Dorsett, D. A., and Hoyle, G., J. Neurobiol., 4: 207, 1973) surrounded by a Helmholtz coil system, intracellular recordings were made from neurons in the pedal, pleural, and cerebral ganglia while animals were subjected to the geomagnetic field and to imposed earth-strength fields generated by the coil. The results indicate that one large, reidentifiable neuron (left pedal neuron 5) consistently shows enhanced electrical activity in response to changes in ambient earth-strength magnetic fields. Although responses are often subtle, the effect is repeatable and has been elicited in more than 30 animals. Similar increases in electrical activity have not been observed in other pedal neurons exposed to identical magnetic stimuli.

The magnetically sensitive neuron does not have any apparent motor function, fails to respond to conventional chemical or tactile stimuli, and is inhibited during escape swimming. In addition, the whitish pigmentation of left pedal neuron 5 is characteristic of cells that contain neuroactive peptides and function in modulating behavioral state (Masinovsky, B. P., Lloyd, P. E., and Willows, A. O. D., J. Neurobiol., 16: 27, 1985).

243.2 COMPARATIVE SURVEY OF A SMALL-AXON TRACT IN THE CERVICAL CONNECTIVE OF FLIES (DIPTERA). D. G. King, Dept. of Zoology and Dept. of Anatomy, Southern Illinois Univ., Carbondale, IL 62901.

The cervical connective of flies encompasses several thousand axons whose cross-sectional areas can range across more than three orders of magnitude within a single specimen. These axons display a consistent spatial arrangement which suggests homology from species to species among diverse families. Most larger axons are located in a dorsomedial motor quadrant, with smaller ascending axons ventrally and laterally. In many species a few bilaterally paired axons and axon tracts are individually identifiable on the basis of position and relative size (King, Soc. Neurosci. Abstr. 9:834, 10:51; Benson & King, Soc. Neurosci. Abstr. 11:626). One such identifiable tract contains several hundred very small axons (diameter < 0.5μm) grouped into a coherent ventro-medial bundle. In transverse section this tract appears as an irregular region located beside the midline near the ventral margin of the connective. With toluidine blue, this tract stains deeply and thus contrasts with the larger axons in surrounding regions.

The ventromedial small-axon tract is conspicuous in some species from each of the three dipteran suborders, suggesting that it forms part of the basic (ancestral) structural pattern for flies. The tract is especially prominent in some tipulids, syrphids, muscids and calliphorids. Nevertheless, a similar tract is not apparent in all species. Particularly in small flies, a clearly-defined ventromedial tract may not be distinguishable because most other cervical connective axons are also similarly small. However, no ventromedial tract appears even in some larger flies whose cervical connectives contain mostly larger axons. In these flies--including some asilids, rhagionids, bombyliids, conopids, and glossinids--a homologous tract either does not occur or consists of many fewer axons. (Alternatively, homologous axons could be larger or could be diffusely arranged rather than coherently grouped.)

The ventromedial small-axon tract corresponds in position and axon size to the tarsal chemoreceptor projection reported in Phormia regina (Calliphoridae) by Edgecomb (1986 Ph.D. Dissertation, Dept. of Entomol., Purdue Univ.) and by Edgecomb & Murdock (Soc. Neurosci. Abstr. 12:863), although the functional identity of this tract has not yet been explored in other species. Obviously, tarsal chemoreception might be less important to robber flies, which are visually-guided aerial predators, than to house flies, which identify their food by walking in it. However, evolutionary change is constrained not only by adaptive utility but also by ontogenetic potential, so comparative study reveals not only patterns of functional specialization but also degrees of freedom available for genetic determination of neural organization.

(Supported in part by NIH grant NS18542 and by SIU School of Medicine. Ray Venezia provided skillful technical assistance.)

243.3 MICROGEOGRAPHIC VARIATION IN THE ACOUSTIC COMMUNICATION SYSTEM OF THE CRICKET FROG. W. Wilczynski and M. J. Ryan*. Dept. of Psych. and Dept. of Zool., Univ. of Texas, Austin, TX, 78712.

Nevo and Capranica (Evol. Biol., 19: 147, 1985) previously demonstrated large-scale geographic variation in call characteristics within two subspecies of cricket frogs, Acris crepitans crepitans and A. c. blanchardi. We have begun an investigation of the microgeographic variation in both the calls and the physiological properties of the peripheral auditory system of these subspecies to assess the coevolution of signal production and reception and its impact on individuals' behavioral selectivity for subspecific calls. Males of both subspecies produce a pulsatile call in which all energy lies above 2.0 kHz. The calls are grouped into subspecies-specific temporal patterns that differ mainly in the call pattern at the end of each call group. However, this part of the call group seems mainly important in intermale aggressive displays (W. Wagner, unpublished). The largest and most consistent difference between subspecies calls is the dominant frequency: the overall mean for A. c. crepitans is 3.91 kHz, while for A. c. blanchardi the mean is 3.54 kHz. The spectral composition changes abruptly in a zone of parapatry in East Texas where the eastern (crepitans) and western (blanchardi) subspecies meet near the Trinity River. In addition, a population of cricket frogs in a relic, East Texas-like habitat in Central Texas near Bastrop, Texas, surrounded by A. c. blanchardi populations has a call with spectral characteristics similar to those of the East Texas A. c. crepitans (mean dominant frequency for this population is 3.77 kHz). Regression analysis shows that call frequency is negatively correlated with male size within each subspecies (for head width regressed on dominant frequency, $r^2 = .335$). However, residual analysis shows that body size alone does not account for subspecific differences in dominant frequency. Furthermore, there is no evidence that character displacement has exaggerated spectral or temporal differences in the parapatric zone. Single unit recordings in the eighth cranial nerve show that in a Central Texas population of A. c. blanchardi auditory fibers innervating the basilar papilla have best frequencies ($\bar{X}$ = 3.50 kHz) that match the dominant frequency of calls from the same population ($\bar{X}$ = 3.56 kHz). Studies are now underway to determine whether a similar match occurs in A. c. crepitans or in the anomalous population near Bastrop, TX, and whether differential peripheral tuning of the basilar papilla contributes to phonotaxis selectivity by individuals for calls from their own subspecies and geographic location.

We thank W. Wagner for assistance during this study. Supported by NSF grant BNS 8606289.

243.4 AUDITORY SENSITIVITY OF HYPOTHALAMIC NEURONS IN RANA PIPIENS. J. D. Allison and W. Wilczynski. Dept. of Psychology, Univ. of Texas, Austin, TX. 78712.

Recent anatomical evidence has suggested the existence of several pathways from auditory centers in this genus to the ventral hypothalamus, an area known to be involved in sex steroid secretion (Neary and Wilczynski, Neurosci. Letters, 71:142-146, 1986). We investigated the physiological response properties of cells in this area to broadband noise bursts or mating calls, played at 77dB SPL. Single units were isolated using standard techniques and the spontaneous firing rate for each was determined using a gated electronic counter. Auditory sensitivity was defined as a 20% or more change in firing rate from the spontaneous firing rate (Urano and Gorbman, J. Comp. Physiol., 141:163-171, 1981). Recording sites were confirmed by application of HRP.

Results indicate that a number of cells in the ventral hypothalamus are sensitive to auditory stimuli. The mean spontaneous firing rate for these sensitive cells was 10.45 spikes/s. Thirty-nine percent of the hypothalamic units isolated changed their firing rate when stimulated with a mating call; 25% showed an excitatory response while 14% were inhibited by the stimulus. The average firing rate change for active units was 2.9 spikes/s (a 27% change). Noise bursts altered the response of 46% of the isolated units; 29.8% were excited by the stimulus and 17% were inhibited. The average change in firing rate for these units was 4.57 spikes/s (a 43.8% change). The difference in mean change of firing rate between the two stimuli was not significant ($t=1.32; p>.05$). However, the variance in firing rate change for the populations was found to be significantly smaller for those cells stimulated with the mating call ($F=6.07; p<.01$). Treatment with human chorionic gonadotropin in intact and castrated animals by injection for 3 successive days prior to recording did not affect spontaneous firing rate or acoustic sensitivity, even though the ventral hypothalamus is a known testosterone binding site (Kelley et al., Brain Res., 140:287-305, 1978). The evidence presented suggests the ventral hypothalamus to be a possible site of sensory-endocrine interactions involved with reproductive behavior in the genus Rana.

We thank J. H. Fox and B. E. McClelland for assistance with this study. Supported by NSF grants BNS 8406221 and NSF BNS 8606289.

243.5 ACOUSTICAL AND NEURAL ASPECTS OF HEARING IN THE BROWN LONG-EARED BAT PLECOTUS AURITUS. R.B. Coles, A. Guppy, M.B. Anderson* & P. Schlegel*. Zoologisches Institut, Universitat Munchen, West Germany & Dept. Zoology, University of Aberdeen, Scotland, U.K.

The frequency sensitivity of hearing in Plecotus auritus was determined by recording averaged evoked potentials and auditory neurones in the inferior colliculus. Using free-field stimuli, the neural audiogram shows good threshold sensitivity in the range 7-50 kHz; above this range there is a steep loss of sensitivity (100dB/octave) with an upper hearing limit near 65 kHz. Auditory sensitivity decreases at 55dB/octave below 7kHz, with a low frequency limit near 2kHz. A remarkable feature of the audiogram of P. auritus is extremely high sensitivity to sound in the range 8-18kHz where thresholds reach -20dB SPL (re.20μPa). Tuning curves and spatial receptive field properties were also examined in midbrain neurones.

The acoustical properties of the external ear show that pressure gain at the eardrum reaches 20dB between 8-20kHz. The gain of the pinna increases rapidly above 6kHz, to a peak of 16dB between 8-12kHz, then decreasing to an average of 8dB up to 80kHz. The gain of the pinna is comparable to a finite-length acoustic horn. The directional properties of the external ear are determined by sound diffraction at the pinna mouth (average radius 12mm). In elevation, the acoustic axis of the pinna moves upwards by 30° between 9-20kHz resulting from asymmetry of the pinna. In azimuth, the acoustic axis is 25° from the midline up to 35-40kHz, and tends to move towards the midline at higher frequencies. Acoustically, the tragus influences the depth and location of nulls below the horizon.

The upper frequency region of good hearing sensitivity is correlated with the main sonar band between 35-45kHz, whereas the more sensitive lower frequency region corresponds to social signals such as a loud long-sweep call used in the field, with energy in the audio range down to 12kHz.

The highly sensitive low frequency hearing of P. auritus is also found in Megadermatid bats and suitable for prey detection by passive listening and location, without the use of echolocation. An important adaptation for this type of foraging behaviour is a large pinna which amplifies sound and provides directionality at low frequencies.

243.6 RANGE JITTER DISCRIMINATION OF FILTERED ECHOES BY ECHOLOCATING BATS. C. F. Moss and H.-U. Schnitzler*. Department of Biology, University of Tübingen, D-7400 Tübingen, F. R. Germany.

Echolocating bats use the time delay between emitted sounds and returning echoes to determine the distance to an object. The big brown brown bat, Eptesicus fuscus, can measure the time delay of sonar echoes with an accuracy of less than 1 μsec, corresponding to a distance discrimination of less than a mm (Simmons, Science, 204: 1336-1338, 1979). Exactly how the bat achieves this remarkable degree of precision is not yet well understood, and therefore, we aimed at evaluating what information in the returning echo is important to the bat's performance in a timing accuracy task.

Six big brown bats were trained in a yes-no procedure to discriminate between two phantom targets, one that simulated a stationary target that returned at a fixed delay and another that simulated a jittering target that underwent small step changes in delay. Bats emitted sonar sounds into a microphone and listened to simulated echoes played through a loudspeaker located at a distance of 1 m. When presented with sounds returned at a variable delay, bats learned to indicate a "yes" (jitter present) response by approaching a platform to the left, and when presented with sounds returned at a fixed delay, they learned to indicate a "no" (jitter absent) response by approaching a platform to the right. The magnitude of the jitter was reduced from 100 μsec to zero in small steps to estimate the smallest jitter the bat can reliably detect. Percentage hits (yes response, jitter present) and false alarms (yes response, jitter absent) were calculated for individual animals at each jitter magnitude tested.

Eptesicus fuscus emits a frequency modulated sonar sound whose 1st harmonic sweeps from approximately 55 to 25 kHz in 2 ms. Sound energy is also present in the 2nd and 3rd harmonics, contributing to a broadband signal which is well suited to transmit information in the time domain. In this study, range jitter discrimination was estimated under conditions in which the echo information available to the bat was manipulated. Baseline performance with natural echoes was compared to that with filtered echoes. The results indicate that the low frequency portion (40-25 kHz) of the 1st harmonic alone is sufficient for the bat to discriminate temporal jitter of less than 0.5 μsec. These findings will be discussed in terms of receiver models that can best account for the behavioral data.

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243.7 ECHOLOCATION IN BATS: ECHO DELAY AND ECHO SPECTRUM BOTH CONVERGE ONTO THE TARGET RANGE AXIS IN PERCEPTION. J.A. Simmons, E.G. Freedman, S.B. Stevenson, and L. Chen. Hunter Laboratory of Psychology, Brown University, Providence, RI 02912.

Echolocating bats (Eptesicus fuscus) can perceive the distance to individual sonar targets with an acuity of a fraction of a millimeter from the time-delay of echoes. In terms of delay, this acuity is in the region of half a microsecond. To successfully capture a flying insect at the end of a pursuit maneuver, the required target-range acuity is only 1 to 2 cm, however, so it appears as though bats have considerably more target-ranging acuity than is strictly necessary for intercepting prey. What is the significance of this "excess" acuity?

Bats also can perceive the difference in distance to the different parts of a complex target that extends along the range axis, again, with an acuity of a fraction of a millimeter. Flying insects have dimensions of several centimeters and might subtend a horizontal or vertical angle of a few degrees at the distances of 0.5 to 1 m relevant to pursuit. Because the minimum perceived image-width is about 3 degrees horizontally and 6 degrees vertically, insects probably do not have "shape" in these dimensions. The fine acuity for the range distribution of a complex target must constitute the primary basis for perceiving the shape of objects as small as insects.

The echoes from two parts of a small, complex target overlap in time by an amount dependent on the separation of the target elements along the range axis. Physiological data from several laboratories, a model of peripheral auditory encoding of echo waveforms, and behavioral data all indicate that the distance between two target elements is represented by the spectrum of echoes if the time separation of the corresponding echo components is smaller than 100 to 200 microsec. Nevertheless, the bat perceives complex targets as being extended along the range axis, with each target component referred to its correct absolute range from the bat. The auditory system's spectral code for the time separation of echo components from complex targets must converge onto the same display as the delay of echoes for target range. This could be accomplished by neural computation of the time separation from the location and spacing of spectral notches along the frequency axis, followed by a mapping of this computed time separation onto the echo delay representation of target range. It could also occur more directly in the creation of the echo delay representation itself if the spectrum of echoes can contribute to the spectrogram correlation process that appears to underlie target ranging. To support fine range perception from echo spectra, the bat has the capacity to perceive echo delay with a correspondingly fine acuity, even though time delay as such might not have to be perceived with this acuity under natural conditions.

243.8 RUFF TREATMENT AND SOUND LOCALIZATION. T.A. Haresign* and A. Moiseff. Dept. of Physiology and Neurobiology, Univ. of Conn., Storrs, CT 06268.

The barn owl, Tyto alba, uses a bicoordinate system for localizing sounds in space. Interaural time difference is used mainly to determine the azimuth of a sound in space and the interaural intensity difference is used to determine elevation. Time differences are proportional to the interaural distance and the angle of the sound relative to the aural axis. Interaural intensity difference is the result of bilateral asymmetries that exist in the ear openings and the facial ruff. The owl processes these binaural cues to construct a map of auditory space in the nervous system. To confirm the importance of the facial ruff in sound localization the feathers comprising the ruff were trimmed during their initial outgrowth. The cutting was done so that the feather length never exceeded one quarter of the adult size. These birds were tested in free field conditions as adults.

Individual birds showed varying degrees of localization ability. All of the birds could localize azimuth correctly, but vertical localization was incorrect. The effects varied from random elevation errors to systematic errors where the owls consistently localized above the actual target.

This data is interesting in light of monaural occlusion studies (Knudsen, E. I. et al., J. Neurosci. 4:1001 1984). In those experiments owls whose auditory experience was altered during development by monaural ear plugging adapted to the altered cues as evidenced by their ability to accurately localize sounds in spite of the altered cues. In our study, the birds were unable to adapt to the ruff treatment.

Currently, experiments are underway using earphones to present auditory stimuli to the owls. Preliminary results indicate that treated owls don't respond correctly to earphone stimuli known to elicit accurate behavior in untreated birds. This may reflect a limitation in the adaptive capabilities of the owl's auditory system.

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243.9

WITHDRAWN

243.10 AUDITORY SELECTIVITY IN THE SONG-CONTROL NUCLEUS HVc APPEARS WITH THE ONSET OF PLASTIC SONG. Susan F. Volman and Masakazu Konishi. Division of Biology 216-76, Caltech, Pasadena, CA 91125.

In adult white-crowned sparrows, auditory responses in the song-control nucleus HVc are selective for an individual bird's own song (Margoliash, J. Neurosci., 1986). Juvenile birds (4-8 months old) that have been exposed to an appropriate song model ("tutor song"), but have not yet sung, show no evidence of neuronal selectivity (Volman and Konishi, Soc. Neurosci. Absts., 1986). We have now quantified song selectivity in HVc neurons of 10 birds, 8-10 months old, during the plastic-song phase of song development. Plastic song has many of the phrase elements of full adult song, but is more variable and often lacks some of the fine structure. We recorded the vocalizations of these birds every 2-4 days and recorded electrophysiologically from HVc as soon after development of plastic song as possible (approximately 2-10 days).

The responses of multi-unit clusters and single units were recorded at 50 sites in these birds. Auditory stimuli were tutor song, 2-4 examples of the birds' own plastic songs, other birds' songs, and tone bursts and other synthetic sounds. Song selectivity was clearly apparent by at least 2 criteria: 1) forward song elicited a greater neuronal response than reverse song at 95% of the recording sites when a bird's plastic song was played, and at 93% of the sites to the tutor song. 2) Alien song, from another subspecies of white-crown, was a poorer stimulus than plastic song at 97% of the sites and than tutor song at 76%. Although tutor song was usually a good stimulus, there was a slight but significant preference for the plastic songs. This preference was most likely to be seen in birds whose plastic song was least similar to the tutor song, including 3 birds in which glass beads, inserted between the two internal tympaniform membranes of the syrinx, successfully frequency shifted the song.

In adult birds a small proportion of neurons in HVc can be classified as "song specific" in that they respond almost exclusively to particular combinations of phrases present in a bird's song (Margoliash, J. Neurosci., 1983). In pre-singing birds we had encountered a few neurons with similar spectral/temporal stimulus specificities. Song-specific units, tuned to the plastic song and/or the tutor song, were also present in birds singing plastic song, and with apparently greater frequency than in pre-singing birds, although these proportions are difficult to quantify. In adult and plastic-song birds, clear auditory responses can be recorded in almost any electrode penetration through HVc. In juvenile birds, by contrast, particularly in the lateral part of the nucleus, auditory responses are more likely to be inhibitory or weak and inconsistent. Thus onset of singing is coincident with an increase in responsiveness as well as with the appearance of selectivity in HVC.

243.11 CHANGES IN FIRING RATE ASSOCIATED WITH HORMONE ADMINISTRATION IN DOVE MIDBRAIN NEURONS. M. Havens and M.-F. Cheng. Rutgers University, Institute of Animal Behavior, Newark, NJ 07102

The distribution of cells which concentrate estrogen in the female ring dove has been described (J. Comp. Neurol. 167:83-104, 1976). There are nuclei which contain a relatively large number of estrogen concentrating neurons. One of these nuclei, the midbrain nucleus intercollicularis (ICo), plays a major role in the control of estrogen dependent female nest coo display. This display is a critical behavior for successful breeding in ring doves. Previous work in our laboratory has demonstrated that ICo lesions disrupt nest coo display and that estrodiol implants in ICo reinstate the behavior in ovariectomized birds.

We have begun to investigate the functional effects of hormones on midbrain neurons in and around ICo. Extracellular field potentials were recorded from single midbrain neurons in ovariectomized doves until a well-isolated, stable cell was encountered in ICo. The electrode was then cemented in place and a second electrode lowered into the brain. After two to four electrodes had been cemented in place, the bird received an im injection of estradiol (50 µg). The spontaneous firing rate and responses to auditory stimulation were then recorded at 10 min intervals for 1 hr. The next day the bird was again anesthetized, given a second 50 µg injection of estradiol and recordings were made as on the first day. On the third day the recording procedure was repeated after which recordings were made from the opposite side of the brain using movable electrodes. In this way recordings were made before hormone exposure, during exposure to a hormone regime which would normally facilitate nest coo display in an ovariectomized bird and after hormone exposure.

Most isolated cells were not present after the first day of recording, however, several have been followed for two days and some for three days. Profound increases and decreases in spontaneous firing rate was observed in many of the neurons after hormone exposure. Approximately half of the cells increased their spontaneous firing rate while half decreased. However, increases in firing rate were more transitory than decreases in that increases typically lasted for 10 to 20 min of a recording hour while suppressions in firing rate typically lasted for longer than 30 min and in the case of several neurons have lasted up to 72 hr. One of the most consistent observations has been that the firing rate of these neurons is affected in less than 20 min after hormone injection on the first day and in many cases in less than 10 min. This observation raises the question of the mechanism of action of estradiol on these neurons.

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243.12 AUDITORY LOCALIZATION BEHAVIOR IN CATS DEPRIVED OF VISION. J.P. Rauschecker* and U. Kniepert* (SPON: V. Braitenberg). Max-Planck-Institut für biologische Kybernetik, D-7400 Tübingen, West Germany.

Prolonged binocular deprivation of pattern vision leads to an increase of auditory responsiveness in superior colliculus (SC) of rats and cats (Vidyasagar, Nature, 275: 140-141, 1978; Rauschecker and Harris, Exp. Brain Res., 50: 69-83, 1983). We have asked whether the higher incidence of auditory-responsive neurons in SC has actual consequences on the behavioral performance of cats that were deprived of vision either from birth or later in life. Since the superior colliculus is known to participate in localization behavior, we have used an experimental paradigm that tested the animals for their ability to localize a sound source in space.

Three normal cats and five cats that were deprived of vision from birth by binocular lid suture were studied. All animals were over 3 months of age. Using operant techniques the cats were trained to run from a startbox in the center of a circular arena (3 m diameter) straight towards one of 8 loudspeakers. All speakers were at zero elevation with respect to the animals' head and in equally spaced azimuthal positions behind the wall of the arena. For testing they were addressed in random order. Acoustic stimuli consisted of tone pips (40 msec, 10 kHz, 75 dB SPL). The experiments were performed in a sound-attenuated chamber and were surveyed and evaluated using a video camera system equipped with a fish-eye lens. Sound localization was measured with a resolution of 5 deg.

In general, both groups showed better performance for straight-ahead positions of the auditory target than for lateral or rear positions. However, for all 8 positions the deprived cats were consistently better in localizing the sound source than the controls. Sound localization errors were up to twice as large in normal as in visually deprived cats. Two of the normal controls were additionally tested in the dark using an infrared-sensitive camera, and no difference was found. The same animals were then lid-sutured and retested after 3 months. A slight improvement in sound localization accuracy was found after this late deprivation, which was however much less pronounced than in the early deprived animals.

243.13 SUPERIOR COLLICULUS FUNCTION IN VISUALLY ELICITED "FEAR" AND IN SCANNING MOVEMENTS IN SYRIAN HAMSTERS. G.E. Schneider, L.S. Carman*, and S. Ayres*. (SPON: W. Rosenblith) Dept. of Brain and Cognitive Sciences,M.I.T., Cambridge, MA 02139.

Most studies of behavioral functions of the superior colliculus (SC) have focused on its role in orienting toward visual stimuli. However, recent studies of hamsters using ablation and electrical stimulation indicate that the SC may be important for visually elicited fear responses. In the present study, 20 adult Syrian hamsters with adult or neonatal bilateral lesions were examined in a battery of visual tasks. Adult lesions included (i) transection of the brachium of SC (BSC), (ii) ibotenic acid lesions of pretectum (PT), (iii) ablation of visual cortex (VC), and (iv) control lesions (EXP) involving exposure of the midbrain but with damage limited to the caudomedial hemisphere (such exposures were used in making BSC and PT lesions). Neonatal lesions of the SC were inflicted with heat on the day after birth.

The most reliable deficit in animals with tectal damage was seen when the task involved visually elicited "fear". Thus, normal hamsters, and animals with VC, PT, and EXP lesions, responded to a visual "loom" stimulus by running or freezing, while those with BSC or neonatal SC lesions exhibited few, if any, such responsess. These "fear" deficits occured even in hamsters which showed some orienting abilities when presented with seeds.

"Scanning time"--the amount of time a hamster spent making scanning movements as he emerged from a tunnel into an open arena with baited visual targets--was also highly correlated with SC damage. Animals with BSC or neonatal SC lesions did not scan before entering the arena, while all other lesion groups showed extensive scanning. Other authors have suggested that scanning may be critical for detecting or orienting toward stimuli, but the hamsters in this task showed no correlation between amount of scanning and accuracy of target approach. Similarly, hamsters presented with seeds showed no scanning before turning to take a seed. These observations suggest to us that scanning in the open arena is not used to locate the food, but rather to survey the environment for novel and potentially dangerous visual stimuli. Ablation of the SC eliminates this visual fear response.

Using the data from all of the animals, amount of scanning and intensity of response to a threatening stimulus were positively correlated ($p<.01$, $r_s = .76$). It is relevant to note that, in normals, scanning and response to a threatening visual stimulus are both robust during initial testing, then both quickly habituate. The other animals with intact SC showed little or no habituation of scanning behavior. It is possible that habituation in these cases was impaired because of hippocampal lesions inflicted during the exposure of the midbrain. (Supported by NIH grants EY 00126, EY 02621, and by a Poitras graduate fellowship.)

244.1 THE EFFECTS OF ELETROMYOGRAPHIC BIOFEEDBACK ON REACTION TIME AND MOVEMENT TIME. R.B. Schultz*, B.R. Etnyre, and J.M. MacArthur*. Rice University, Houston, TX 77030

The effects of electromyographic (EMG) biofeedback on Reaction Time (RT) and Movement Time (MT) were investigated utilizing 45 male subjects from a university population. All subjects performed RT and MT tasks. Both experimental groups were exposed to EMG biofeedback, one experimental group received written information explaining the purpose of the EMG biofeedback. Significant differences in the mean RT's in the first block of 25 trials compared with the subsequent three blocks of trials supported a learning effect. No other effects for RT were significant. A significant difference between MT's for the EMG-Only group and the Control group were revealed with no difference between the Control and EMG-Biofeedback groups. The differences between experimental groups may have been due to strategy alteration, anxiety and motivation.

244.2 FORCE FEEDBACK DYNAMICS DURING HUMAN MUSCLE FATIGUE. R.F. Kirsch* and W.Z. Rymer Northwestern University Medical School and Rehab. Instit. of Chicago, Chicago, IL 60611.

We have begun to examine the actions of force-sensitive reflex pathways in man under dynamic conditions. As in a previous study (Kirsch and Rymer, J. Neurophysiol. 57: 1987), muscle fatigue is being used to reduce contractile force of the elbow joint musculature, a disturbance which should be compensated by a force regulator. In our present study, a random (bandlimited Gaussian white noise) angular perturbation is being used for two primary reasons. First, the random perturbation is quite unpredictable, greatly reducing the contamination of results by voluntary intervention. Secondly, efficient mathematical algorithms can be employed to characterize the dynamic behavior of the system.

Subjects are seated and secured in a chair such that the elbow of their dominant arm rotates about the same axis as a large torque motor, which is used to apply a controlled angular perturbation. Joint angle and torque are sampled via computer, as are surface EMG signals from each of the two heads of biceps, brachioradialis, and triceps. The subject is asked to achieve a certain background torque level (ranging from 10 to 35% of max) and to maintain this mean level during application of the 8 sec random perturbation of ±2 deg about a mean elbow angle of 90 deg. A set of such trials are performed before and after a set of repeated fatiguing contractions. Post-fatigue trials began 10 minutes after completion of the fatiguing exercise to minimize the metabolic effects of fatigue on the EMG signal.

Our results are based on comparisons of pre- and post-fatigue torque and EMG responses. We reasoned that a force regulator would act to maintain the force response of a weakened muscle to an applied perturbation by removing inhibition and thus generating a larger EMG response. In 3 subjects studied to date, substantial fatigue, as evidenced by large shifts in the slope of the isometric torque-EMG relationship (mean shift across all subjects of 150.3%) was induced, but dynamic variations in torque during the perturbation were virtually identical before and after fatigue for matched mean torque levels. Stiffness frequency responses identified as the transfer function between angle and torque (which was modified to remove actuator dynamics) were likewise quite similar. This is in agreement with past work (Hunter and Kearney, J. Biomech. 16:985-991, 1983). On the other hand, incremental EMG, calculated as the root-mean-square (RMS) value of the EMG signal during the perturbation minus the isometric RMS value, increased significantly for matched isometric EMG levels in both lateral biceps and brachioradialis of all three subjects (average shifts of 42.4 and 40.9%, respectively). Interestingly, the incremental EMG signal of medial biceps remained constant or decreased slightly in 2 of the 3 subjects (it was contaminated with noise in the third subject).

In summary, the dynamic character of elbow joint torque was essentially unmodified by fatigue, while the size of the reflex EMG response to the perturbation was increased significantly. This behavior is consistent with the actions of a force regulatory system. Work continues on detailing the dynamics of this system and determining its role in normal motor control.

Work supported by NIH grant NS-19332 and Sensory Motor Performance Program.

244.3 CAVEATS OF TREMOR RESEARCH: A COMPUTER MODEL. R.J. Elble. Dept. of Medicine, Southern Ill. Univ. Sch. of Med., Springfield, IL 62708.

We have shown that physiologic tremor is composed of two distinct oscillations, mechanical-reflex and 8- to 12-Hz. The mechanical-reflex oscillation may assume a wide range of frequencies, determined by limb mechanics and reflex loop dynamics. By contrast, the 8- to 12-Hz oscillation has a mean frequency that is largely independent of limb inertia and stiffness. Similarly, the sine qua non of essential tremor is an oscillation in the range of 5 to 12 Hz which has a mean frequency that is independent of limb mechanics and reflex latencies. This pathologic oscillation is distinct from the normal mechanical-reflex oscillation exhibited by controls and patients alike. These properties suggest that the 8- to 12-Hz physiologic and essential tremors are produced by nonlinear central oscillators coupled to the stretch reflex. To conceptualize the complexities of this hypothesis, we developed a computer model containing the following elements: 1) a nonlinear (Van der Pol) central oscillator, 2) a second-order critically damped skeletal muscle model, 3) second-order lightly damped limb mechanics, and 4) first-order spindle and golgi tendon organ feedback. To construct this model, differential equations were written for each component, based on previous laboratory studies by other authors. These equations were then integrated into a classic feedback loop arrangement and solved on a personal computer using the Euler method. Components of this model were studied individually and together in a closed loop arrangement whereby the strength of feedback to the central oscillator could be varied. This model illustrates the following important caveats of tremor research: 1) Torque or displacement perturbations to the mechanical-reflex system produce phase resetting at a fixed latency determined by the reflex loop time, but similar perturbations to the nonlinear central oscillator produce resetting at a variable latency determined by the strength of the perturbation. 2) The nonlinear central oscillator has an inherent preferred frequency that is independent of reflex loop dynamics, but strong sensory feedback to the cental oscillator can produce frequency entrainment at the mechanical-reflex frequency. We (J. Neurol. Neurosurg. Psychiat. 1987, in press) and other authors have utilized brief torque perturbations to the wrist and time-locked computer averaging of EMG and wrist angle data to examine reflex involvement in physiologic and essential tremor. However, such analysis assumes a linear behavior for the various components of tremor. Our laboratory data indicate that this assumption is incorrect for the 8- to 12-Hz physiologic and essential tremors, and our computer model illustrates the complex phase resetting and frequency entrainment produced by system nonlinearities. (Supported by NINCDS grant RO1-NS20973)

244.4 A MUSCULOSKELETAL MODEL OF THE HUMAN LOWER EXTREMITY FOR PREDICTING MUSCULOTENDON TORQUES IN THE SAGITTAL PLANE. M.G. Hoy, M.E. Gordon, and F.E. Zajac. Mech. Eng. Dept., Stanford Univ. and Rehab. Res. & Dev. Ctr., VA Med. Ctr., Palo Alto, CA 94304.

Musculotendon actuators control human movement by generating torques which act on the body segments. How actuators are coordinated in control of a specific movement may depend on the ability of each actuator to generate torque at certain joint angles. To understand the torque-producing ability of each actuator, the geometry of the musculoskeletal system and the length-dependence of musculotendon force must be considered. Musculoskeletal geometry determines moment arm, and therefore the torque generated by a given musculotendon force. How musculotendon force varies with musculotendon length, however, depends on the relative length of tendon in series with muscle (Zajac *et al.*, Proc. 8th Conf. IEEE Eng. Med. Biol. Soc., 1986). Effective human tendon lengths, which include the length of tendon both internal and external to the muscle, and their effect on human musculotendon force and torque have not been previously reported.

To determine the isometric torque-producing capacity of lower extremity actuators, we modeled the trunk, thigh, shank, and foot as planar segments articulated at the hip, knee, and ankle. Eighteen actuators were each represented by one or more straight line segments. We used coordinate data reported by Brand *et al.* (J. Biomech. Eng. 104: 304, 1982) to describe the attachment sites of actuators on the skeletal segments, and to calculate musculotendon length and moment arm for each actuator. Musculotendon force was calculated using a dimensionless actuator model that is scaled to individual actuators by four parameters: maximum muscle strength, optimal muscle fiber length (l^M_o), pinnation angle at l^M_o, and tendon slack length (l^T_s) (Zajac *et al.*, 1986). Tendon slack lengths were chosen by assuming that passive force is developed in muscle only at fiber lengths greater than l^M_o, then selecting l^T_s so that the joint angle at which l^M_o occurred coincided with the onset of *in vivo* passive torque at the joint.

With our model, we show that musculotendon torque depends on actuator-specific musculoskeletal geometry and musculotendon parameters, and that tendon slack length has a profound influence on isometric musculotendon torque. We found that the joint angle of peak isometric torque and the range of joint angles over which each actuator produced torque were unique, even among actuators generating torque in the same direction at a joint. Neglecting the effect of tendon in analyses of musculotendon torque may lead to erroneous predictions of musculotendon function not only in static situations but during movement as well.

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244.5 ABOUT THE KINEMATICS IN THE CONTROL OF STEPPING. A. Beuter, H. Flashner*, and A. Arabyan*. Kinanthropologie, Université du Québec à Montréal, C.P. 8888, Succ. "A", P.Q., H3C 3P8 and Mechanical Engineering, University of Southern California, OHE 430, Los Angeles, CA 90089.

It has been suggested that movement planning is done in terms of endpoint coordinates (Flash, 1983) and that inverse Kinematics is used to transform the endpoint positions into the corresponding time sequence of joint angles (Hollerbach, 1985). Although efforts have been made to model endpoint coordinates in planar, multijointed limb movements, no direct evidence of an inverse kinematics process has been offered sofar. Beuter et al., (1986) and Flashner et al., (1987) have suggested that the speed of operation and the variability observed in stepping prevented the implementation of a full inverse kinematics solution. They showed that in stepping only a small number of cartesian space parameters were necessary to specify the foot path (i.e., step length, height and duration), and suggested that the trajectory required to perform the task would be better analyzed in joint space. Therefore the goal of this investigation was to express joint trajectories in terms of simple functions and small numbers of undetermined parameters. Experimental data were collected on six subjects stepping repeatedly at a natural cadence over a low obstacle. Subjects' performances were videotaped in the sagittal plane at 60 pfs and digitized using a motion analysis system. The trajectories of the hip and knee joints of a typical subject were modeled using different classes of functions including polynomials, cosine, harmonics, even polynoms and cycloids by the means of least squares approximation. Results show that the qualitative characteristics of the trajectories are preserved by all approximations with a small number of basis functions. Deviations from the experimental data in low order approximations usually occurred at the end of the swing phase when extensor muscles are activated in anticipation for landing. Results indicate that under normal operation, free-swinging leg movements in stepping are performed in an open loop mode and that only a limited amount of information stored in the nervous system is required for completing the motion. In other words, it appears that the nervous system does not need to implement a full inverse kinematics solution in the control of stepping.

244.6 ABSENCE OF DIRECTION-SPECIFIC RATE CODING AMONG LOW THRESHOLD MOTOR UNITS OF FIRST DORSAL INTEROSSEUS. R.M. Enoka, G.A. Robinson and A.R. Kossev*. Dept. of Exercise & Sport Sciences, Univ. of Arizona, Tucson, AZ 85721.

There has been an interest in the differential behavior of single motor units in multifunctional muscles, such as when a muscle contributes to one action or another. We have addressed this issue by examining motor-unit rate coding in the first dorsal interosseus muscle (FDI) of human subjects during isometric, ramp increases in force. It is known that the force exerted by FDI is modulated significantly by rate coding. We were interested in the patterns of rate coding when FDI acted as a prime mover (abduction force) and as a synergist (flexion force).

Motor-unit potentials were measured with a branched, bipolar electrode (Gydikov et al., Electromyogr. Clin. Neurophysiol., 26:273, 1986) made of 2 stainless steel, insulated wires, each 50 μm in diameter. The electrode contained 3 leading-off areas (~ 500 μm each); 2 on one wire, 2-3 mm apart, and 1 on the other wire. The electrode, about 25 cm in length, was inserted subcutaneously so that both ends of the electrode emerged from the skin with the leading-off areas in close proximity to the surface of the muscle. The leading-off areas could, therefore, be positioned to maximize the selectivity of the measurement. The index finger of each subject was placed in a splint, and the hand was positioned so that the force exerted by FDI in the abduction and flexion directions was sensed by separate transducers located at the first interphalangeal joint.

Target forces were displayed on an oscilloscope, and in response to verbal commands, subjects performed slow (2-4 s) ramp increases in force ($\bar{X} \pm SD$ = 4.11 $\pm$ 3.26 N/s, range = 0.94 - 13.88 N/s) to the target levels. The target forces ($\bar{X} \pm SD$ = 13.8 $\pm$ 8.5%, range = 5.4 - 32.8% of maximum) were set at a value that was just above threshold ($\bar{X} \pm SD$ = 8.7 $\pm$ 5.6%, range = 2.6 - 21.6%), but sufficient to sustain a low, steady motor-unit discharge. There were no marked differences in the recruitment thresholds of individual motor units in the flexion and abduction directions. In all instances, interimpulse intervals were initially long and declined as the target force was approached. This reliance on rate coding is similar to that reported previously for FDI (Milner-Brown et al., J. Physiol. (Lond.), 230: 371, 1973) but not for other muscles, such as biceps brachii (Kukulka & Clamann, Brain Res., 219: 45, 1981). For each motor unit, qualitatively similar discharge patterns were observed in both the abduction and flexion directions. These data confirm other reports on the absence of a direction effect on motor unit behavior, at least among low threshold, FDI motor units.

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244.7 FATIGUE-INDUCED CHANGES IN THE THRESHOLD FORCES OF RECRUITMENT AND DERECRUITMENT FOR LOW THRESHOLD MOTOR UNITS OF FIRST DORSAL INTEROSSEUS. A.R. Kossev*, G.A. Robinson and R.M. Enoka (SPON: A. Kaszniak). Dept. of Exercise and Sport Sciences, Univ. of Arizona, Tucson, AZ 85721.

Fatigue modulates motor-unit behavior in human subjects, such as reducing discharge rate during maximal efforts. However, recruitment thresholds of motor units during isometric, ramp increases in force, which are known to depend on activation history, are not thought to be affected by fatigue, at least that associated with a 120-s sustained contraction (Denier van der Gon et al., J. Physiol. (Lond.), 359: 107, 1985). Since this conclusion was based on submaximal efforts, we examined the effects of more substantial fatigue on the recruitment (F_r) and derecruitment (F_d) threshold forces of motor units.

The experimental techniques are described in the accompanying poster (Enoka et al.). The protocol involved three sequences (n ~ 12) of a ramp increase, plateau, and ramp decrease in isometric force. The first and third sequences were used to determine F_r and F_d in both the abduction and flexion directions. Each phase of the task (ramp, plateau, and ramp) lasted 2-3 s, and there was a 5-s rest between each performance. The oscilloscope-displayed target force (plateau) was just above threshold (see preceding poster). The second sequence comprised the fatigue test; the ramps took 2 s each, the plateau lasted 10 s, the target force was 50% of maximum, and there was no rest period between each performance. The subjects repeated this sequence until they could no longer attain the target values.

The protocol was designed to compare F_r and F_d of single motor units during and after the fatigue test to those obtained before the test (control). The data revealed: (1) F_r declined over the course of the fatigue test. The relative change seemed inversely related to initial F_r; (2) despite attempts to maintain smooth ramp profiles, there was marked variability in F_r and F_d during the fatigue test; (3) although the fatigue test was performed only in the abduction direction, postfatigue-test F_r and F_d values in both directions were greater than control values. The fatigue test induced a similar degree of fatigue in FDI whether the muscle acted as a prime mover (abduction) or as a synergist (flexion); (4) the typical reliance of FDI motor units on rate coding to modulate force was less apparent after the fatigue test in that some units did display the traditional discharge rate profiles while others did not. These data provide further evidence of a fatigue-related effect on motor-unit behavior in conscious human subjects; specifically, fatigue caused an increase in F_r and F_d of low-threshold motor units in FDI during slow, ramp changes in isometric force.

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244.8 DISSOCIATION OF H-REFLEX AND ELECTROMYOGRAPHIC CHANGES PRIOR TO VOLUNTARY MOVEMENT. K.C. Hayes and J.A. Hendry*. Dept. of Physiology, Univ. Western Ontario, London, Ontario, Canada N6A 5C1.

This study was designed to test the hypothesis that post synaptic inhibition of the alpha motoneuron pool is the primary mechanism underlying the electromyographic "premovement silent period" (PSP) that precedes phasic activation of a tonically contracted muscle. Hoffmann (H) reflexes were evoked from the soleus muscle of nine healthy adult subjects as they maintained tonic contraction of triceps surae prior to a rapid plantar flexion reaction time (RT) task. PSPs were observed in all subjects, with an overall frequency of 78% of the RT trials. PSPs preceded the voluntary plantar flexion response by 60-80 ms and had an average duration of 54±22 ms. The amplitude of H-reflexes evoked during the PSP was not diminished, indicating that postsynaptic inhibition of the alpha motoneuron pool is unlikely to contribute to the PSP. The normalized mean amplitude of H-reflexes evoked during the PSP was, in fact, significantly ($p \leq .05$) greater ($\bar{x}$=44%) than the mean of H-reflexes evoked during background contraction ($\bar{x}$=35%). These results support the alternate hypothesis of a reduced output from supraspinal centers underlying the PSP. A reduction in descending excitatory input to the motoneuron pool may be coupled with reduced presynaptic inhibition of Ia afferent terminals that, in turn, contributes to increased peripheral reflex excitability. The functional significance of these mechanisms would be their potential for clearing the spinal centers to allow synchronous motor unit recruitment and at the same time allowing for an elevated gain or reduced threshold of the segmental stretch reflex.

244.9 EXCITATION FROM THE SURAL NERVE IS PREFERENTIALLY DISTRIBUTED TO THE MEDIAL GASTROCNEMIUS PORTION OF THE TRICEPS SURAE MOTONEURON POOL. L. LaBella, J. Kehler*, and D.A. McCrea. Department of Physiology, University of Manitoba, Winnipeg, Canada, R3E 0W3.

Postsynaptic potentials (PSPs) were recorded intracellularly in 115 triceps surae motoneurons of 10 chloralose-anaesthetized adult cats with intact spinal cords, upon electrical stimulation of the ipsilateral sural and perforant hindlimb nerves.

With twice threshold (2T) stimulation of the sural nerve, excitatory PSPs (EPSPs) with latencies typically between 2.0 and 2.5 ms, were the predominant effect in 95% of all medial gastrocnemius (MG) motoneurons tested. These EPSPs occurred with a minimum central latency of 1.8 ms suggesting a trisynaptic linkage of a minimum of two interneurons. Only in a few MG cells was the EPSP followed by an inhibitory potential (IPSP), and in only one cell was an IPSP the sole effect. Unlike these effects recorded in MG motoneurons, 2T stimulation of sural led to either inhibition or no potential change in most lateral gastrocnemius (LG) and soleus (SOL) cells tested. Thus, EPSPs were the predominant effect in only 15 and 30% of LG and SOL cells respectively. The minimum central latency for excitation was 2.5 ms, suggesting the presence of at least three interneurons. In contrast to the apparently pure excitation normally produced in MG cells, EPSPs in LG and SOL rarely occurred without subsequent inhibition. The results indicate that sural excitation occurs preferentially in the MG portion of the triceps surae motoneuronal pool, and at a preferentially shorter latency. In addition, it appears that this distribution is independent of motoneuron "type" as assessed by consideration of a variety of motoneuron membrane electrical properties. Insofar as membrane properties reflect motoneurons of different motor unit type, this data contradicts reports that the incidence of sural EPSPs is greater in those cells belonging to faster units. Stimulation of the perforant nerve at 5T produced predominant inhibition in 71% of all triceps surae motoneurons studied. Thus, the effects were consistent with an "FRA-like" synaptic input from this nerve. However, of the small number of cells which did receive excitation from this nerve, the majority were MG (7 of 8). These EPSPs occurred more frequently at 5T than at lower stimulation strengths and once again, the polarity of PSPs appears to be independent of motoneuron type.

Taken together, these findings suggest that at least some peripheral excitation may be differentially distributed among motoneurons belonging to the same functional pool. Whether or not this represents an organizing principle in the regulation of muscle synergy requires further investigation, but it appears that the ordering of cutaneous effects in ankle extensor motoneurons is not necessarily according to motoneuron "type", as has been previously reported.

244.10 COMPARISON OF PHYSIOLOGICAL AND HISTOCHEMICAL PROPERTIES OF MOTOR UNITS FOLLOWING CROSS-REINNERVATION OF ANTAGONISTIC MUSCLES IN THE CAT HINDLIMB. C.K. Thomas, T. Gordon, R.B. Stein and S. Erdebil*. (SPON: M. Warenycia), Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alberta, T6G 2H7.

We have shown that the normal size relationships between muscle unit force and axon size returns after self- and cross-reinnervation of triceps surae muscles (Gordon & Stein, J. Neurophysiol., 48: 1175, 1982; Gordon et al., J. Physiol., 374: 443, 1987). This re-ordering of motor units involves considerable reorganization since nerves supply muscle fibers which formerly belonged to several different motor units. In this correlative physiological and histochemical study of reinnervated lateral (LG), medial (MG) gastrocnemius and soleus muscles after cross- reinnervation by flexor motoneurons, we have examined the basis for the return of the size principle. Eighteen to 24 months after cross-union of the tibial and common peroneal nerves of 2-6 month old cats, motor units were classified into types, FF, FR, and S and muscle fibers classified as the corresponding FG, FOG and SO fiber types on the basis of histochemical staining.

As previously shown, motor unit force was well correlated with the size of the innervating motor axon but the force of different unit types overlapped considerably more than normal. The reinnervated motor units also showed a higher degree of fatiguability with muscles containing a high proportion of motor units which were intermediate between FR and FF units in fatiguability. Motor units with fatigue indexes below 0.5 were included with FF units and those with indexes above 0.5 were included with the FR units for comparison with histochemical fiber types.

SO and FOG muscle fibers were found in all reinnervated muscles but FG fibers were only found in reinnervated MG and LG muscles, consistent with previous findings of resistance of soleus muscles to complete conversion. Type grouping of muscle fibers was characteristic of the reinnervated muscles. Reinnervated SO fiber size increased significantly to become similar to FOG and FG fibers and calculation of the relative innervation ratio by the relative proportion of muscle fiber types to corresponding motor unit types indicated that the muscle unit size increased in S units. Nonetheless, the force generated by S motor units remained smaller relative to FR with the gradation of mean force in the reinnervated muscles consistent with the normal S<FR<FF despite overlap between groups. Since mean force varies over a 10-fold range and mean fiber area and relative innervation ratio vary over less than a 2-fold range, these data suggest that force per cross-sectional area (specific force) of the muscle fibers is respecified after reinnervation.

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244.11 MOTOR UNIT FORCE, INNERVATION RATIO AND MUSCLE FIBER SIZE IN NORMAL AND REINNERVATED MUSCLE. J.E. Totosy de Zepetnek*, H.V. Zung*, and T. Gordon. (SPON. P. Bawa). Dept. of Pharmacology. University of Alberta, Edmonton, Alberta, Canada. T6G 2H7.

Motor units in reinnervated muscle develop force in the normal range and force is well correlated with axon size (Gordon and Stein, J. Neurophysiol. 48:1175, 1982). Since axons reinnervate muscle fibers that formerly belonged to several different motor units, this return of the size principle requires extensive readjustment of motor unit force to match axon size. We have examined 1) the basis for the recovery of the normal force range and for the return of the normal size relationship between force and axon size and 2) whether reinnervated motor unit force is better predicted by type or by the size of the innervating motoneuron.

Four to six months after section and resuture of the common peroneal nerve, isolated single motor units from the rat tibialis anterior muscle were classified physiologically as per Burke et al. (J. Physiol. 234: 749, 1973) then histochemically following repetitive fatiguing stimulation to glycogen deplete the muscle unit. Glycogen-depleted fibres were detected using periodic acid-Schiff (PAS) stained sections from which we could directly measure the number of fibers (innervation ratio) and total muscle fiber cross-sectional area for each unit.

Examination of reinnervated motor units revealed that although the range of maximum force development was normal (10-250 mN) there was greater overlap between motor unit types in both force and fatigability. On the other hand, in both normal and reinnervated motor units fully analysed to date (1 FF, 3 FI and 4 FR), force was well correlated with muscle fiber cross-sectional area and innervation ratio such that force varied directly as a function of total unit area (slope (m) from a log-log plot =1.02±0.13, r=0.95, n=8). In contrast, there was no correlation between intrinsic force (motor unit force per muscle unit area) and tetanic force (m=0.06±0.11, r=0.20, n=8). Thus for any reinnervated fast motor unit, subtype is a poor predictor of motor unit force. These data suggest that motor units form a continuum with force depending on total cross-sectional area.

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244.12 ORGANIZATION OF MOTOR UNIT PROPERTIES IN THE CAT TRICEPS SURAE MUSCLES AFTER PARTIAL DENERVATION. T. Gordon, and R. Orozco. Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alta., Canada T6G 2H7.

Axonal sprouting is a well recognised compensatory response of undamaged neurons to injury or loss of neighbouring motoneurons, but the degree to which this sprouting enlarges muscle units is unclear. In partially denervated rat hindlimb muscles, a condition in which terminal sprouting predominates, a 5-fold expansion of unit size has been described. Because this corresponds with the size of neonatal motor units, Brown et al. (Ann. Rev. Neurosci. 4:17, 1981) suggested that the neonatal muscle unit reflects the maximum capacity of a motoneuron. However, larger expansions in the cat flexor digitorum longus muscles (Hatcher et al. Exp. Brain Res. 60:590, 1985) indicate that this may not be a general prediction.

In this study, we eliminated 7%-94% of the innervation of triceps surae muscles by section of one of the 2 contributing ventral roots, L7 or S1. Since proportions of nerves which exit in either root vary widely between animals but not bilaterally within animals, measurement of charge delivered to each root in response to stimulation of the peripheral nerves, and the force developed in the muscles in response to stimulation of the L7 and S1 roots, provided direct measures of the extent of partial denervation and of the compensatory response.

Two to twelve months after section of one ventral root, force in the medial and lateral gastrocnemius muscles had recovered so that the intact root had fully compensated for the partial denervation. Force elicited in the intact root on the experimental side was altered in direct proportion to the loss of innervation, while root charge was unchanged. Soleus muscles, in contrast, failed to recover contralateral levels of force.

1) Complete compensatory enlargement of muscle units in the MG and LG muscles without alteration in axonal size and 2) incomplete force compensation in soleus was confirmed by isolation and characterization of single motor unit. (n=322 and 127 for experimental & control units from 5 and 16 cats respectively). Unit force was normally correlated with axonal size, and graded according to type with S < FR < FI=FF, but there was a greater overlap between types in force and fatiguability.

We suggest that nodal sprouting, which is more common in fast-twitch muscles, allows effective reinnervation of partially denervated muscles and that the limited sprouting described previously could be due to the limitations of terminal sprouting rather than due to limited motoneuron capacity.

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244.13 CONTRACTILE PROPERTIES OF THENAR MOTOR UNITS IN NORMAL AND QUADRIPLEGIC SUBJECTS. B. Calancie, T. Gordon, R.B. Stein, L.A. Davis* and B. Dolphin*, Depts. of Physiology, Pharmacology and Surgery, University of Alberta, Edmonton, Alberta, Canada.

Microstimulation of motor axons was used to obtain the mechanical characteristics (twitch tension, contraction time, and half-relaxation time) of single motor units in the opponens pollicis muscle of normal (n=3) and quadriplegic (n=3) subjects. The quadriplegic subjects are being studied to assess the effect of chronic electrical stimulation on muscle properties.

A bipolar needle electrode was placed in the opponens pollicis at a point approximately mid-way along and ventral to the first metacarpal. EMG was measured with 2 surface disc electrodes 8 mm in diameter placed over the thenar muscles and separated by about 3 cm. The thumb contacted a force transducer at the distal end of the first phalange. Threshold stimulation via the bipolar needle of a single motor unit caused an all-or-none increment in surface EMG which, along with the force record, was averaged on an LSI 11/23 computer.

To our surprise, motor unit twitch amplitudes in one quadriplegic subject were larger on average than units in control subjects. The range, mean and standard deviations were 5 to 69.1 mN, 30.5 $\pm$ 14.5 mN for the quadriplegic units (n=40), and 2.4 to 76.8 mN, 18.0 $\pm$ 14.7 mN for the control units (n=61). Contraction times were similar in range and mean value (51.7 to 127.3 ms, 71.0 $\pm$ 12.5 ms; 51.7 to 113 ms, 74.4 $\pm$ 14.0 ms), as were half-relaxation times (36.0 to 121.2 ms, 66.9 $\pm$ 21.2 ms; 32.5 to 95 ms, 59.5 $\pm$ 16.5 ms).

One possible explanation for the large twitch tensions is that some motoneurons to opponens pollicis in the quadriplegic subject may have been destroyed by the spinal lesion, and that surviving motoneurons sprouted to innervate a larger number of muscle fibers than seen in normal subjects. The frequent spasms occurring in the subject appear to have been sufficient to prevent substantial fiber atrophy, but not sufficient to maintain the fatigue resistance of these motor units, which is low.

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244.14 **QUANTITATIVE ANALYSIS OF THE TEMPORAL RELATIONSHIPS AMONG HINDLIMB MOTONEURONS DURING FICTIVE LOCOMOTION IN THE CAT.** D.J. Kriellaars*, R.M. Brownstone, B.R. Noga and L.M. Jordan. Dept. of Physiology, University of Manitoba, Winnipeg, Canada, R3E OW3.

The nature of the central neuronal organization for mammalian locomotion is not well established. Controversy remains on whether or not a strict alternation of activity exists between flexors and extensors as predicted by a half-centre organization. Some researchers have suggested that the neuronal organization for locomotion has a multipartite nature (Stein, pp.245-253, in Comparative Neurobiology: Modes of Communications in the Nervous System, Ed: Cohen & Strumwasser, 1985.) from the observation that motoneuron populations for pluriarticular muscles (posterior biceps, semitendinosus, flexor digitorum longus) exhibit activity in both flexion and extension (i.e. are double bursting) or have activity which is "sandwiched" between extension and flexion. In paralyzed precollicular-postmammillary decerebrate cats, fictive locomotion was elicited by electrical stimulation of the mesencephalic locomotor region. Intracellular records of two lumbar motoneurons and bilateral electroneurograms (ENGs) from a variety of hindlimb peripheral nerves were simultaneously digitized. Dual intracellular records allow direct observation of the relationship of central pattern generator-related synaptic events in contrasting species of motoneurons. During locomotion, motoneurons exhibited membrane potential oscillations with locomotor periodicity called locomotor drive potentials (LDPs). The relationships among flexor and extensor LDPs and ENGs were statistically and quantitatively assessed by plotting the start time of flexor activity against the stop time of extensor activity and vice versa. We utilized the first derivative of the LDPs which proved to be a sensitive technique to detect coincident inflexion points in the simultaneously recorded motoneurons and their relationship to ENGs. The data indicate that the motoneuronal activity could be accounted for on the basis of a strict alternating output from the spinal central pattern generator. Observations from dual motoneuron records indicate that the excitatory drive creating a flexor LDP directly covaries with the inhibition of an extensor LDP. Comparison of double burst motoneurons with pure flexors or extensors demonstrates that these motoneuron pools receive both extensor and flexor excitatory drives and indicate that the extensor inhibitory drive may terminate the brief burst of activity during the flexion phase. These observations support the spinal module system for locomotion (Jordan et al, Soc. Neurosci. Abstr., Vol. 12, Part 2, p.877, 1986).

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244.15 **EVIDENCE FOR A SPINAL MECHANISM REGULATING THE AFTERHYPERPOLARIZATION AMPLITUDE IN LUMBAR MOTONEURONES DURING FICTIVE LOCOMOTION IN THE CAT.** R.M. Brownstone, L.M. Jordan, D.J. Kriellaars *, B.R. Noga. Dept. Physiol., Univ. Manitoba, Winnipeg, Canada, R3E OW3.

It has previously been shown (Brownstone et al, Soc. Neurosci. Abstr., Vol. 12, Part 2, p.877, 1986) that the afterhyperpolarization (AHP) is reduced or eliminated during repetitive firing of cat alpha-motoneurones during fictive locomotion. It is now shown that this reduction in the AHP is not an all-or-none effect. At high firing rates, the AHP is completely abolished. The frequency-current (f-I) relation is then eliminated; i.e. there is no relation between current injected into the cell and the rate at which it fires. However, at lower firing rates, the AHP is reduced in amplitude rather than eliminated completely. At this time, the f-I relation is not abolished, but rather attains an increased gain as compared with the control, indicating that the AHP conductance is reduced rather than shunted. In some cells, the AHP may be modulated within each step cycle, producing a consistent change in firing pattern within each burst.

The reduction in the AHP is associated with locomotor-related spiking in the motoneurone. In a cell that is rhythmically active during locomotion but not firing, the AHP witnessed following short pulse current-evoked spikes is larger in amplitude during the depolarized phase of the locomotor drive potential (LDP) and smaller during the hyperpolarized phase. However, when the cell exhibits rhythmic spiking during the LDP, the AHP is reduced in amplitude.

The reduction in the AHP is related to the depolarized phase of the LDP. In the majority of cells into which short current pulses were injected to produce action potentials throughout the step cycle, there was a reduction in the AHP only during the depolarized phase. Also, when sustained depolarizing current was injected into the cell such that repetitive firing occurred during both the depolarized and hyperpolarized phases, there was a marked reduction in the AHP amplitude during the depolarized phase. The AHPs in the hyperpolarized phase were similar in amplitude to those elicited before the locomotor trial.

These data lead us to suggest that the mechanism responsible for reducing the amplitude of the AHP is intimately related to the central pattern generator for locomotion, and therefore likely resides within the spinal cord.

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244.16 LOCUSTS LOAD COMPENSATE USING SEVERAL DISCRETE MOTOR STRATEGIES THAT CAN BE ELICITED, IN PART, BY STIMULATION OF A SINGLE SENSE ORGAN. S.N. Zill and K.A. Jepson*. Dept. of Cellular and Structural Biology, Univ. of Colo. Med. Sch., Denver, CO 80262.

Many animals show patterns of coordinated motor responses to load compensate and maintain postural stability when the substrate upon which they are standing is displaced. Human subjects, for example, respond with several discrete types of strategies when standing upon a moveable platform (Horak and Nashner, J. Neurophysiol. 55:1369, 1986): small and intermediate movements of the platform produce swaying at the ankle or hip joints, while large displacements produce 'steps', that is, movements of a whole leg to shift the base of support under the center of body mass. The particular leg sense organs that can mediate these different responses have not been identified in vertebrates. We have applied a similar paradigm to examine load compensatory responses of an invertebrate, the locust. Our results suggest that locusts use either joint swaying or leg 'stepping' strategies in load compensation and that a single identified joint receptor, the femoral chordotonal organ, may contribute to the mediation of both responses.

Locusts were restrained and myogram wires placed in the extensor and flexor tibiae muscles of both hindlegs. Animals were then released into a cage which was mounted on a swivel joint and driven by a motor. The motor produced repetitive displacements (rotations through a 45° arc at 0.7 Hz) of the cage and the substrate upon which the animal stood. Freely standing animals actively using the hindleg in postural support showed two general patterns of muscle activity in response to displacements of the cage. In one pattern, termed the resistance mode, tibial motoneurons fired repetitively at fixed phases in cycles of displacement. In another pattern, termed the flexion mode, animals moved the femoro-tibial joint to complete flexion and tonically fired the flexor muscle. The latter strategy apparently places the joint in a position to maximize stability of body support.

Both general types of responses can also be elicited by stimulating a single joint receptor of the hindleg, the femoral chordotonal organ, in both restrained and freely moving preparations. The present study has demonstrated that insects can load compensate and that a single proprioceptive sense organ can mediate load compensatory postural adaptations in several different modes of reflex action. We are currently examining the interneurons of the central nervous system that can mediate and modulate these reflex actions.

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244.17 **KINEMATIC STRATEGIES OF WIPING IN SPINAL FROGS.** S. F. Giszter* and J. McIntyre* (SPON: E. Bizzi). Dept. Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Previous workers have shown that spinal frogs are able to wipe irritations from the body surface in a coordinated way. Because frogs adjust movements based on differing body configurations and stimulus positions, the spinal frog may be a model system to study the physiological mechanisms underlying coordinate transformations and trajectory formation. We have performed a detailed examination of wiping behaviors to answer the following questions about the kinematic strategies and control used to accomplish the wiping task: 1) How repeatable is performance for movements with similar target location and initial postures? 2) Are wiping trajectories continuously or discretely adjusted in response to variations in target location over the body and variations in body position? 3) What kind of adjustments are made in response to perturbations?

Bullfrogs (rana catesbaiana) were transected at the level of the fourth ventricle, while in leopard frogs (rana pipiens) the CNS anterior to the fourth ventricle was removed. Wipes were elicited with a vibrating stylus or glassfibre patches soaked in 5% sulphuric acid. Wiping in the two species were qualitatively similar. Movements were measured by digitizing limb positions in videotapes (30Hz sample rate), and using a Watsmart infrared camera system to obtain orientations of reference LED arrays attached to the frogs (100Hz sample rate).

1) To assess performance for similar targets we recorded wiping by one hindlimb to a stimulus applied to the contralateral hindlimb. In this behavior both legs move to an aiming position from which the effector limb moves to wipe the target limb. The unperturbed spinal frog reproduces these endpoint aiming positions in successive wipes within a range of several millimetres (body length ~15cm).

2) To evaluate aiming adjustments the aiming positions of the hindlimb for wiping to locations distributed over the back and forelimb were examined for continuous variations of target location and body configuration. A small discrete set of stereotyped endpoint locations and wipe directions were used by the wiping limb as opposed to a continuously varying adjustment to the continuously varied target location.

3) We also examined hindlimb to hindlimb wiping for evidence of adjustment to disturbances. Small unimposed variations of limb locations about the average aiming position showed no interlimb correlations. When large perturbations were made to the target limb in hindlimb/hindlimb wipes these resulted in terminated wiping movements rather than adjusted trajectories.

These results suggest spinal frogs use a discrete number of kinematic strategies to achieve successful wiping throughout the workspace. These strategies are performed with high endpoint accuracy relative to the requirements of the task. It seems that the spinal frog chains together a set of movements selected from a finite repertoire.

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244.18 **THE INTERACTION BETWEEN SARCOMERE LENGTH CHANGE AND KNEE JOINT KINEMATICS DURING ISOMETRIC TORQUE PRODUCTION IN THE FROG.** R.L. Lieber and J.L. Boakes*. Division of Orthopaedics and Rehabilitation, University of California and Veterans Administration Medical Centers, San Diego, CA 92161.

INTRODUCTION: The purpose of this study was to measure sarcomere length, joint torque, and joint kinematics directly in order to determine the nature of the interaction between muscle properties and joint kinematics during isometric torque production.

METHODS: The dorsal head of the semitendinosis muscle from the grassfrog (Rana pipiens) was chosen for this study due to its well-established sarcomere length-tension relationship (Gordon et al. 1966). The muscle-bone complex, consisting of the pelvis, femur, tibia, and semitendinosis muscle, was placed into a chamber containing chilled frog Ringer's solution (14°C). In 10 specimens, the hip was immobilized at 90° of flexion and the knee manipulated through its range of motion from 0-160° of flexion at 10° intervals while measuring sarcomere length using laser light diffraction (Lieber et al. 1984).

Knee joint torque was determined at 10° intervals by, first, directly stimulating the semitendinosis muscle using bipolar electrodes placed along the muscle length and then calculating knee joint center of rotation at 10° intervals using methods similar to those described by Panjabi (1979).

RESULTS: A linear relationship was observed between sarcomere length and knee joint angle with sarcomere length ranging from 3.5 um at full knee extension to 2.5 um at 160° of knee flexion The regression equation fit to the data indicated that for each 10° increase in joint angle, sarcomere length decreased by about 70 nm.

Semitendinosis lever arm remained relatively constant (about 3 mm) throughout the range 0-160° with two small peaks at about 35° and 105° of flexion. Knee joint torque (calculated as the vector product of lever arm and measured contractile force) increased from 30% maximum at full knee extension, peaked at 140° of flexion, and then decreased to 75% of maximum at 160°.

DISCUSSION: The main result of this study was that maximum joint torque occurred at an angle (140°) which was neither the angle at which muscle force was maximum (160°) nor the angle at which the effective lever arm was maximum (90°). At the optimal knee joint angle (140°), sarcomere length was only 2.6 um corresponding to a muscle force of only 70% maximum. Optimal joint angle thus results from the interaction between muscle properties and joint kinematics and not either property alone.

REFERENCES: Gordon et al. (1966). J. Physiol. 184:170-192. Lieber et al. (1984). Biophys. J. 45:1009-1117. Panjabi, M.M. (1979). J. Biomech. 12:911-920. Supported by the Veterans Administration and NIH grants AM25501, AM26344 and AR35192.

244.19 **Role of Digastric in Jaw Opening While Chewing Gum of Different Size and Hardness at Various Frequencies.** B. Bishop, O. Plesh* and W. McCall. Depts. of Physiol. and Oral Medicine, State Univ. of New York at Buffalo, Buffalo, NY, 14214

Only fragmentary information exists concerning mechanisms controlling the initiation, extent and termination of jaw opening (JO) and the timing and level of activity in the digastric muscle during chewing. Thus, JO and digastric activity were assessed when pieces of gum of different hardness and size were chewed at different frequencies to obtain new information about control of JO and digastric activity by sensory feedback. Jaw movements in 3 planes were recorded with a Kinesiograph; digastric and medial pterygoid EMGs were detected from implanted wires; and anterior temporalis and masseter surface EMGs were recorded from the right side while adults with normal dentition chewed in time with a metronome set randomly at 46, 66, 100 and 160 BPM. Gums of two hardnesses (soft or hard), and sizes (10 x 18 mm and 0.7 gm, or 20 x 36 mm and 1.4 gm) were chewed on the right in random sequence at each frequency.

Onset of digastric activity never started before cessation of closer activity. When chewing at high frequencies (100 or 160 BPM), JO followed onset of digastric activity but at low frequencies (46 or 66 BPM), JO preceded digastric activity. During the delay the closer muscles were silent showing that the delayed opening was not due to residual closer activity. During slow chewing digastric activity ceased prior to or at peak opening but at high frequencies it ceased after peak opening. Burst duration of digastric activity, although surprisingly stable (238+59 ms) over all experimental conditions, was longer on soft gum than on the hard gum, regardless of gum size. In contrast, peak amplitude of digastric activity was greater on the large gum than on the small gum regardless of gum hardness. Gape was independent of chewing frequency, burst duration, and gum hardness. However, like the peak amplitude of digastric activity, gape was greater when chewing the large gum.

In summary, the onset of digastric activity with respect to opening depends on chewing frequency, whereas digastric burst duration remains relatively stable regardless of chewing frequency, gum size or gum hardness. Both peak amplitude of digastric activity and gape are larger when chewing the larger size gum. We conclude that even though the digastric muscle is a major jaw opening muscle, its activity does not correlate closely with initiation, extent or termination of mandibular opening. Concurrent behavior of all masticatory muscles determines timing and path of jaw movements. Supported by NIDR 5R01DE0671703.

244.20 LARYNGEAL MUSCLE ACTIVATION AND TIMING DURING RESPIRATION, VOCALIZATION, SPEECH AND SWALLOW. C. L. Ludlow, S. Sedory*, M. Fujita* and G. Schulz*. Human Motor Control Section, NINCDS, Bldg.10/5N226, Bethesda, MD 20892.

Activation and timing patterns of two intrinsic laryngeal muscles on each side were contrasted during speech and vocalization with activation patterns during respiration and swallow. Six normal adults, three female, consented to intrinsic laryngeal electromyography. Percutaneous concentric needles were employed to record from the thyroarytenoid (TA) and cricothyroid (CT) muscles on the left and right sides with simultaneous recording of respiratory and laryngeal movements and voice. The experimental conditions included quiet respiration, swallow, vocalization (extended vowel production) and speech (sentence repetition). The percent activation relative to the maximum peak activation during inspiration was computed for vocalization, speech and swallow. Similarly, the percent activation relative to the maximum peak during swallow was computed for respiration, vocalization and speech. Activation onset and offset times for each of the muscles during swallow, vocalization and speech were identified (when the level became > than 150% of the inspiration maximum for onset and < 150% above the minimum inspiration level for offset). The speed of activation was the peak of the first derivative of the rectified signal after averaging with a sliding window of 40 ms.

Mean activations were greater for the TA and CT muscles during inspiration. The peak activation times for the CT and TAs did not differ during either respiratory cycle, suggesting coordinated activation of these two muscles during respiration. The percent activation relative to inspiration was greatest during swallow for the TAs and during speech for the CTs. TA and CT activation onset times were most similar during swallow and phonation when the two muscles tended to act together. The greatest difference in TA and CT onset times occurred during speech when CT activation preceded TA activation. The peak velocity of activation was greatest early during phonation and swallow and later during speech, when associated with syllable stress. The percent increase in activation over inspiration levels were greater for the CTs than the TAs during speech and vocalization. The results demonstrated that laryngeal muscle activation during speech involves separate activation of the CT and TA muscles and a more complex activation pattern. These findings indicated why speech is more likely to be affected by motor control disorders of the laryngeal musculature, than phonation or swallow.

245.1 ANTIBODIES TO DYNORPHIN A (1-13) BUT NOT β-ENDORPHIN INHIBIT ELECTRICALLY-ELICITED FEEDING IN THE RAT. K.D. Carr, T.H. Bak*, T.L. Gioannini* and E.J. Simon. Department of Psychiatry, New York University Medical Center, New York, NY 10016.

The anorectic action of opioid antagonists has been confirmed in numerous animal models including the feeding elicited by lateral hypothalamic electrical stimulation in the rat (Carr & Simon, 1983, 1984; Jenck, Gratton & Wise, 1986). In the present study, highly specific antibodies to dynorphin A (1-13) and β-endorphin were administered ICV to determine the effect of selective peptide inactivation on electrically-elicited feeding. Over an 18 day period, each of seven rats was tested six times; twice under control conditions and twice following administration of each of the antisera. In each test session, stimulation intensity was fixed at 100 μa and four estimates of frequency threshold were obtained in serial order. Mean brain stimulation frequency threshold for eliciting feeding following vehicle infusions was 18.9 ±0.9 pulses per second (pps). Antibodies to dynorphin A (1-13) produced a significant increase in threshold to 28.4 ±2.7 pps ($p<.01$, $t=4.98$, matched pair, two-tailed test). Mean threshold following administration of β-endorphin antibodies was 20.9 ±2.4 pps ($p>.20$, $t=1.17$). Within test sessions serial estimates of feeding threshold increased progressively after administration of dynorphin antibodies (linear trend: $p<.01$, $F(1,18)=12.76$). This pattern is similar to that observed following systemic administration of naloxone (linear trend: $p<.01$, $F(1,27)=21.6$). Central dynorphin A, or one of the other opioid peptides derived from prodynorphin, recognized by the antiserum used, may be involved in the control of ingestive behavior. The anorectic action of naloxone may result from antagonism of dynorphinergic transmission, although a role for other opioid peptides cannot be ruled out.

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245.2 SACCHARIN AVAILABILITY NOT PREFERENCE PROMOTES CROSS-TOLERANCE TO MORPHINE IN RANDOMLY BRED RATS. A. Acevedo-Cruz*, R. Knurowski*, and D. Novin (SPON: D.A. VanderWeele). Dept. of Psychology and Brain Research Institute, Univ. of California, Los Angeles, CA 90024.

The observation that ingestion of a palatable glucose-saccharin solution decreases the sensitivity of the analgesic effects of morphine in the rat has led to the suggestion that highly preferred sweet solutions elicit the release of endogenous opioids and lead to the development of cross-tolerance to morphine (Bergman et al., Beh. Neural Biol. 44: 347, 1985). Rats bred for high rates of hypothalamic self-stimulation show a marked preference for saccharin and also develop tolerance to morphine after exposure to saccharin solutions. Conversely, randomly bred rats show moderate preference for saccharin and an attenuated cross-tolerance to the opiate. Since albino rats show large individual differences in their preference for saccharin, and since it has been assumed that preference itself is the decisive factor in the development of morphine cross-tolerance, we tested whether rats with very different preferences for saccharin would differ in the development of the cross-tolerance. Rats with high (HI) and low (LO) preference for a 3mM saccharin solution were identified from a larger pool of rats and their sensitivity to the analgesic effects of morphine assessed in a hot plate test (HPL) after 21 days of access to the solution. A group of rats that had access only to water (W) served as control. Rats were housed in group cages of 4 rats each. Mean daily group intakes were: 310.7 (HI), 157.1 (LO), and 168.9 g (W). HPL values were obtained before (baseline), and at 15 and 30 min after the injection of saline or 2.5 mg/kg morphine. Both the HI and the LO groups showed cross-tolerance to the analgesic effects of morphine as compared to the W group. The HI and LO groups did not differ in their response to morphine. These results suggest that it is the ingestion of saccharin, regardless of preference, that leads to the development of cross-tolerance to morphine. Furthermore, an above-normal ingestion of fluid is not necessary to develop the tolerance, since the LO group became tolerant and ingested the same amount of fluid as the W group.

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245.3 EFFECTS OF DOPAMINE AND OPIATE RECEPTOR BLOCKADE ON MORPHINE AND AMPHETAMINE INDUCED FEEDING: INTERACTIONS WITH SWEETNESS. K.R.Evans* and F.J.Vaccarino. (SPON: N. Mrosovsky). Dept. of Psychology, Univ. of Toronto, Toronto, Canada, M5S 1A1.

Previous studies have shown that morphine- (MOR) and d-amphetamine- (AMP) induced feeding can be differentiated when the treated animals are given the choice of sugar, sweetened chow (5% sugar) or unsweetened chow; MOR-treated animals increase their intake of all foods while AMP only increases the intake of sugar. The present studies examine possible interactions between the opiate and dopamine (DA) systems with regard to the differential MOR and AMP effects by combining their treatments with the DA antagonist alpha-flupenthixol (FLU) or the opiate antagonist naloxone (NX).

In the first experiment, rats were tested for MOR- (2.0 mg/Kg) and AMP- (0.25 mg/Kg) induced eating following treatment with FLU in doses of 0.0, 0.05, 0.10 or 0.20 mg/Kg. Intake of sugar, 5.0% sugar/chow mixture and chow was recorded for 1.5 h following drug treatment. Both AMP and MOR increased intake of sugar, which was under all conditions preferred to the other tastes, but MOR also produced small increases in intake of sweetened chow. FLU attenuated AMP-induced feeding at all doses, though only the highest dose attenuated MOR-induced feeding. 0.05 mg/Kg FLU increased intake of sweetened chow in MOR-treated animals. None of the doses of FLU had any effect on SAL-treated animals. In the second experiment rats were injected with NX in doses of 0, 0.125, 0.50 and 1.0 mg/Kg immediately prior to MOR- and AMP- treatment. NX attenuated MOR and AMP induced feeding at all doses. NX decreased feeding in SAL-treated animals only at the 1.00 mg/Kg dose.

Results suggest that the MOR-induced feeding is generally excitatory, whereas AMP has a more specific effect, possibly related to stimulus relevancy (ie. specific increases in sugar intake). Both MOR and AMP-induced feeding seem to require the integrity of DAergic and opiate systems, respectively. Further, since neither FLU or NX had any effect on the ability to discriminate between tastes or on their normal preferences, it is unlikely that endogenous DA or opiate mechanisms control these aspects of feeding behaviour.

245.4 MU OPIOID RECEPTORS IN THE AMYGDALA CONTRIBUTE TO THE CONTROL OF FEEDING. B.A. Gosnell. Dept of Physiology, University of Texas Health Science Center, Dallas, Texas, 75235

Opioid peptides stimulate feeding when injected into the lateral cerebral ventricle or directly into certain regions of the brain. In these experiments, highly selective opioid agonists were injected into the amygdala of rats to determine whether opioid peptides and receptors in this structure are involved in the regulation of food intake. The amygdala has been shown to contain moderate to high densities of opioid-containing fibers and cell bodies (Khachaturian et al., Trends Neurosci. 8:111-119, 1985).

Guide cannulas (unilateral) were implanted with the tips directed toward the central nucleus of the amygdala (AMYG) in male Sprague-Dawley rats. Injections (0.5 μl volume) were made via a cannula which extended 0.5 mm beyond the guide cannula. The peptides tested were Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (DAGO), [D-Ser^2,Leu^5]enkephalin-Thr^6 (DSLET) and dynorphin A (DYN). These peptides represent selective agonists of mu, delta and kappa opioid receptors, respectively.

In the first experiment, DAGO, DYN and DSLET were injected at doses from 0.3 to 3 nmol. DAGO (3 nmol) significantly increased 4 hr food intake (2.6±0.8 g vs 0.5±0.4 g for NaCl-treated controls); DYN and DSLET had no effect. In the second experiment, DAGO increased intake at doses of 1 and 3 nmol. In a third experiment, injections of DAGO and DYN into the AMYG were compared to injections into the medial hypothalamus (HYPO). As expected, DYN (2 nmol) increased 2 and 4 hr intake when injected into the HYPO but not when injected into the AMYG. DAGO (2 nmol), however, stimulated intake when injected into either area. In the fourth experiment, bilateral injections of DAGO into the AMYG (3 nmol/side) were found to be no more effective than unilateral injections. Finally, the feeding effects of DAGO (3 nmol) were blocked by naloxone (25 μg, injected into the AMYG 1 hr after DAGO) and by β-chlornaltrexamine (1 μg, injected into the AMYG 4 hrs before DAGO).

Stanley et al. reported that AMYG injections of [D-Ala^2,Met^5]enkephalinamide stimulated food intake (Soc. Neurosci. Abstr. 10:1103, 1984). The present results support this finding and suggest that mu rather than kappa or delta opioid receptors in the AMYG contribute to the control of feeding. Further, the AMYG appears to differ from the HYPO in relation to the receptor types involved.

Supported by NIH grants NS23565 and RR07175.

245.5 THE EFFECT OF THE MU OPIOID LIGAND, [D-Ala^{2}MePhe^4,-Gly-ol^5] ENKEPHALIN (DAGO) ON FOOD INTAKE IN DIABETIC AND NON-DIABETIC RATS. A.S. Levine, M. Grace*, B.A. Gosnell, C.J. Billington*, Neuroendocrine Research Laboratory, VA Medical Center and Department of Food Science and Nutrition, University of Minnesota, Minneapolis/St. Paul, MN, 55417.

Glucose is known to alter the responsiveness of laboratory animals and humans to a number of opioid effects including nociception, smooth muscle contractility, behavioral tolerance and energy intake. Agonists of the mu, delta and kappa opioid receptor have been shown to increase short-term food intake after intraventricular administration. In the present study we evaluated the effect of central administration of the mu opioid ligand, DAGO, on food intake in diabetic and non-diabetic rats. Male rats were injected with streptozotocin to induce diabetes and cannulas were placed in the right lateral ventricle. In the first study, saline, 1 nmol, 3 nmol or 10 nmol DAGO were injected into a group of diabetic and non-diabetic controls. DAGO failed to stimulate feeding in the non-diabetic rats and only slightly stimulated feeding in the diabetic rats following the initial injection (diabetics: saline = 0.6 ± 0.6 ; 1 nmol = 3.1 ± 0.6; 3 nmol = 3.4 ± 0.4; 10 nmol = 4.9 ± 1.6 g/4 hr ($p < 0.05$); non-diabetics: saline = 0.9 ± 0.5; 1 nmol = 1.1 ± 0.6; 3 nmol = 2.5 ± 1.0; 10 nmol = 1.7 ± 0.5 g/4 hr). After repeated administration the diabetic rats appeared to be more sensitive to the administration of DAGO compared with the control rats. To study this phenomenon in greater detail we injected a different group of diabetic and non-diabetic animals with saline or 10 nmol DAGO (n = 11 per group) and injected them with drug or vehicle twice daily at 0900 hr for a period of five days. Food intake was measured on days 1, 2 and 5; four hours after injection of DAGO. On days 3 and 4 animals were injected with drug or vehicle but food intake was not measured. The effect of repeated injections of DAGO on 4 hour food intake was much more pronounced in the diabetic rats compared to their controls (diabetic rats: day 1 = 1.3 ± 0.6; day 2 = 3.7 ± 0.5; day 5 = 6.8 ± 1.0 g/4 hr. non-diabetic rats: day 1 = 2.4 ± 0.5; day 2 = 0.8 ± 0.4; day 5 = 3.3 ± 0.7 g/4 hr). Saline injection did not alter food intake over the 5 day period in either diabetic or non-diabetic animals. The results of these studies indicate that the mu opioid ligand, DAGO, stimulates feeding in a more reliable fashion after repeated injection. In addition, this "sensitization" is more pronounced in diabetic rats compared with non-diabetic controls. These data further support the concept that the effect of opioids on food intake are different in diabetic animals compared to their euglycemic controls. This work was supported by NIDA 1R01-DA03999-01 and the VA.

245.6 NALOXONE IMPAIRS ACQUISITION IN AN ACTIVE AVOIDANCE PARADIGM IN RATS. G.A. Olson, F. Gundy*, A.J. Kastin, M.F. Pignatiello*, and R.D. Olson. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148 and V.A. Medical Center, New Orleans, LA 70146.

Although most studies have demonstrated the facilitory effect of naloxone on memory, few have used the active avoidance paradigm. Those that have used it produced contradictory evidence, with findings of enhancement, interference, or no effect with naloxone. No clear-cut variables, such as doseage, task, or species accounted for the differences.

In this study 24 Sprague-Dawley-derived albino rats aged 84-106 days and weighing 342-479 g were placed in a traditional escape/avoidance, bar-press paradigm with a 15 sec warning signal (light), a shock intensity calibrated at 0.511 mA and maximum duration of 30 sec, and a mean ITI of 60 sec (ranging 48-72 sec). Rats were administered either naloxone (0.01 mg/kg) or diluent IP. Injections were given immediately before placing the rats in the apparatus for 10 min of habituation, after which conditioning began. Twenty-five trials were administered each day for 3 consecutive days, followed by a day with 5 warm-up trials and then 25 extinction trials. In extinction half of each group received naloxone and half diluent.

Naloxone impaired acquisition, as indicated by significant day by drug interactions in the ANOVAs for measures of number of avoidances and number and latency of bar presses (avoidances and escapes). In general, the naloxone group showed little learning, but the diluent group did learn by the third day. Extinction results were confounded by the lack of learning by the naloxone group and were not affected by the drug given during extinction itself.

To determine if naloxone simply lowered the pain threshold, thus affecting learning indirectly, a tail-flick test was performed after the same dose of naloxone or diluent (n = 20). All rats were given four trials starting 10 min after the injection; the most deviant score was discarded, and the mean of the remaining three was used. There were no significant differences between the two groups, suggesting that there was no alteration of the pain threshold to account for the differential learning.

Thus, a small dose of naloxone given before training interfered with the acquisition of an active avoidance response in rats.

245.7 ENHANCEMENT OF SUCROSE INTAKE BY THE KAPPA OPIOID AGONIST U-50,488H PERSISTS BEYOND THE PERIOD OF DRUG EXPOSURE. W.C. Lynch and G. Burns.* Department of Psychology, Montana State University, Bozeman, MT 59717.

Opioid agonists are known to facilitate food intake motivated by deprivation, taste, and various other treatments (For review see Levine, A.S., et al. Br. Res. Bull., 14: 663-672, 1985). Suggested interpretations include the possibility that opioids directly stimulate hunger, that they enhance food palatability, or that they reinforce taste preference learning. The present experiment examined the acquisition of a preference for sucrose solutions in naive rats. One purpose was to determine whether mu or kappa agonists given in repeated association with sucrose intake might facilitate preference learning. A second purpose was to determine whether or not drug-induced facilitation of intake persists beyond the period of drug exposure. Forty naive male Holtzman albino rats were randomly assigned to 4 groups. Each day for 15 days (5/week), all animals received a 30 min 2-bottle intake test in which they could select either sucrose (20%, w/v) or tap water. Animals were nondeprived and received injections 30 min before each intake test. Group U-50L received a low dose (0.1mg/kg, sc) of the kappa-selective agonist U-50,488H; group U-50H received a high dose (1.0 mg/kg, sc) of the same drug; group MOR received a moderate dose of morphine sulfate (1.0 mg/kg, sc); and group SAL received a 1 ml/kg (sc) injection of normal saline vehicle. After 10 days of testing, all animals were shifted to SAL injections and intake testing continued for 5 more days.

Preliminary analyses indicate that both morphine and the high dose of U-50,488H inhibit sucrose intake, whereas the low dose of U-50,488H consistently facilitates intake. More importantly, following drug withdrawal the 4 groups maintain these acquired preference differences. This suggests that the low dose of U-50,488H facilitates sucrose intake through an effect on learning and that an established preference is maintained in the absence of further drug treatment. Analysis of within session lick patterns on the 10th day of testing showed that U-50L treatment facilitates intake (relative to SAL) only during the 3rd through the 7th min of the session. This confirms previous reports that opioid agonists affect the continuation rather than the initiation of intake, perhaps due to enhanced gustatory feedback. The failure of U-50H to facilitate intake suggests the importance of receptor selectivity. At the higher dose, U-50,488H inhibited intake in a manner similar to that seen with the mu agonist morphine. Perhaps at this higher dose U-50,488H binds sufficiently to mu receptors to produce morphine-like inhibitory effects that counteract its facilitative effect on the development of preference.

This work was supported in part by a grant to WL from the NSF/MONTS EPSCoR Program (ISP 8011449).

245.8 CHANGES IN IN VIVO OPIATE RECEPTOR BINDING WITH FEEDING IN THE RAT. E.A. Stein. Dept. of Biology, Marquette Univ., Milwaukee, WI, 53233.

It has become apparent that one or more endogenous opioid peptides plays an as yet unspecified role in mechanisms related to consummatory behavior. Opioid antagonists have been shown to suppress feeding (and drinking) when administered centrally or peripherally while agonists generally increase feeding. Central drug administration as well as measurements of peptide levels have begun to map those central sites mediating this behavior. To extend this inquiry, we have utilized an in vivo receptor autoradiography procedure to further delineate those areas which show altered opiate receptor binding after this behavior.

Male Holtzman rats are implanted with chronic jugular catheters and then assigned to either an ad lib feeding group or a 23 hr food deprived (water available ad lib) group for 7 to 10 d. On the day of sacrifice, water is removed from all cages and ½ of the ad lib group is given non-caloric, sweetened chow pellets to which they had been previously exposed. Likewise, ½ of the deprived group were given their daily food, while ½ received no food. Thus four groups were defined: ad lib, ad lib plus palatable, 23 hr deprived, and 23 hr deprived plus food. All animals received .002 mg/kg 15,16 ^{3}H Diprenorphine (Dpr) through the chronic connulae, permitted to eat for 15 min, then returned to their home cage for 5 min to complete nonspecific binding work out. Rats were then decapitated, brains rapidly removed, stored at -70°C until sectioned. Sections (20μ) were cut at -15°C, mounted onto glass slides, dried on a slide warmer and exposed to ^{3}H sensitive film (LKB Ultrafilm) for 4-6 weeks in standard x-ray cassettes (Wolf).

Autoradiograms were analyzed by the ROD method of Gallistel et al., using a Spatial Data 850 Image Analyzer. Grouped data were subjected to analyses of variance followed by student Neuman Kuels analysis. Areas analyzed include the cingulate, frontal cortex, nucleus accumbens, caudate, diagonal band, olfactory tubercle and pirform cortex. Data will be discussed with respect to the modulation of feeding and/or reinforcement mechanisms by opioids.

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245.9 ATYPICAL ENDOGENOUS OPIOID SYSTEMS AND AN AUTO-ADDICTION OPIOID MODEL OF ANOREXIA NERVOSA Mary Ann Marrazzi, Dept. Pharmacology, Wayne State University School of Medicine, Detroit, Michigan 48201

We have proposed that an atypical opioid system in the BALB/C mouse may resemble that in anorexia nervosa patients and may be involved in the etiology of the disorder (Buck & Marrazzi, 1987). Morphine causes sedation and increases food intake and blood glucose in most species including normal human subjects. In contrast, in BALB/C mice morphine decreases food intake and blood glucose and increases motor activity. This combination of hyperactivity and anorexia also characterize anorexia nervosa. These atypical morphine responses are very interesting in light of a critical role of endogenous opioids in the pathophysiology of anorexia nervosa proposed by our auto-addiction opioid model (Marrazzi & Luby, Int. J. Eating Disorders 5:191- 208, 1986). According to this hypothesis, an initial period of dieting induces the release of endogenous opioids, which then induce a positively reinforcing elation and an addiction to dieting. Endogenous opioids may play a dual role in responding to starvation. They increase food intake to correct it, and they adapt for survival until it is corrected. The latter may involve a down-regulation of function to an essential minimum. If these two responses become uncoupled and addiction occurs to only one component, eating disorders could result. Different balances of these responses may result in different responses to morphine in typical and atypical species, reflecting differences in the underlying opioid systems. Some of the atypical opioid systems may result in a biological predispositon to anorexia nervosa. There is also evidence of opioid release with starvation and for a disturbance of endogenous opioids in anorexia nervosa. The clinical picture of anorexia nervosa resembles that observed in addictive disorders. Narcotic antagonists may be therapeutically useful in interrupting the addictive cycle (Luby, Marrazzi & Kinzie, J. Clin. Psychopharmacol. 7:52-53, 1987 and companion abstract).

In this context, the atypical endogenous opioid systems will be further characterized using strain comparisons and the selective kappa subtype of opioid agonist, U50,488. A spectrum of endogenous opioid systems have been previously described in mice with reference to other opiate actions.

245.10 TREATMENT OF EATING DISORDERS WITH NARCOTIC ANTAGONISTS. Elliot D. Luby* and Mary Ann Marrazzi (Spon. Helene C. Rauch), Depts. of Psychiatry and Pharmacology, Wayne State University School of Medicine, and Dept. of Psychiatry, Harper-Grace Hospitals, Detroit, Michigan 48201.

The auto-addiction opioid model of chronic anorexia nervosa that we have proposed (Marrazzi, M.A. and Luby, E.D., Int. J. Eating Disorders 5:191-208, 1986) suggests that narcotic antagonists may be useful to interrupt the addictive cycle in the treatment of eating disorders. Our initial studies look promising and will be presented here. According to the auto-addiction model, endogenous opioids are released during an initial period of dieting which then cause a positively reinforcing elation and a self-perpetuating addiction. The clinical picture in anorexia nervosa resembles that of addictive disorders. Elevated total endogenous opioid activity in the CSF of anorexia nervosa patients has been reported. Evidence also suggests endogenous opioids are released during food restriction. Although the endogenous opioids increase food intake, they may play a dual role in the response to starvation. They also adapt the organism for survival until the starvation is corrected, by down-regulating function to an essential minimum. This is based on a number of parallels of opiate actions and known adaptations to starvation. If these two responses become uncoupled and addiction occurs to only one of them, eating disorders could result. Addiction to the elation and/or down-regulation without the concommitant opioid drive to eat could result in anorexia nervosa. Addiction to the opioid drive to eat could result in bulimia. Thus, opiate blockade may be useful to interrupt the addictive cycle in the treatment of both anorexia and bulimia. Our initial sutdies with long-acting orally active narcotic antagonists in both anorexia nervosa and bulimia will be reported.

245.11 FEEDING BEHAVIOR IN ADULT RATS EXPOSED NEONATALLY TO OPIATE DRUGS. J. A. Stuckey*, T. R. Insel, and D. C. Jimerson. Laboratory of Clinical Science, NIMH, Bethesda, MD 20892.

Previous studies have shown that neonatal treatments of rats with opiates produce changes in opiate receptors that persist into adulthood (Tsang and Ng, 1980; Handelmann and Quiron, 1983). These receptor changes are associated with changes in behaviors which are thought to be mediated in part by endogenous opiates (Zagon and McLaughlin, 1980; Dow-Edwards and Handelman, 1985). Neonatal treatment of rats with morphine has been shown to accelerate the appearance of naloxone induced suppresion of feeding in rat pups (Aroyewun and Barr, 1983). In this study we are investigating the effects of neonatal treatment of rats with a variety of opiate drugs on adult feeding behavior.

On the day of birth, male pups were randomly assigned to litters of 7-8 pups per litter. On days 1-7 pups received subcutaneous injections of morphine, tifluodom, or naltrexone (0.5 mg/kg dissolved in saline) or saline as vehicle control. Treatments were randomized among the litters, with 7-8 pups receiving each treatment. On day 21 the pups were weaned, and on day 42 placed in individual cages. Rats were adapted to an 18 h food deprivation schedule in which they received a test diet of evaporated milk diluted 1:1 with a 10% w/v sucrose solution for one hour, followed by 5h access to solid pellets as maintanance diet. After adaptation to the testing schedule, rats received s.c. injections of saline or naloxone dissolved in saline 30 min prior to access to the test diet.

After saline injection, the thirty minute intakes were not significantly different between the neonatally treated groups, although the animals treated with morphine neonatally ate slightly more than the animals treated neonatally with saline: saline (19.3 +/- 1.1), morphine (22.3 +/- 1.6), naltrexone (20.2 +/- 1.1), tifluodom (20.8 +/- 1.3), (values are ml +/- SEM). There was a significant difference between groups in percent inhibition of 30 minute intakes by naloxone, $F(3,26)=4.82, p<0.01$, and a significant effect of dose of naloxone, $F(1,26)=13.73, p<0.01$. With 0.1 mg/kg naloxone, both the morphine group (37 +/- 5) and the tifluodom group (35 +/- 5) were significantly different from the saline group (22 +/- 7), $p<0.05$, while with 1.0 mg/kg naloxone only the morphine group (54 +/- 8) was significantly different from the saline group (33 +/- 5), $p<0.05$, (values are percent inhibition +/- SEM).

Neonatal pretreatment with morphine and tifluodom appear to have long term effects on naloxone's inhibition of feeding in adults. Further tests of feeding behavior using other paradigms are being conducted with rats treated neonatally with opiate drugs to determine the full extent to which such treatments may effect feeding behavior. At the completion of behavioral testing brain opiate receptors will be assayed to determine if potential alterations in opiate receptors are correlated with changes in feeding behavior.

245.12 COMPARATIVE EFFECTS OF CCK-8 AND ANALOGS ON ANORECTIC ACTIVITY AND IN VITRO GALL BLADDER CONTRACTION. J. Blosser, S. Augello-Vaisey, M. Barantes, J. Comstock, D. Gawlak, M. Towne, R. Simmons, J. Rosamond. Pharmaceutical Division, Pennwalt Corporation, Rochester, NY 14623.

Cholecystokinin octapeptide (CCK-8) is a potent anorectic peptide which exerts a variety of peripheral effects, including contraction of smooth muscle in gall bladder and intestine and secretion of pancreatic enzymes. Although it is known that certain structural features of CCK-8, such as tyrosine-O-sulfate and the C-terminal heptapeptide are required to elicit these varied responses, possible differences in receptor specificity have not been well explored. We have prepared a series of 13 monosubstituted CCK-8 derivatives comprised of sequential D-amino acid substitutions at each position, or other replacements of the methionines and the N-terminal aspartic acid residue. The present study has compared the potency of these analogs to inhibit food intake with their potency to stimulate guinea pig gall bladder contraction in vitro. Anorectic potency was measured in male Sprague-Dawley rats, trained to eat powdered chow on a 21 hour fast, 3 hour feeding schedule. Different doses of each peptide were administered ip and the dose which inhibited food intake by 50% for the first half hour of feeding was calculated with the aid of the ALLFIT multiple curve fitting program. Analogs with D-amino acid substitutions in positions 1 or 2 (counting from the N-terminus) retained good anorectic activity (ED50 = 1-8 nmol/kg). Single D-amino acid substitutions in positions 3 through 8 showed decreased anorectic activity relative to CCK-8 (9-50 fold). Substitution of methionine in position 3 with methionine sulfoxide did not alter anorectic potency. However, a similar substitution of methionine in the 6 position decreased activity 5 fold while an aminohexanoic acid substitution enhanced anorectic activity 8-fold. In general, similar changes were observed in in vitro gall bladder contractile potencies and a positive correlation could be shown between anorectic and in vitro gall bladder contraction activities ($r=0.86$, $p<.05$, $n=14$). A notable exception was an analog with a succinic acid substituted for aspartic acid in the position 1 which was 80 times more potent in inhibiting food intake but only equipotent to CCK-8 in eliciting gall bladder contraction (EC50=3.5 nM). This apparent disparity may be due, in part, to a greater stability of the succinyl derivative, as evidenced by the greater resistance of succinyl derivatives to aminopeptidase activity in vitro. In conclusion, a positive correlation could be demonstrated between the ability to inhibit food intake and to stimulate gall bladder contraction of the series of mono substituted CCK-8 derivatives. It appears that receptors which mediate these diverse effects are highly similar in their structural specificity.

245.13 CHOLECYSTOKININ ADMINISTRATION DOES NOT INFLUENCE TASTE-EVOKED ACTIVITY IN RAT NTS. B. K. Giza*, T. R. Scott and R. F. Antonucci* (SPON:L. C. Skeen). Dept. of Psych. and Inst. for Neurosci., Univ. of Delaware, Newark, DE 19716.

Physiological factors associated with satiety have been shown to influence taste-evoked neural activity at several levels of the nervous system. Gastric distension, hyperglycemia and moderate hyperinsulinemia are all associated with a decrease in responsiveness to sugars and, less reliably, to other taste qualities. Cholecystokinin (CCK) is a gut hormone and putative neurotransmitter that has been found to induce satiety in a variety of species, including the human. While its effects are known to be vagally mediated, the mechanism and site of CCK's action are unknown. There has been speculation that the satiety effect induced by its exogenous administration could be mediated by alterations in taste sensitivity. We monitored multiunit taste-evoked activity in the nucleus tractus solitarius (NTS) of anesthetized rats during intravenous administration of either plasma (controls) or either 2 or 6 ug/kg CCK-8, quantities sufficient to depress feeding significantly in behavior tests. Taste stimuli were 1.0 M glucose, 1.0 M fructose, 0.1 M NaCl, 0.03 M HCl and 0.01 M quinine HCl. We found no significant changes in responsiveness to any stimulus in the 30 min period following CCK administration. Possible interpretations of this result include 1)the dose of CCK may have been inadequate. While behaviorally salient, 2.0 ug/kg is a small experimental dose. At 6.0 ug/kg, however, CCK probably approaches its threshold for induction of malaise, and so higher doses are to be avoided. Gosnell and Hsaio found no effect of CCK on chorda tympani activity even at 5.0 ug/kg, but a modest response facilitation at 10 ug/kg. The broad physiological effects of a dose of this magnitude, however, make conclusions tenuous. 2)There could be an effect on taste responses that is not apparent in NTS. This is improbable in the rat where NTS is an obligatory synapse and also receives centrifugal projections from both thalamo-cortical and ventral forebrain taste areas. 3)CCK may be a satiety factor whose mechanism does not involve the taste system. 4)CCK may not be a true satiety factor. Evidence supporting its candidacy is strong, yet there are studies whose results suggesting that CCK suppresses feeding only as a consequence of the malaise induced by the quantities administered in many experiments.

245.14 INTERACTION OF CCK RECEPTOR AGONISTS AND ANTAGONISTS WITH PERIPHERAL TYPE CCK RECEPTORS IN THE BRAINSTEM AND PYLORIC SPHINCTER: EVIDENCE FOR DISTINCT RECEPTOR SUBPOPULATIONS. T.H. Moran, L. Shnayder*, R.T. Jensen*, T.K. Sawyer* and P.R. McHugh. Department of Psychiatry, Johns Hopkins Univ., Baltimore, MD, 21205, Digestive Diseases Branch, National Institutes of Health, Bethesda, MD, 20892, Biopolymer Chemistry, Upjohn Co., Kalamazoo, MI, 49001.

We have previously demonstrated the differentiation of central and peripheral type CCK receptors. Binding to peripheral (Type A) receptors is sulphate dependent. In the rat, these recpetors are found in the pancreas, pyloric sphincter, vagal trunk and branches and within the brainstem in the nucleus tractus solitarious (NTS), area postrema (AP) complex. In the present study, we have examined the ability of CCK agonists and antagonists to inhibit the binding of 125I CCK-8 to CCK receptors in the forebrain (Type B receptors), in the brainstem NTS and AP (Type A) and in the pyloric sphincter (Type A). The agonists were a series of CCK analogs with substitutions at various points in the peptide chain. While the potencies of the five agonists varied, two distinguished Type A from Type B receptors, but all showed the same affinity for brainstem and pyloric receptors. A different pattern of results was found for the CCK antagonists. Of the four proglumide derivatives, the potencies (IC50's) for inhibiting binding were uniformly higher for the Type A receptor. Furthermore, compounds 1453 and 1398 demonstrated significantly higher affinities for the pyloric receptor than for the brainstem receptor (1453: pylorus 160 nM, NTS-AP 2.8 uM; 1398: pylorus 180nM, NTS-AP 4.2 uM). The IC50's for 1409 demonstrated an 8 fold higher affinity for the pyloric receptor (120 nM vs 960nM), while 1287 showed an equal affinity (10 uM) for the two sites. The benodiazapine derivative, L-364 (Merck) clearly differentiated peripheral from brain CCK receptors and demonstrated a somewhat greater affinity for the pyloric receptor. Together, these results affirm the Type A / Type B receptor distinction and suggest that peripheral (Type A) CCK receptors represent at least two further subtypes differentiated on the bases of affinities for CCK antagonists. This distinction may allow the identification of the site of action for the satiety effect of CCK. (supported by AM-19302)

245.15 POTENCY OF L-364,718 ON THE BEHAVIORAL ACTIONS OF PERIPHERALLY ADMINISTERED CHOLECYSTOKININ. S. Khosla* and J. Crawley, (Spon: J.N. Crawley) Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20892

A newly synthesized antagonist of cholecystokinin, 3S(-)-N-(2, 3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1, 4-benzodiazepine-3-yl)-1H-indole-2-carboxamide (L-364,718), was tested for its ability to block the actions of intraperitoneally administered cholecystokinin (CCK) octapeptide sulfate on feeding and exploratory behaviors. CCK (10 µg/kg i.p.) significantly reduced consumption of a palatable cookie mash to 40% of saline vehicle control levels in a 30-minute free-feeding session, and induced pauses of behavioral inactivity averaging 83 seconds/5 minute session, in male, Swiss-Webster mice fasted overnight. L-364,718 significantly blocked the reductions in feeding and exploration induced by CCK, at doses of the antagonist from 500 ng/kg to 10 mg/kg i.p. Time course analysis showed that the antagonism by L-364,718 (10 µg/kg i.p.) of the behavioral actions of CCK (10 µg/kg i.p.) persisted for at least 60 minutes. These data are consistent with the inital reports by Chang et al.[1] and Lotti et al.[2], indicating that L-364,718 is a potent antagonist of CCK at peripheral CCK receptors.

[1] Chang et al., JPET 30:212-217, 1986
[2] Lotti et al., LIFE SCIENCES 39:1631-1638, 1986

245.16 THE EFFECT OF ALTERATION OF HORMONAL STATUS OF MALE AND FEMALE RATS ON CHOLECYSTOKININ'S SATIETY EFFECT: PITUITARY IMPLANTS AND NEONATAL STEROID HORMONE TREATMENT. S.A. Wager-Srdar*, J.E. Briggs*, and A.S. Levine (SPON: D. Krahn). Neuroendocrine Res Lab, VA Medical Center and Dept of Food Science and Nutrition, University of Minnesota, Minneapolis/St. Paul, MN 55417.

Several gut-brain peptides are putative satiety factors. It has been reported that reproductive state of the rat affects responsiveness to the short term satiety effects of glucagon, bombesin and cholecystokinin. Cholecystokinin (CCK) is the most extensively studied of the gut petides. Studies utilizing cycling and ovariectomized female rats show that the reliability of CCK's satiating effect is modulated by the level of sex hormones present and the sensitivity of the female rats to CCK's satiating effect varies during lactation.

To further examine the influence of sex hormonal status on CCK's satiating effect, levels of prolactin and gonadal hormones of rats were experimentally altered. Pituitaries from littermate donors were implanted under the kidney capsules of male and female rats which moderately elevated prolactin levels in recipient animals (4-6x). Moderately elevated prolactin did not alter the responsiveness of pituitary implanted male and female rats to CCK compared to sex matched sham operated rats. Another group of female littermates were injected with estradiol on neonatal day 3 and 5. Adult female rats had elevated prolactin levels, delayed sexual maturation and prolonged or anestrous cycles. Neonatal administration of steroid hormones may alter the differentiation of sexually dymorphic regions of the brain. Age-matched cycling and neonatal estrogenized rats were sensitive to CCK's satiating effect.

Effect of CCK (10 µg/kg) on Food Intake

Males	↓	($p < 0.05$)
Females (sham-operated, cycling)	-	N.S.
Females (pituitary implants)	-	N.S.
Cycling females	↓↓	($p < 0.05$)
Neonatal estrogenized females	↓↓	($p < 0.05$)

Sham operated cycling female rats were insensitive to CCK's effect and their response was significantly different from other groups studied ($F(5,84) = 4.642$, $p < 0.05$). The findings of these preliminary studies re-confirm that responsiveness to CCK's satiating effect is altered by reproductive status. It does not appear that moderate elevation of prolactin levels effects this response but more extensive studies are required to confirm these findings.

245.17 OXYTOCIN SECRETION AND DECREASED GASTRIC EMPTYING RATES PARALLEL THE INHIBITION OF FEEDING PRODUCED BY LITHIUM (LiCl) AND CHOLECYSTOKININ (CCK). Monica J. McCann*, Joseph G. Verbalis, and Edward M. Stricker. Department of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Many chemical agents produce a decrease in food intake when administered to rats. However, it is not always clear whether the decrease in food intake results from satiety or from visceral illness. Consequently, we have initiated studies to determine whether biological measures, plasma oxytocin (OT) levels and gastric emptying rates, could discriminate between these two states. More specifically, the effect of LiCl and CCK on OT secretion was compared with plasms OT levels observed in association with feeding.

We found that CCK (10 ug/kg) and LiCl (3-6 mEq/kg) led to 5-10-fold elevations in plasma OT levels. These elevations were far greater than those observed after ingestion of a large meal or distention of the stomach with isotonic saline. Two- to three-fold increases in plasma OT levels also were observed after lower doses of these agents. Thus, high levels of plasma OT appear to be associated with conditions presumed to reflect visceral illness, whereas low stimulation of OT release does not discriminate between satiety and gastric illness. Gastric emptying rates were slowed both by LiCl and CCK, as is observed after infusions of calories into the intestines. Thus, it is unlikely that changes in gastric emptying rates discriminate between illness and satiety.

A second series of experiments examined the extent to which these biological measures predicted food intake in rats. A range of inhibition of feeding was produced by varying the administered dose of LiCl or by giving CCK to animals that had been totally or partially vagotomized by surgery. Peak plasma OT levels after injection of LiCl or CCK were related exponentially to the reductions in food intake ($r = 0.78$). CCK was less able to affect food intake, OT secretion, or gastric emptying when an afferent vagotomy was produced by systemic injection of the neurotoxin, capsaicin. However, in all animals, the inhibition of feeding always lasted much longer than the induced elevation in plasma OT whereas the reduced rates of gastric emptying appeared to have the same time course as the inhibition of food intake.

To summarize, OT secretion and gastric emptying each correlate strongly with food intake in rats, and these interrelated biological measures can be used to study the control of food intake. However, peak changes in plasma OT predict the degree of inhibition of food intake, whereas gastric emptying rates predict the duration of inhibition.

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245.18 HINDBRAIN SINGLE UNIT RESPONSES TO GASTRIC DISTENSION AND CHOLECYSTOKININ IN NORMAL AND CAPSAICIN TREATED RATS. S Ritter, RC Ritter, WR Ewart*, DL Wingate*. College of Veterinary Medicine, Washington State University, Pullman WA 99164. WOI Regional Program in Veterinary Medicine, University of Idaho, Moscow, ID 83843 and *Gastrointestinal Science Research Unit, London Hospital Medical College, London E1 2AJ, England

Termination of ingestion involves both mechanical and chemical signals from the gastrointestinal tract. Some of these signals are conveyed to the brain by vagal sensory neurons and there is interaction or integration of various sorts of vagal sensory information in the dorsal hindbrain (Ewart and Wingate, 1984). The neurotoxin, capsaicin, destroys small diameter sensory neurons, including a subpopulation of vagal sensory neurons (Dinh and Ritter, 1986). Recently, Ritter and Ladenheim (1985) reported that capsaicin pretreatment attenuates CCK-induced suppression of food intake but fails to attenuate intake suppression by gastric preloads. These findings, along with other data from our laboratories, suggests that capsaicin might reduce sensitivity to some gastrointestinal sensations while not impairing sensation of others. In order to test this hypothesis, we recorded from single neurons in the nucleus of the solitary tract (NTS) and the dorsal motor nucleus of the vagus (DMV) of rats previously treated with capsaicin or its injection vehicle. Recording was begun 2 weeks after capsaicin treatment. We searched for units in an area from obex to 250 um rostral to obex and 200 um lateral to obex on either side. The maximum depth of our search was 1.1 mm down from the surface. We selected spontaneously active single units whose firing rate was increased or decreased by at least 50% during a 2 ml, 30 sec gastric distension. We then tested these units for their response to near gastric arterial infusion of saline (0.2 ml), CCK-8 (8 and/or 80 ng/0.2 ml) and gastrin (G17). We have recorded 16 units from capsaicin treated rats, 27 units from vehicle treated rats and 7 units from rats receiving no pretreatment. In normal and vehicle treated rats, neurons responding to gastric distension were readily identified and nearly all of these units also changed their firing rate in response to CCK-8. In capsaicin treated rats, gastric distension responsive units were also readily identified. However, most of these units did not respond to CCK or exhibited an attenuated response. Thus, it appears that pharmacologically separable populations of gastrointestinal afferents interact postsynaptically in the dorsal hindbrain. These results are consistent with anatomical findings indicating that capsaicin damages a subpopulation of vagal afferent neurons and support previous data indicating that capsaicin attenuates the behavioural response to CCK but not the response to gastric distension. Supported by NIH Grant RO1 NS20561 and the Fogerty Center.

245.19 EFFECT OF CHOLECYSTOKININ, BOMBESIN, AND GASTRIN ON GASTRIC EMPTYING AND SATIETY IN THE RAT. K.L. Conover*, S.M. Collins* and H.P. Weingarten (SPON: G.K. Smith). Department of Psychology and Intestinal Disease Research Unit, McMaster Univ. Hamilton Ontario Canada L8S 4K1.

The ability of four gut peptides to inhibit gastric emptying and induce satiety was assessed in the rat. Rate of gastric emptying was measured using a double-sampling procedure after an intragastric infusion of 10 mls of 0.9% saline (Conover et al., Physiol Behav 39: 303-308, 1987). Satiety was assessed in normally feeding rats eating a 15% sucrose solution.

Cholecystokinin octapeptide (CCK-8) in a dose range that retarded gastric emptying (1.4 to 5.6 ug/kg) also suppressed eating. This finding supports the suggestion that inhibition of gastric emptying mediates CCK-induced satiety. However, doses of secretin (14.3 and 28.6 ug/kg) which retarded emptying to a greater extent than 1.4 ug/kg CCK-8 were less effective than CCK in inhibiting eating. Thus, decreased emptying by itself cannot account fully for the satiety action of CCK-8. Bombesin (4 ug/kg) suppressed eating but had no effect on emptying suggesting that the satiety action of this peptide is not mediated via gastric emptying mechanisms. Finally, pentagastrin (in doses as high as 100 ug/kg) did not affect either satiety or the rate of gastric emptying.

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245.20 EFFECTS OF BOMBESIN ADMINISTRATION ON TASTE-ELICITED BEHAVIORS OF INTACT AND CHRONIC DECEREBRATE RATS. Francis W. Flynn Dept. of Psychology and Neuroscience Program, University of Wyoming, Laramie, WY, 82071

Bombesin (BBS) is a tetradecapeptide which, when administered either systemically or into the cerebral ventricles, has a suppressive effect on food intake. One way by which BBS may inhibit feeding is to reduce the reinforcing oral sensory properties of the food. To evaluate this possibility, the effects of BBS administration on taste reactivity responses and intraoral intake of sucrose and water were measured in intact rats. Method. Intact rats (N=6) were fitted with intraoral cannula and maintained on 4 daily tube-fed 8 ml meals. One hr following the morning meal, the rats were removed from their home cage and injected (ip) with either 10 ug/kg BBS or an equal volume of isotonic saline. The rat's intraoral cannula were then attached to lengths of PE tubing. The rat was then placed into the clear plastic test chamber and allowed to adapt for 15 min. An infusion pump maintained a constant rate of delivery (0.8 ml/min) of either sucrose (0.1 M) or distilled water. During the first minute of the intraoral infusion, taste-elicited oral motor responses were videotaped for subsequent taste reactivity analysis. The intraoral infusion continued until the taste was rejected from the rat's mouth. The amount consumed was then computed by multiplying the rate of infusion x time spent ingesting. Results: Intraoral sucrose intake volume decreased (6.0 ± 0.7 ml) following BBS injection, $p < .05$. BBS injection also significantly suppressed intraoral water intake (3.8 ± 1.8 ml) under these test conditions, $p < .05$. There was no significant effect of BBS injection on the number of ingestive or aversive taste reactivity responses elicited by either 0.1 M sucrose or water. This suggests that BBS suppresses intake without causing an immediate reduction in the oral stimulating properties of the stimulus. In Experiment 2, the effect of ip BBS (10 ug/kg) and saline (0.15 M) injections on intraoral sucrose (0.1 M) intake was measured in tube-fed supracollicular decerebrate rats using the same taste delivery procedure as above. Decerebrate rats (N=4) ingested significantly less 0.1 M sucrose following BBS injection (1.0 ± 0.2 ml) than following saline injection (5.2 ± 2 ml), $p < .05$. These results show that caudal brainstem mechanisms, in isolation of the forebrain, are sufficient to mediate the anorectic effects of BBS.

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246.1 SIDE EFFECTS RATINGS IN GERIATRIC DEPRESSION TREATED WITH NORTRIPTYLINE. R.C.Young, G.S. Alexopoulos,* M. O'Boyle,* A. Dhar,* H. Kutt.* The New York Hospital and Cornell University Medical College, White Plains, N.Y. 10605.

The pharmacotherapy of geriatric depression can be accompanied by subjective side effects. This is true even when drugs having a favorable side effect profile, such as nortriptyline (NT), are used. We assessed subjective side effects in geriatric inpatients treated with NT.

Thirty-four patients with major depressive illness were studied. All were aged > 60 years and met DSM III criteria for major depression. They were in stable physical health. During a 5-7 day evaluation period they were treated with benzodiazepines p.r.n. for anxiety and insomnia. Before, and at weekly intervals during treatment, subjective side effects were recorded with the modified Asberg Scale (Asberg et al, 1970; Ziegler et al, 1978). Depressive symptoms were rated using the 21-item Hamilton Scale (Hamilton, 1960). The patients were treated with NT at an average final dose of 1.2 mg/kg $\pm$ 0.3 mg/kg for 6 weeks. Plasma drug concentrations were assessed during treatment by high performance liquid chromatography.

Both total side effects scale scores (TS) and the sum of scores for anticholinergic items (AC) initially increased (weeks 1 and 2) and then decreased during treatment. TS and AC were each positively and significantly correlated with Hamilton scores during treatment (r=.10 to .57, and .22 to .46, respectively). Correlations between both TS and AC and NT dose (mg/kg) and NT plasma concentrations were low and not significant. Significant positive correlations between both TS and AC and plasma 10-hydroxylated NT concentrations were noted at weeks 5 and 6 (r= .38 to .54, and .42 to .56, respectively.

These preliminary findings suggest that both depressive state and drug effects are determinants of subjective side effect in geriatric depressives. Further investigation of depressive state, hydroxylated metabolite concentrations, and subjective and objective measures of toxicity in the elderly is warranted.

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246.2 EFFECT OF ACUTE AND CHRONIC ANTIDEPRESSANT TREATMENT ON SUBSTANCE P CONTENT IN RAT BRAIN. H. Mitsushio*, M. Takashima*, N. Mataga*, and M. Toru* (SPON: R. S. Schmidt). Div. of Mental Disorder Research, N.C.N.P. Nat'l Inst. of Neuroscience, Tokyo 187, JAPAN

Several psychotropic drugs affect substance P (SP) content in the striatum and substantia nigra. Treatment with acute apomorphine, acute d-amphetamine, and chronic antipsychotics decreases, while subacute methamphetamine and chronic lithium increases SP contents in the striatum and substantia nigra. We have found that chronic carbamazepine administration also increased SP content in these areas. Since both carbamazepine and lithium can prevent the oscillation of manic-depressive illness, we were interested in the effects of antidepressant drugs themselves on SP contents.

For the acute study, male Wistar rats were injected 10 or 20 mg/kg (i.p.) of imipramine, desipramine, clomipramine, amoxapine or mianserin, and decapitated 1.0 hr later. For the chronic study, rats were fed the food containing three different doses of these antidepressants (0.25, 0.5 and 1.0 g of imipramine, desipramine, or clomipramine in 1.0 kg of food, and 0.2, 0.4, and 0.8 g of amoxapine or mianserin in 1.0 kg of food) for 40 days.

In contrast to lithium and carbamazepine, chronic treatment with all the antidepressants decreased SP content in the striatum and substantia nigra. Acute treatment caused different effects: imipramine and desipramine decreased SP content only in the striatum; clomipramine and amoxapine didn't alter SP content in either area; and mianserin decreased SP content in both areas. Among the five antidepressants tested, clomipramine and amoxapine decreased SP content only after chronic treatment. This effect is similar to that of antipsychotics. Since these two antidepressants are 10-fold more potent in blocking the D_2 receptor than the other three, the mechanism by which chronic but not acute treatment decreases SP content may be due to a D_2 receptor blocking action. This explanation, however, can not be applied to the other three antidepressants, which also decreased SP content after chronic treatment, as they have little D_2 receptor blocking action. Since chronic treatment with antidepressants has been reported to increase the sensitivity of cingulate cortical neurons to iontophoretically applied SP (R.S.G. Jones et al. Neuropharm. 24, 627-633, 1985), the decrease in SP content after chronic treatment with antidepressants may correspond to the reduction in SP release. Therefore, the chronic treatment of antidepressants may attenuate SP synaptic transmission. The present findings suggest the possibility that alterations in SP neurotransmission may play a role in the pathophysiology of manic-depressive illness.

246.3 LEARNED HELPLESSNESS: WHAT CRITERION SHOULD BE USED IN ANIMAL MODELS ? R.E.Musty, M. Jordan*, and R. H. Lenox Dept. of Psychology and Dept.of Psychiatry, University of Vermont, Burlington, VT 05405

Exposure to inescapable shock leads to learned helplessness LH). Some investigators have used total failure to escape as a criterion for LH (e.g., Overmeier & Seligman, J. Comp. Physiol. Psychol., 1967, 63, 23.), others have used elevated escape latencies (e.g., Sherman et al., Biochem. Behav., 1982, 16, 449. Many laboratories have reported difficulty obtaining LH in rodents using the latter approach. Rats were given preshock (2.0 ma, 5 sec., 80 trials) in one half of a shuttle box. Rats were then tested in a the shuttle box with the mid-wall removed [1.3 ma for 20 trials, each was composed of a 5 sec. 70 db 4000 Hz conditioned stimulus (CS) followed by 40 sec. of shock]. A fixed ratio-2 (FR-2) response was required to escape or avoid shock . Control rats were tested without previous preshock.

An escape deficit (ED) criterion was defined as an escape response of greater than 20 sec. latency on greater than or equal to 13/20 trials. Approx. 40 % of the preshocked rats were "helpless" and about 20 % of the control rats were "helpless" (Fishers exact test, df=1, p< .05, one-tailed). These percentages for the ED criterion are similar to those in the published literature. Profiles of escape latencies over all 20 trials for each animal were examined and 3 profiles emerged: 1. rats which escaped in the first 5 trials, but did not escape more than two times thereafter, 2. rats which responded throughout the 20 trials with highly variable escape latencies and 3. rats which performed at consistently short latencies. A second criterion was then developed and examined: 1. continuous (total) failure (CF) was defined as failing to escape within 45 sec trial in the first 5 trials and at least 80% failure to escape in trials 6-20. Using this criterion, 20 % of the preshocked animals were "helpless" and 10 percent of the controls were "helpless" (Fisher's exact test, df=1, p< .12, one tailed). "Helpless" rats, using the ED criterion includes profiles 1 and 2, but the CF criterion includes only profile 1. Thus, CF may be more descriptive of a homogeneous type of "helplessness". We hypothesized that reducing observed random escape responses and raising task difficulty would yield more reliable LH. Thus, a barrier with a 3 x 3 in. open door was placed between the two halves of the shuttle box. When rats were tested in this situation, either in the preshock or control conditions, the percentage of "helplessness" using the ED criterion increased in both groups, but using the CF criterion, the percentage of "helplessness" increased about threefold in the preshocked group only. These data suggest that the criterion for LH as well as the relative task difficulty can significantly effect the frequency of observed LH and the homogenity of the LH population. Supported by PHS-RO1-MH41571.

246.4 REINFORCING PROPERTIES OF MAGNESIUM AS DEMONSTRATED BY CONDITIONED PLACE PREFERENCE IN MICE. S. I. Lawley* and K. M. Kantak. Laboratory of Behavioral Neuroscience, Department of Psychology, Boston University, Boston, MA 02215.

Recent studies from our laboratory indicate that magnesium has stimulant-like behavioral properties. Because magnesium does have stimulant-like qualities, it was hypothesized that it might also have reinforcing properties as other stimulants do. Our previous studies indicate that magnesium will substitute for cocaine in a conditioned place preference (CPP) procedure. Thus we used the conditioned place preference paradigm in mice to investigate the possibility that magnesium may have its own rewarding properties. Wooden boxes with two dissimilar arms and a central, neutral area were used to condition animals. Side preference was determined on the third day of habituation by that arm in which the animal spent more than 50% of the total time spent in either arm. During the 15-minute test period the number of visits the animal made to each arm was recorded. Magnesium chloride ($MgCl_2$) was injected sc and the animals were conditioned to the less preferred side. Saline was paired on alternate days to the more preferred side. After conditioning the side preference of the animals was again determined in the drug free state. The following day ip injections of cocaine (5.0 mg/kg) were administered to determine if substitution for magnesium conditioning would occur. There were three separate doses of $MgCl_2$ used to condition the animals: 15, 30, and 125 mg/kg. We also used two different lengths of conditioning: a) a conditioning period of 8 days with 4 pairings per drug and b) a conditioning period of 16 days with 8 pairings per drug. With the conditioning period of 8 days, 54% of the animals showed evidence of conditioning as seen in their shift in preference. All these animals maintained their conditioned side preference with cocaine administration, indicating substitution. Following 16 days of conditioning only 22% of the animals showed evidence of conditioning to all doses of $MgCl_2$. These animals also maintained conditioned side preference with cocaine administration. Analysis of total time in the two arms indicates there are no general sedative effects of $MgCl_2$ which could obscure reinforcing effects. These data support the idea that magnesium may have some reinforcing properties as seen in its ability to switch side preference in some animals. This is further supported by the number of visits made by the animals to the preferred sides after conditioning. Cocaine will support magnesium conditioning and thus similar mechanism of action is indicated. But because magnesium appears to have a limited ability to support CPP, it may have a lower abuse liability than cocaine.

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246.5 ENHANCEMENT OF SEROTONERGIC FUNCTION BY LITHIUM IN AFFECTIVE DISORDER PATIENTS. L.H. Price, D.S. Charney, P. Delgado*, G.R. Heninger, Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Studies in laboratory animals indicate that lithium has significant effects on serotonergic (5HT) function. Although these findings have been invoked to explain lithium's ability to augment the therapeutic actions of tricyclic (TCA) and monoamine oxidase inhibitor (MAOI) antidepressants, few clinical studies have investigated lithium's effects on central 5HT function in patients. It has been shown that the prolactin response to I.V. tryptophan is blunted in depressed patients compared with healthy controls, while treatment with some, but not all, antidepressant drugs enhances the response. One prior study has demonstrated an enhanced response in healthy subjects treated with lithium. The present investigation utilized the prolactin response to tryptophan to determine the nature and time course of lithium's effects on 5HT function in patients. METHODS: Twenty patients with DSM-III affective disorders gave voluntary informed consent to participate. After a minimum 2-week placebo period (at least 3 weeks psychotropic drug-free), patients received tryptophan 7 grams I.V. infused at a constant rate over a 20-minute period. Samples for plasma prolactin were obtained at intervals before and after the tryptophan infusion. Subjective mood and cardiovascular measures were also obtained. The test procedure was repeated in 13 patients after <14 days and in 11 patients after >28 days of active lithium treatment; 4 patients had repeat testing at both time points. Prolactin was measured by radioimmunoassay. RESULTS: As in previous studies, tryptophan caused significant increases in prolactin. The prolactin response was significantly enhanced after short-term lithium treatment, but enhancement after long-term lithium treatment did not reach statistical significance. CONCLUSION: Like long-term treatment with TCAs and MAOIs, short-term lithium treatment increases the prolactin response to tryptophan in affective disorder patients. Long-term lithium treatment has equivocal effects in such patients, whereas a clear enhancement is seen in healthy subjects. These findings are consistent with the hypothesis that lithium augmentation of TCAs involves changes in 5HT neurotransmission, but suggest that homeostatic responses of the 5HT system to long-term lithium treatment may differ in affective disorder patients and healthy subjects.

246.6 INVOLVEMENT OF VARIOUS 5-HT RECEPTOR SUBTYPES IN THE EFFECTS OF SEROTONIN ANTAGONISTS ON PAG STIMULATION-INDUCED AVERSION. F. Jenck, A.L.M. van Delft* and C.L.E. Broekkamp*. CNS Pharmacology Department, ORGANON International, OSS - The Netherlands.

Serotonin has been suggested to act in the brain through activation of different receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2, 5-HT_3). In order to get insight in the functional role exerted by these receptor subtypes in the brain, the effects of serotonin antagonists with differential selectivity for the various subclasses of 5-HT receptors have been tested on aversive brain stimulation. Neurostimulation was applied to the dorsal part of the periaqueductal gray matter (PAG), one of the main cerebral structures subserving brain negative reinforcement.

Rats were trained, in a rectangular cage, to stop an aversive PAG stimulation by escaping from one compartment to the opposite compartment. The minimal current necessary to induce such an escape response was determined: stimulation intensity was held constant and the stimulation frequency was varied in order to measure the frequency threshold inducing escape. This frequency threshold was determined before and following intraperitoneal injection of mianserin, Org 3770, metergoline, ketanserine, pirenperone, (-)pindolol and isapirone (TVXQ 7821).

Mianserin (0.032-32 mg/kg), Org 3770 (0.1-32 mg/kg) and metergoline (0.1-10 mg/kg), which block nearly all 5-HT receptor subtypes decreased the threshold for escape. Opposite effects were obtained with the 5-HT_2 selective antagonists ketanserine (0.32-10 mg/kg) and pirenperone (0.032-1.0 mg/kg) which increased the threshold for escape responses. (-)Pindolol (0.32-3.2 mg/kg) and isapirone (1.0-32 mg/kg), which have selectivity, within the serotonin system, for the 5-HT_{1A} and 5-HT_{1B} subtypes of receptors, did not have any effects. Control experiments were performed with the dopamine antagonist haloperidol, the α_1 antagonist prazosin and the α_2 antagonist yohimbine.

Taken together, these data suggest that the various 5-HT receptor subtypes differentially contribute to the control of central aversive systems in rats:

1) blockade of the 5-HT_2 receptor subtype seems to contribute to inhibit the central aversive systems.
2) blockade of 5-HT_{1A} and 5-HT_{1B} receptor subtypes by (-)pindolol and isapirone does not influence central aversion.
3) the facilitatory effects of acute injection of mianserin, Org 3770 and metergoline on the central aversive systems may be mediated via 5-HT_{1C} or 5-HT_3 receptor subtypes in the brain.

246.7 **Behavioral effects of Thyroidectomy and Antidepressant Therapy.** Laurel A. Freed*, Alexandra Fingesten*, Thomas H. Milhorat and Diana L. Dow-Edwards . (Spon:M. E. Friedlander) Lab. of Cerebral Metabolism, Dept. of Neurosurgery, SUNY Health Science Center at Brooklyn, NY, 11203.

We have found that the metabolic response to imipramine can be altered by varying the thyroid status of the animal (Dow-Edwards, et al., in press). In order to determine whether the behavioral responses to imipramine would also be altered by varying the thyroid state, we thyro-parathyroidectomized (TX) or Sham operated (SO) adult male Sprague-Dawley rats and then housed them under standard laboratory conditions for 2 weeks. On day 14, daily ip injections of either imipramine (10 mg/kg, Sigma) or saline were begun and continued for 2 weeks. Four groups of animals were then evaluated: SO-saline; SO-imipramine; TX-saline; and TX-imipramine. On day 28 post-op 42 rats underwent a 2 day period of testing to determine immobility in the Porsolt test (Porsolt et al., Nature, 1977, vol. 266, p.730-732). The opaque container had 18cm of 30°C water. The length of immobility occuring in the second test period (a maximum of 5 minutes) was recorded. Another 18 rats were examined in an open-field apparatus for a period of 15 minutes. The number of grid lines crossed, number of rearings and duration of wall climbing were recorded. The rats were then sacrificed by decapitation for analysis of plasma T_3 and T_4 levels. Other rats were given imipramine acutely and the Porsolt scores determined.

The results show that T_3 ($p = 0.0004$, ANOVA) and T_4 ($p < 0.001$, ANOVA) levels were significantly decreased compared to control values verifying the completeness of the TX. Chronic imipramine therapy had no effect on the immobility score as determined in the Porsolt test, yet it did significantly decrease the number of gridlines crossed in the open field ($p = 0.010$, ANOVA). TX significantly decreased ($p = 0.05$, ANOVA) the length of time of immobility in the Porsolt test much as acute imipramine did. TX also significantly increased ($p = 0.002$, ANOVA) the incidences of free rearing in the open field. Therefore, chronic imipramine and TX appear to have opposing effects upon the behaviorial indexes measured.

246.8 PRODRUGS OF 2-PHENYLETHYLAMINE: COMPARISON OF THEIR EFFECTS ON LOCOMOTOR ACTIVITY AND ON CONCENTRATIONS OF BIOGENIC AMINES IN BRAIN. D.J. Stewart, A.J. Greenshaw*, G.B. Baker and R.T. Coutts*. PMHAC Research Unit and Neurochemical Research Unit, Department of Psychiatry, University of Alberta, Edmonton, Alberta, CANADA T6G 2B7

The trace amine 2-phenylethylamine (PEA) may play an important role in normal central nervous system function and has been implicated in the etiology of affective disorders. By producing specific elevations of this trace amine with prodrugs, it may be possible to assess its mechanisms of action, particularly with reference to neurotransmitter amine systems. The study reported here investigated the behavioural and neurochemical effects of acute administration of two known prodrugs of PEA, N-cyanoethyl-PEA (CEPEA) and N-propargyl-PEA (PGPEA), in rats.

Three doses of each prodrug were used (0.03, 0.10 and 0.30 mmol/kg, i.p.) and behaviour was measured in a computer-controlled infra-red photobeam activity monitor for 60 min post-injection. Following this, the rats were killed by decapitation and the brains were removed and rapidly dissected into the following areas: frontal cortex, hypothalamus, nucleus accumbens, caudate nucleus and hippocampus. The remaining portions of the brain were combined, termed "the remainder", and retained for analysis of the prodrugs and PEA. High pressure liquid chromatography with electrochemical detection was used for analysis of concentrations of catecholamines and serotonin (5-hydroxytryptamine) and their acid metabolites and electron-capture gas chromatography was employed for analysis of the prodrugs and PEA following derivatization with pentafluorobenzoyl chloride or pentafluorobenzenesulfonyl chloride.

PGPEA induced a dose-related increase in motor activity, whereas CEPEA had a sedative effect. PEA and prodrug levels were significantly higher in the remainder sample after PGPEA than after CEPEA. Both drugs induced dose-related decreases in noradrenaline in the hypothalamus, hippocampus and caudate nucleus and in dopamine in the caudate nucleus and nucleus accumbens. At the highest dose of each prodrug, the ratio of the acid metabolite 5-hydroxyindole-3-acetic acid (5HIAA) to serotonin was increased in the caudate nucleus following PGPEA and in all areas except the hypothalamus following CEPEA. The behavioural and neurochemical effects of the acute administration of these two prodrugs are discussed with reference to brain concentrations of prodrug and PEA and effects on monoamine oxidase activity.

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246.9 ANTI-DEPRESSANTS IN AN ANIMAL MODEL OF ANXIETY. S.R. Bodnoff, B. Suranyi-Cadotte, D.H. Aitken*, R. Quirion, and M. J. Meaney. Douglas Hospital Research Center, Dept. of Psychiatry, McGill Univ., Montreal, Canada H4H 1R3.

Both clinical and anecdotal evidence suggest that anti-depressants, when administered chronically, but not acutely, possess intrinsic anxiolytic properties. In the present series of experiments, the anxiolytic property of the tricyclic anti-depressants, desipramine (DMI) and amitriptyline (AMI) were compared with two known anxiolytics, diazepam and adinazolam in an animal model of anxiety. Food-deprived animals were placed into a novel environment containing food. Novelty produces fear or anxiety which suppresses consummatory behavior. Drugs that reduce fear of novelty decrease the latency for food-deprived animals to begin eating.

Adult, male Long Evans rats were food-deprived for 48 hours. One hour prior to behavioral testing, rats received a single i.p. injection of diazepam (2 mg/kg), adinazolam (20 mg/kg), DMI (10 mg/kg), AMI (10 mg/kg) or vehicle. The behavioral testing involved placing rats into individual plexiglas cages lined with beta chips and 12 evenly-spaced food pellets and recording the latency for animals to begin eating. The results showed that chronic (21 days), but not acute injections of the anti-depressants significantly reduced the latency for animals to begin eating in the novel environment, although the percentage decrease relative to controls was not as great as found with typical anxiolytics. The development of the anxiolytic property of the anti-depressants was examined in a time course study in which animals were injected for 1,7,14, or 21 days with either diazepam, DMI, or vehicle. Only animals treated for 2 or 3 weeks with DMI showed a significant decrease in latency to begin eating. Finally, the role of the benzodiazepine receptor (BZR) in mediating the anxiolytic property of the anti-depressants was evaluated using the BZR antagonist Ro15-1788 (20 mg/kg). Animals were injected for 3 weeks with diazepam, DMI, or vehicle. Again, the anti-depressants significantly reduced the latency to begin eating in the novel environment. Although the BZR antagonist fully blocked the anxiolytic property of diazepam, it had no effect when given in conjunction with DMI. These data suggest that the BZR is not directly involved in mediating the anxiolytic effect of the anti-depressants.

246.10 BEHAVIORAL AND NEUROENDOCRINE RESPONSES TO THE 5-HT_{1B} AGONIST m-CPP IN RATS. F. Matos*, M. Shlaftein*, P.M. Whitaker-Azmitia and E. Edwards. (SPON. A. Orr) Department of Psychiatry, SUNY at Stony Brook, New York 11794.

m-Chlorophenylpiperazine (m-CPP), a metabolite of the anti-depressant drug trazodone has been recently characterized as a serotonin agonist with specificity for the 5-HT_{1B} receptor subtype in rodents. The present experiments examine the effect of acute treatment of rats with m-CPP on three parameters whose changes are consistent with the stimulatory action of 5-HT receptors: locomotor activity, 5-HT syndrome, increases in plasma corticosterone.

Locomotor activity was measured with a photocell actimeter one hour after the administration of m-CPP (3 mg/kg, i.p.). Activity was measured for 40 minutes. Activity in m-CPP treated rats was significantly reduced (126 $\pm$ 20 saline controls vs 33 $\pm$ 5 m-CPP; values represent the mean number $\pm$ S.D. of light beams crossed during a 40 minute recording session).

m-CPP induced a behavioral response typically observed after treatment with serotonin precursors and other serotonin agonists. Behavioral scores were recorded for headweaving, forepaw treading, hindlimb abduction, and Straub tail in twenty minute sessions. Headweaving was enhanced ($p < .01$) during the period of 4 - 20 minutes. Hindlimb abduction ($p < .05$ at eight minutes), forepaw treading ($p < .01$ during the period of 10 - 15 minutes) and Straub tail ($p < .01$ during the period of 4 - 10 minutes) were all enhanced in a dose dependent fashion with injection dosage ranging from 1.5 - 12 mg/kg body weight.

m-CPP injection caused an increase in the secretion of corticosterone (6 mg/kg injected i.p.). This increase (+75%) was observed in m-CPP treated rats 24 hours after injection.

Data from these experiments provide evidence for m-CPP effects being mediated through an interaction with 5-HT receptors and thus support the use of this compound as a tool in studies aimed at elucidating the functional role of serotonin in the central nervous system.

246.11 SELECTIVE 5-HT_2 ANTAGONISTS RESULT IN ANTIDEPRESSANT-LIKE ACTIVITY ON A BEHAVIORAL SCREEN FOR ANTIDEPRESSANT DRUGS. G.J. Marek, A. LI*, and L.S. Seiden. Dept. of Pharmacol. & Physiol. Sciences, The University of Chicago, Chicago, IL 60637.

Over the last few years, antidepressant drugs have been demonstrated to have a unique and characteristic effect on rats performing under a differential-reinforcement-of-low-rate 72 sec. (DRL 72-s) schedule of reinforcement. Only antidepressant drugs and electroconvulsive shock increase the reinforcement rate, decrease the response rate and enhance temporal discrimination on the DRL 72-s schedule, an operant schedule of reinforcement (McGuire & Seiden, 1980; Seiden & O'Donnell, 1985; Seiden et al., 1985).

Since antagonism of 5-hydroxytryptamine (5-HT) receptors is one of the most potent actions of several atypical antidepressant drugs such as trazodone and mianserin, we have examined a large series of 5-HT antagonists with varying selectivity for the 5-HT_2 vs. the 5-HT_1 receptor: ketanserin, ritanserin, pipamperone, pizotifen, cyproheptadine, trazodone, cianserin, mianserin, LY53857, metergoline and methysergide. Antidepressant-like activity (increased reinforcement rate, decreased response rate and enhanced temporal discrimination) was seen with administration of 5-HT_2 selective antagonists such as ritanserin, ketanserin, and pipamperone. A lack of effect was seen on the DRL 72-s schedule following administration of non-selective 5-HT antagonists such as methysergide and metergoline. A significant correlation was seen between the ability of these drugs to increase the reinforcement rate on the DRL 72-s schedule and their selectivity for the 5-HT_2 vs the 5-HT_1 receptor (r_s=+0.91, binding data from Leysen et al., 1981). Significant correlations were not seen for the alpha-1 adrenoreceptor (r_s=+0.43), alpha-2 adrenoceptor (r_s=-0.33), histamine-1 receptor (r_s=+0.12) or the dopamine-2 receptor (r_s=-0.07). These results support the hypothesis that the antidepressant-like activity of trazodone and mianserin on the DRL 72-s schedule may be related to selective antagonism of 5-HT_2 receptors. This suggests that the therapeutic action of some atypical antidepressants like trazodone and mianserin could be related to selective antagonism of 5-HT_2 receptors. This research is supported by PHS MH-11191; RSA MH-10562 (L. Seiden) and MSTP Grant GM-07281 (Marek).

246.12 DOWN-REGULATION OF 5-HT_2 RECEPTORS ATTENUATES THE EFFECTS OF TRAZODONE ON RATS PERFORMING ON BEHAVIORAL SCREEN FOR ANTI-DEPRESSANTS. A. Li*, G.J. Marek, L.C. Nguyen*, and L.S. Seiden. (SPON: M. Kleven). Dept. of Pharmacol. & Physiol. Sciences, The University of Chicago, Chicago, IL 60637.

The differential-reinforcement-of-low-rate 72-second (DRL 72-s) schedule of water reinforcement is an effective screen for anti-depressants (O'Donnell & Seiden, 1982; Seiden, Dahms & Shaughnessy, 1985). Only antidepressant drugs and electroconvulsive shock increase reinforcement rates, decrease response rates, and enhance temporal discrimination of rats performing on this schedule. 5-HT antagonists with 100-1000 fold greater affinity for the 5-HT_2 over the 5-HT_1 receptor such as ketanserin, ritanserin, pipamperone, mianserin, and trazodone are similar to anti-depressant drugs in their effects on DRL-scheduled behavior (Marek, Li, Seiden, 1987). Administration of methysergide or metergoline which antagonizes both 5-HT_1 and 5-HT_2 receptors does not affect performance on the DRL 72-s schedule (Marek, Li, Seiden, 1987). Mianserin and trazodone have already been shown to be clinically effective in the treatment of affective disorders. Recently, there have been encouraging results indicating that ritanserin may also be an effective antidepressant. Thus, selective antagonism of the 5-HT_2 over the 5-HT_1 receptor may result in a clinically effective treatment of depression. The effect of trazodone on DRL-scheduled behavior was measured before and during repeated administration of trazodone (10 mg/kg, daily for 14 days). Repeated administration of trazodone down-regulates 5-HT_2 receptors (Riblet and Taylor, 1981). The reinforcement rate increasing effect of trazodone was decreased from day 5 to day 14 of the repeated administration of trazodone. In another experiment, the effects of trazodone were measured before and during a regimen of methysergide injections (10 mg/kg daily for 40 days) that is known to specifically down-regulate 5-HT_2 receptors without affecting 5-HT_1 or B-receptors (Blackshear et al., 1983; Peroutka and Snyder, 1980). Trazodone (5 mg/kg) was tested on days 1, 7, 14, 27, and 38 of the regimen of methysergide injections. Trazodone significantly increased the reinforcement rate on day 1 but not on days 7, 14, 27, or 38. This suggests that the regimen of repeated administration of methysergide attenuated the reinforcement rate increasing effects of trazodone. The results from both of these experiments provide additional evidence that the 5-HT_2 receptor site plays a role in the effects of trazodone on rats performing on the DRL 72-s schedule. This research is supported by PHS MH-11191; RSA MH-10562 (L. Seiden), and MSTP Grant GM-07281 (G. Marek).

246.13 AN ELECTROPHYSIOLOGICAL COMPARISON OF DESIPRAMINE AND THE POTENTIAL ANTIDEPRESSANT, MDL 19,660. S.M. Sorensen, J.M. Zwolshen*, M.W. Dudley, J.M. Kane*, and F.P. Miller. Merrell Dow Research Institute, Cincinnati, OH

MDL19,660(5-(4-chlorophenyl)-2,4-dihydro-2,4-dimethyl-3H-1,2,4-triazole-3-thione) shows potent antidepressant-like activity in behavioral tests but does not inhibit amine uptake or monoamine oxidase activity and is devoid of receptor antagonist activity. In this study, we compared the effects of desipramine (DMI) and MDL 19,660 on the sensitivity of rat cerebellar Purkinje (P) neurons to iontophoretically applied norepinephrine (NE). The sensitivity of these neurons to NE is diminished following chronic administration of DMI and this is thought to be due to the desensitization of beta receptors which is seen following chronic administration with many antidepressant agents (Yeh and Woodward, JPET, 226:126-134, 1983). These experiments were undertaken to determine whether MDL 19,660 also caused a decrease in the sensitivity of P neurons to NE.

Neither compound, administered acutely at doses up to 10 mg/kg i.p., altered the spontaneous firing rate of P neurons or the depression in rate caused by NE. MDL 19,660 did, however, significantly reduce the NE enhancement of GABA responses after acute administration, whereas DMI did not. MDL 19,660 also produced a slight decrease in the response of the P neuron to iontophoresced GABA itself.

When administered chronically (10 mg/kg/day i.p. x 21 days), neither compound altered spontaneous or NE-evoked slowing of P neurons. Both compounds did, however, produce a significant decrease in NE enhancement of GABA inhibition following chronic administration. This subsensitivity to NE was more pronounced in animals chronically treated with MDL 19,660 than for DMI.

The results indicate that MDL 19,660 has a profile which is similar to that of DMI in this experimental system with some important differences. First, MDL 19,660 produced a more marked desensitization to NE enhancement of GABA inhibition than DMI following chronic administration. Second, MDL 19,660 caused a significant decrease in this NE sensitivity after even a single administration, whereas DMI was effective only after chronic administration. The data are consistent with the hypothesis that MDL 19,660 has potential as an antidepressant and further suggest that the compound may have a faster onset of therapeutic activity than DMI.

AMYGDALA AND LIMBIC CORTEX

247.1 THE ORGANIZATION OF THE CANINE CENTRAL NUCLEUS OF THE AMYGDALA. M.L. Estes and C.H. Block, Departments of Pathology and Brain and Vascular Research, The Research Institute of The Cleveland Clinic Foundation, Cleveland, OH 44106.

The central nucleus of the amygdala (CNA) is a limbic structure that is involved in the integration of autonomic function and emotional behavior. A variety of peptides and neurotransmitters have been identified within this nucleus in the rat (Wray and Hoffman, 1983) and rabbit (Block, et al. 1984). Since neuroactive substances are part of both the intrinsic and extrinsic circuitry of the amygdaloid complex, the following studies were conducted to examine the organization of these substances in the canine brain.

Serial frozen sections were cut at 30 μm in the coronal plane and were processed for immunocytochemical localization of vasoactive intestinal polyeptide (VIP), neurotensin (NT), methionine enkephalin (ENK), cholecystokinin octapeptide (CCK), and substance P (SP).

The CNA in the dog lies in the dorsal aspect of the temporal lobe and is bordered by the anterior, lateral, basolateral, and medial amygdaloid nuclei. It is comprised of two major cell groups, the medial (mCNA) and lateral (lCNA). The mCNA is larger in area than the lCNA, occupying the region caudal to the anterior amygdaloid area and extending to the amygdalohippocampal area. Cells of the mCNA can be further divided into three zones. The dorsal and ventral zones are outlined by the fibers of the longitudinal association bundle and stria terminalis and form a capsule around a cell-sparse internal zone. The cells of the dorsal mCNA are round in shape, moderately dark-staining, and have a centrally-located nuclei. They are approximately 20 μm in diameter. The neurons of the ventral mCNA are pale-staining and approximately 14 μm in diameter. The few cells within the internal zone are a mixture of both cell types of the dorsal and ventral group. The lCNA lies lateral and dorsal to the mCNA and appears to be continuous with the putamen, although it does not contain any of the large cells of that complex. The cells of the lCNA are small (approximately 8 μm in diameter), oval, and pale-staining. Fibers containing VIP and NT were primarily localized to the ventral mCNA, whereas ENK-containing fibers surrounded the ventral zone. SP and CCK fibers outlined the ventral aspect of mCNA. Caudally, VIP and SP appeared to be contained within the fibers of the stria terminalis. In addition, ENK - and NT - containing cells were found in the mCNA. CCK - , ENK - ,and SP - containing fibers outlined the lCNA and infiltrated the putamen.

The presence and the anatomical organization of the canine CNA suggest that the neuropeptides may be part of the systems involved in autonomic integration, as demonstrated in other species.

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247.2 THE EFFECTS OF EXTREMELY LOW-LEVEL KINDLING STIMULATION UPON THE SOCIAL BEHAVIOR AND AMYGDALOID SLOW-WAVE ACTIVITY IN A SQUIRREL MONKEY (S. SCIUREUS) AND THE INFLUENCE OF DOPAMINERGIC AGENTS UPON THESE EFFECTS. R. L. Lloyd, A. S. Kling, K. E. Tachiki, M. Ghazizadeh,* O. Ricci,* B. Wexler,* and S. Ruane.* Psychiatry Service, VA Medical Center, Sepulveda/UCLA Department of Psychiatry, CA 91343.

Daily low-level electrical stimulation (150 uA @ 60 Hz for 1 sec) was delivered to the amygdala of a squirrel monkey at an intensity which was below threshold, not only for motor convulsions, but also for electrographic seizures and/or synchrony. By the thirteenth stimulation primary after discharge activity was recorded in the amygdala, and by the 68th stimulation, evidence of somatic convulsions appeared, which were primarily autonomic, but which eventually developed into full partial complex seizures. Changes in the social behavior of the stimulated animal occurred prior to the development of overt convulsions. The effects included a fall in social rank, hyper- and inappropriate aggression, paresthesia, and what appeared to be visual hallucinations. In addition, the duration of primary and secondary after-discharge activity appears to be influenced by whether the animal is in isolation or in the presence of conspecifics. These data speak to the issue of whether abnormal electrical activity within the amygdala can produce abnormal social behavior, in the absence of overt signs of seizure activity. In addition, these data argue against the position that the behavioral pathologies observed in temporal lobe epilepsy are due to underlying neuropathies, and that the presence of the electrical sequelae is merely coincidental.

Finally, abnormal behaviors are enhanced by apomorphine, while a biphasic effect upon both the animal's abnormal behaviors and spontaneous electrical discharge activity is produced by the neuroleptic Tiaspirone. The main effect of Tiaspirone is to reduce both the abnormal electrical and behavioral effects of this novel form of kindling.

247.3 **CARDIAC AND RESPIRATORY RELATIONSHIPS WITH UNIT DISCHARGE IN THE HUMAN AMYGDALA AND HIPPOCAMPUS.** R.C. Frysinger, R.M. Harper, and C.L. Wilson. Depts. of Anatomy and Neurology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1763.

The amygdala and hippocampus are mesial temporal lobe structures with a close anatomical relationship with autonomic and respiratory control systems. Stimulation and recording studies have implicated the amygdala in control of blood pressure (Frysinger et al., *Exp. Neurol.* **83**:604, 1984) and respiration (Zhang et al., *Exp. Neurol.* **91**:193, 1986). In the cat, amygdala neurons discharge phasically with the cardiac and respiratory cycles in a state-dependent fashion, and tonic discharge rates correlate with spontaneous changes in blood pressure and respiratory rate (Frysinger et al., in preparation, 1987). The hippocampus may play a role in cardiovascular regulation (Versteeg et al., *Brain Res.* **292**:317, 1984), and hippocampal neurons discharge in phase with the respiratory cycle (Radna and MacLean, *Brain Res.* **213**:45, 1981). The extent and nature of such correlations in the human have not yet been explored. We therefore examined cardiac and respiratory relationships with neuronal discharge in the amygdala and hippocampus of humans.

We recorded respiratory air flow and ECG simultaneously with neuronal discharge from the amygdala and pes hippocampi of epileptic patients undergoing chronic depth electrode monitoring during assessment for resective surgery. Cross-correlation histograms were used to test for neuronal discharge timing relationships with onset of inspiration or the QRS complex of the ECG. Inspiratory area, respiratory period and heart rate were calculated on a breath-by-breath basis off-line, and linear regression was used to test for tonic rate correlations with unit discharge rates normalized over the breath.

Amygdala neurons showed both tonic rate correlations with respiratory period and inspiratory area (3/6 each) and discharge timing relationships with the cardiac and respiratory cycle (2/6 each). Hippocampal neurons commonly showed discharge timing relationships with the cardiac cycle (5/11) and tonic rate correlations with heart rate (2/11), but respiratory relationships were rare. It was unusual for a neuron in either area to show both a tonic rate correlation and a discharge timing relationship (2/17).

These results suggest that activity in the hippocampus is closely coupled to cardiac control systems, while the amygdala has links to both cardiac and respiratory systems. The finding in this and previous studies that discharge timing relationships and tonic rate correlations tend to be independent of each other suggests the existence of distinct functional subpopulations of neurons subserving the phasic and tonic aspects of forebrain influences on the cardiovascular and respiratory systems.

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247.4 AMYGDALOID PROJECTIONS TO THE PREFRONTAL CORTEX AND ASSOCIATED ROSTRAL STRIATUM: A FLUORESCENCE RETROGRADE TRANSPORT STUDY IN THE RAT. A.J. McDonald. Dept. of Anatomy, Univ. of South Car. Sch. of Med., Columbia, SC 29208.

Studies have shown that the striatal projections of the rat prelimbic and dorsal agranular insular cortex (medial and lateral prefrontal cortex [PFC], respectively) terminate mainly in ventral and dorsomedial portions of the rostral striatum. The amygdala projects to both the PFC and its associated striatal regions. The present study examined the relationship of amygdaloid neurons projecting to these interconnected cortical and striatal fields using the fluorescent retrograde tracers true blue and diamidino yellow. Different tracers were injected into the medial or lateral PFC and their associated rostral striatal regions (nucleus accumbens [NA], dorsomedial caudatoputamen [DMCP], or ventrolateral caudatoputamen [VLCP]. In all NA animals and several DMCP and VLCP animals, knife cuts (1.5-2.0 mm wide) were made just rostral to the striatal injection to ensure that cortically-projecting amygdaloid neurons with axons passing through the striatum would not be artifactually double-labeled by the striatal injections. The pattern of retrograde labeling in the DMCP and VLCP rats with knife cuts appeared to be identical to that seen in the animals that did not receive knife cuts. Medial PFC injections were paired with injections into the NA (n=10) or DMCP (n=14). In the NA group, the topography of amygdaloid neurons labeled by the NA injections (NA cells) was very similar to that of amygdaloid neurons labeled by the medial PFC injections (MPFC cells). MPFC cells and NA cells were found mainly in the medial two-thirds of the basolateral amygdala and the lateral half of the amygdalohippocampal area. Numerous double-labeled neurons were also seen in these regions. Labeling in the DMCP group was similar to that observed in the NA group except that few DMCP cells or double-labeled cells were seen in the lateral nucleus and caudal half of the amygdala. Lateral PFC injections were paired with VLCP injections in twelve rats. Cells in the basolateral amygdala labeled by the lateral PFC injections (LPFC cells) tended to be more laterally situated than those labeled by medial PFC injections. The topography of VLCP cells and double-labeled cells was similar to that of LPFC cells except that very few VLPC or double-labeled cells were seen in the caudal half of the amygdala. The results of this study indicate that amygdaloid neurons projecting to PFC areas and their associated rostral striatal fields have similar topographies. In addition, many amydaloid neurons send axonal branches to both cortex and striatum. These neurons would appear to be able to simultaneously send identical information to the first two links in the prefrontocortical-limbostriatal-ventropallidal pathway. (Supported by NIH grant NS 19733).

247.5 A GOLGI, HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STUDY OF THE LATERAL ENTORHINAL CORTICAL NEURONS OF THE RHESUS MONKEY. A.A. Carboni(1), P.B. Cipolloni(2), C.L. Barnes(2) and W.G. Lavelle(1). (1) Dept. of Surgery, Div. of Otolaryngology/Head and Neck Surgery, UMass. Med. Center, Worcester, MA 01655. (2) Depts. of Anatomy and Neurology,, BU Sch. of Med. Boston, MA. 02118 and ENR VA Hosp., Bedford, MA 01730.

The neuronal types of the entorhinal cortex of the human and lower species have been described with the Golgi method (Cajal, S., Studies on the Cerebral Cortex: Limbic Structures, trans. L.M. Kraft, Chicago: Year Book Publishers, 1955; Lorente de No', R., J. Psychol. Neurol., 45, 381-438, 1933). We have expanded on these Golgi studies and have further characterized some of the neuronal subpopulations by histochemical and immunohistochemical labeling.

In a series of monkeys, the neurons of the lateral entorhinal cortex (LEC) were impregnated by the rapid-Golgi method (Valverde,F., Contemporary Methods in Neuroanatomy, W.J.H. Nauta and S.O.E. Ebbesson (Eds.), New York: Springer-Verlag, 1970) or the Braitenberg method (Braitenberg, et al., Stain Tech. 42: 277-282, 1967). They were also stained for NADPH-diaphorase enzyme, somatostatin, neuropeptide-Y, cholecystokinin, and substance P. Intervening sections were Nissl stained for cytoarchitectonic localization. The characterization of the neuronal types was based upon the number of spines, dendritic array, axonal arbors and chemical staining.

In general, the neurons found in the monkey LEC exhibit more varied forms than do those of the neocortex but, as in the neocortex, can be segregated into spinous and sparsely spinous types (Cipolloni, et al., Neurosci. Absts. 9: 953, 1983). The predominant type of spinous neuron is the typical pyramidal neuron found in all cortical layers except I and IV (lamina dessicans). A subset of the pyramidal type is the biapical pyramidal neuron and is found in layers II and III. Spinous multipolar neurons with radially arrayed dendrites are frequently found in layers II, III and VI. A cell type in the LEC not routinely found in the neocortex is the spinous tripolar of layer VI.

The sparsely spinous types include multipolar cells of varying sizes and complexity, bitufted and bipolar cells oriented either vertically or horizontally, and the neurogliaform neuron. In layer II, another cell type not seen in the neocotex is a sparsely spinous multipolar neuron which has two predominant dendrites coursing obliquely toward the pia and a horizontal axon that appears locally ramified as do the axons of all the sparsely spinous neurons. Certain of these sparsely spinous neurons can be found in all the cortical layers of the LEC and are the only type found in layers I and IV.

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247.6 THE SUBCORTICAL PROJECTIONS OF THE ENTORHINAL CORTEX IN THE RHESUS MONKEY. D.L. Rosene and R.C. Saunders (SPON D. Doudet) Dept. of Anatomy, Boston University School of Medicine, Boston, MA, 02118, and Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

The entorhinal cortex, area 28 on the medial surface of the temporal lobe, has become recognized as a relay from the sensory association areas of the neocortex to the hippocampus. More recently, however, it has been demonstrated that the entorhinal cortex can sustain memory processes after removal of the hippocampal formation (H) and amygdala (A). Connections of area 28 with areas other than the neocortex and the H have, however, been largely ignored. Thus, as part of a larger investigation of the nonhippocampal projections of the entorhinal cortex in the rhesus monkey, we sought to determine, using complementary anterograde and retrograde anatomical tracers, its subcortical projections.

In the thalamus anterograde label was found over the magnocellular part of the medial dorsal nucleus (MDmc), the anterior thalamic nuclei (AntN), the lateral dorsal nucleus and the nucleus centralis latocellularis. Injections of retrograde tracers into the AntN and the MDmc resulted in labeled cells primarily in layer 6 in the sulcal part of area 28 (28S). In addition, there were a few labeled cells in layer 3 of 28S and 28M after the MDmc injection. In the hypothalamus, anterograde label was found over the medial nucleus of the mamillary body (MB) and the lateral hypothalamic area. Retrogradely labeled cells were found primarily in layer 3 of 28S, and to a lesser extent in the medial and caudal part of the entorhinal cortex (28M). All five subdivisions of the entorhinal cortex project to the bed nucleus of the stria terminalis (BNST) and to the intermediate nucleus of the septum. Like the projection to the MB, those to the BNST originate primarily in layer 3. In comparison, the projections to the intermediate septum originate more from layer 5. The entorhinal cortex also projects to the striatum, head of the caudate, and the putamen. These projections appear to arise from layer 5 cells in all subareas of the entorhinal cortex. Furthermore, it is cells in layer 5 which also project to the nucleus basalis of Meynert. In the amygdala, terminal label was found over the lateral basal nucleus, the central nucleus, the cortical nuclei and the cortical transition area. Like the entorhinal projection to the striatum this projection arises from layer 5 cells in all parts of the entorhinal cortex and from layer 3 cells in the prorhinal l cortical area.

The subcortical projections of the entorhinal cortex appear to parallel those from both the A and the H. These projections as well as cortical projections provide a means by which the entorhinal cortex may contribute to memory function independent of the neighboring hippocampus and amygdala.

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247.7 A CYTOARCHITECTONIC ANALYSIS OF THE HUMAN ENTORHINAL CORTEX. R. Insausti[1], T. Tuñón[*2], T. Urrutia[*1], L.M. Gonzalo[*1] and J.M.M. Peñuela[*2]. 1. Dept. Anatomy, Univ. Navarra. 2. Dept. Pathology, Hosp. Navarra, Pamplona, SPAIN.

The entorhinal cortex is a component of the hippocampal formation, present in all mammals, but that reaches maximal development in primates, especially in man. The entorhinal cortex is a very important link between the neocortex and the hippocampus; when this pathway is disrupted (i.e. Alzheimer's disease) there are important functional consequences as memory impairment (Hyman et al., Ann. Neurol. 20:472-481, 1986). Recently, it has been defined several cytoarchitectonic fields in the entorhinal cortex of the macaque monkey (Amaral et al. J. Comp. Neurol. in press, 1987). By using these criteria as a guide, we attempted to find out the homologous fields in the human entorhinal cortex.

Brains from autopsies (age range 22-70 years) were obtained shortly after death and fixed with 10% formalin (three cases); four additional cases were perfused through the carotids or carotid and basilar arteries with 4% paraformaldehyde in phosphate buffer. After a postfixation period of 48 hr. the brains were equilibrated with 20% glycerol in phosphate buffer. The brains were blocked perpendicular to the anterior-posterior commissure line and they were cut transversely in a freezing microtome at 50 µm. A one-in-five series was mounted and stained with thionin. Fiber series were similarly prepared; in addition, acetylcholinesterase, neurofilament and somatostatin series were available in one case. Three additional hemispheres were cut horizontally (n=2) or parasagittally (n=1) at the same thickness. Drawings were made with a camera lucida attached to a stereomicroscope at a magnification of 15 times, on which the different cytoarchitectonic features as presence of the lamina dissecans, islands of cells in layer II, etc. were plotted. Additional two-and-three dimensional reconstructions were obtained for the different cases.

The human entorhinal cortex lies at the ventromedial aspect of the temporal lobe. The rhinal surcus makes the lateral border of the rostral entorhinal cortex, being replaced by the colateral surcus at more caudal levels. The human entorhinal cortex has a less distinct lamination than in the monkey, except for a small medial field. We have been able to distinguish homologous divisions as in the monkey. There are no sharp borders between adjacent fields, but rather gradients that run rostrocaudally: the caudal and lateral fields resemble more closely the neocortical fields. There is no a single cytoarchitectonic feature that defines the whole entorhinal cortex; neither the dense clumps in layer II nor the presence of a lamina dissecans are present all over the entorhinal surface. It is rather the multiplicity of techniques that give a better distinction of the different fields.

247.8 RELATIONS BETWEEN CINGULATE CORTICAL CELL DISCHARGE PATTERNS AND HIPPOCAMPAL FORMATION SLOW WAVE THETA ACTIVITY. L.V. Colom* and B.H. Bland*. SPON: (S.H. Roth) Dept. of Psychology, University of Calgary, Calgary, Alberta, Canada, T2N 1N4

The cingulate cortex, along with the hippocampal formation, receives projections from the medial septal and diagonal band nuclei. The cingulate cortex in turn projects to the hippocampal formation via the subicular and entorhinal cortices. Although the majority of research on slow wave rhythms and cell discharge patterns has been carried out on the septohippocampal system, recent research has indicated that there are theta generators and rhythmic cells in the cingulate and entorhinal cortices as well. In the present study we recorded cells in the cingulate cortex of rats anesthetized with urethane, during the spontaneous occurrence of slow wave theta and large amplitude irregular activity (LIA). Electrode tip locations were marked by iontophoretically ejecting Pontamine Sky Blue. The responses of eight of the total of 16 cells recorded were tested during the systemic administration of eserine and 5 cells during the administration of nicotine. In the urethane condition, all 16 cells showed a clear activation during theta compared to LIA (range 2-100 times greater firing). In 13 of the cells, the increase in firing during theta was nonrhythmic with simple spike firing. Two cells were weakly rhythmic, again with simple spike firing and 1 cell showed a high degree of rhythmicity, firing in simple spike rhythmic bursts. All 8 cells tested with eserine were activated in a similar manner as during spontaneous theta and in contrast to hippocampal theta-on cells, nicotine also activated all 5 cells tested. We conclude that the cingulate cortex contains a population of cells whose discharge patterns are clearly related to the hippocampal formation EEG patterns of slow wave theta and LIA. The majority of the cells in the present study were similar to tonic theta-on cells in the hippocampal formation, although one rhythmic neuron was recorded. Furthermore, the cingulate cortex cells were activated by both eserine and nicotine, in contrast to our previous work on hippocampal formation theta-on cells. The latter cells were activated by muscarinic agonists and depressed by nicotine. The site of action of muscarinic and nicotinic activation of cingulate cortex cells requires further study. (Supported by AHFMR and NSERC)

247.9 QUANTITATIVE ASPECTS OF THE HUMAN CINGULATE CORTEX USING A COMPUTER CONTROLLED IMAGE ANALYZER. G. Schlaug, E. Armstrong, A. Schleicher*, K. Zilles, Department of Anatomy, University of Cologne, D-5000 Cologne 41, FRG and Department of Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

Cytoarchitectonic studies are based on structural inhomogeneities, which are due to differences in cell densities, size, shape and columnar organization. In this study the varying cytoarchitectural complexities within the cingulate cortex have been quantitatively analyzed using a computer controlled image analyzer (Microvideomat 2, C. Zeiss, FRG). A grey level index (GLI) has been measured, which is defined as the ratio of the area covered by cell bodies to the entire area of the measuring field. The transition zone from allo- to isocortex is characterized by shifts in laminar proportions and cellular densities. The isocortex has lower cell densities in the outer main lamina, but higher cell densities in the inner main lamina than the allocortex. Since the GLI values are also a measure of neuropil proportions, these results are discussed as structural correlates of a relative increase in the receptive and processing capacities of the isocortex over the adjacent allocortex.

In contrast to the posterior cingulate cortex, the anterior part lacks an inner granular layer and shows a lower cell density. This has been substantiated by columnar cell counts showing areal specific differences. In order to investigate the causes for these different cell densities, the dendritic arborization of layer V pyramidal neurons were analyzed in a Golgi-study. These cells have a significant increase in primary basilar dendrites in the anterior cingulate cortex, which leads to an increase in neuropil volume and partially explains the lower cell densities.

The isocortical areas of the posterior cingulate cortex (23 and 31) and the adjacent parietal (7) and visual (19) cortices can be differentiated by quantitative criteria. Besides differing GLI-values and laminar proportions, areas 7 and 19 have a higher degree of columnar organization than areas 23 and 31. A method developed to measure radially oriented and periodical cell columns, shows no significant differences in the width and periodicity of the columns, but their delineability from the surrounding neuropil differs among these areas. The columns may present a basic uniform cortical structure, and their differences in delineability may depend on afferent fiber bundles.

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247.10 BEHAVIORAL AND ELECTROPHYSIOLOGICAL EFFECTS OF LESIONS OF THE INSULAR CORTEX IN THE SQUIRREL MONKEY (S. SCIUREUS). A. S. Kling, R. Lloyd, O. Ricci*, M. Ghazizadeh*, B. Wexler* and S. Ruane*. Psychiatry Service, VA Medical Center, Sepulveda/UCLA Department of Psychiatry, CA 91343.

This study is the first report to our knowledge of the effects of bilateral lesions of the insular cortex in primates. Clinical observations and anatomical evidence have suggested that the insula subserves both general and special visceral sensory sensibility. Autonic and somatomotor effects have been observed with electrical stimulation, and deficits in language are seen following stroke in man. Anatomically, the insula receives projections from a variety of limbic structures and projects to both medial and lateral amygdala as well as other structures.

Because of its amygdala projections, we hypothesized that bilateral ablation in a primate species would affect both social/affective behavior and amygdaloid electrical activity. For this study, 3 adult (1 ♂ 2 ♀) S. sciureus were studied for 6 months to 1 year. 1 ♂ and 1 ♀ had a pair of chronic electrodes implanted in the medial and lateral amygdala prior to the insular ablations. Electrical recordings via radiotelemetry were made during presentation of visual, auditory, taste and small stimuli while restrained, and auditory stimuli, food, and a live snake in a social setting. After bilateral ablation of the insular cortex, all 3 subjects exhibited 7-14 days of anorexia and lethargy, followed by (1) omini and hyperphagia, (2) tameness toward handling, (3) excessive genital rubbing by the females, (4) absence of genital display in male, (5) a fall in social rank, (6) decreased social interaction, (7) approach to a live python without fleeing when bitten. Changes in amygdaloid electrical activity include a 25-50% reduction in total power from medial subnuclei to all stimuli. Greater reductions were observed in social versus restrained conditions. In general there was a shift in the distribution of total power from the higher bands to delta and theta. Responses to taste and smell followed the same pattern as auditory and visual stimuli. For the lateral placements, small increases in total power were observed, especially under restrained conditions, to auditory and visual stimuli. The behavioral changes after bilateral insular lesions are strikingly similar to amygdalectomy. Reductions in total power and a shift in frequency distribution recorded from amygdala after the insular cortex lesions are consistent with the predominant role of the medial amygdala in regulation of social/affective behavior and suggests participation of the insular cortex, along with the temporal pole and orbital cortex, in the maintenance of social bonding in primates.

247.11 TOPOGRAPHIC AND LAMINAR ORGANIZATION OF THE CONNECTIONS BETWEEN THE AMIGDALA AND THE ORBITO-INSULO-TEMPORAL CORTEX IN THE CAT. A. Llamas*, F. Clascá* and F. Reinoso-Suárez. Dpt. Morfología, Fac. Medicina, Univ. Autónoma. 28029 Madrid.Spain.

The orbito-insulo-temporal cortex of the cat covers the convexity of the orbital gyrus (GO), and the ventral portions of the anterior and posterior (GSP) sylvian gyri, as well as the lips and bottom of the anterior rhinal, sylvian, and rostral half of the posterior rhinal sulci. This region, which includes the taste area, the granular and agranular insular cortices, the rostral half of areas 35 and 36, and the ventral part of the temporal area (Krettek,J.E. and J.L.Price,J. Comp. Neurol.,172:687,1977) , is heavily interconnected with the amygdala (A). In order to elucidate the detailed topography of these connections, fifty-seven cats received small injections of HRP or HRP-WGA in different portions of OIT or A.

Our rersult indicate that the lateral (AL) and basal (AB) nuclei are the almost exclusive source of amygdaloid projections to OIT. The neurons from which these projections arise are mainly located within the rostral half of these nuclei. AL projects heavily to the caudal two-thirds of OIT. There is a remarkable topographic segregation within AL between neurons projecting to periallocortical or to isocortical areas. AB projects over the whole extension of OIT with a less clear topographical arrangement. The laminar distribution of the axons of AL and AB neurons is different for some OIT regions.

The various OIT sectors show clear differences in their cortico-amygdaloid projections. AB receives projections mainly from the agranular insula and area 35. The central nucleus receives projections from the rostral half of GO, although this nucleus does not project to OIT. The rostral third of OIT sends only very scarce projections to AL. The insular cortex projects heavily to AL, and the distribution of these connections reciprocates the topography of the amygdalo-cortical projections from AL. AL also receives a strong input from areas 35 and 36, but these projections show less topographic segregation. A band of cortex located in the ventrolateral aspect of the GSP strongly projects to AL, while adjacent sectors of this gyrus have nearly no connexions with A. The laminar distribution of the neurons which give rise to the cortico-amigdaloid projections is different for AL and BL, and from one cortical region to another.

The present results confirm and extend previous findings (Llamas,A. et al., Science ,95: 794, 1977; Llamas,A. et al., Neuroscience, 15: 561, 1985) and show that OIT is the most prominent gateway for direct neocortical inputs to A, as well as one of the main targets of amygdalo-cortical connections. The topographic organization of these interconnections may represent an anatomical basis for segregated channels conveying information between the different OIT areas and A. In addition, the connectional heterogeneity shown in this study add support for further parcellation of some nuclei of the cat's amygdaloid complex.

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CELL LINEAGE AND DIFFERENTIATION III

248.1 CONDITIONED MEDIUM RESTORES A NORMAL PHENOTYPE TO JIMPY OLIGODENDROCYTES GROWN IN VITRO. P.E. Knapp, W.P. Bartlett[1] and R.P. Skoff. Dept. Anat. and [1]Cell Biol., Wayne State Univ. Sch. of Med., Detroit, MI 48201 and [1]Dept. Anat., The Milton S. Hershey Medical Center, Hershey, PA 17033.

Using immunocytochemical techniques we have shown that oligodendrocytes (OLs) from normal mouse cerebra express galactocerebroside (GC) and 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP) in cell bodies, processes and large membrane sheets by 7 days in vitro (DIV) (Knapp et al, Dev. Biol., 120:356, 1987). In our system, relatively small numbers of OLs from jimpy (jp) brains are GC+ or CNP+, and jp OLs do not produce the large (>100 um diameter) immunostained membrane sheets which are characteristic of normal cultures. Thus, development and differentiation of OLs is compromised in vitro as well as in vivo. In order to study the interactions of developing jp glia, we grew mixed cerebral cultures from jp brains in normal control medium and in control medium which had been conditioned for 1-2d by cultures consisting primarily of normal astrocytes (CM). CM was either centrifuged (2,000 rpm for 12 min) or passed through a 0.22 um sterile filter before application to jp cultures. After only 7d in CM (8 DIV) jp OLs show marked improvement relative to those grown in control medium. The numbers of both GC+ and CNP+ cells were increased, usually several hundredfold. Growth in the presence of CM had a likewise remarkable effect on the production of immunostained membrane sheets by jp OLs. In these cultures OLs were able to produce and maintain membranes which contained both GC and CNP and which were virtually indistinguishable from those in normal cultures. Our normal OLs produce immunodetectable amounts of myelin proteolipid protein (PLP) after 8-9 DIV, but we do not see PLP incorporation into the membrane sheets even after 4 weeks in vitro. Jp cultures can rarely be immunostained for PLP. When jp OLs are grown in CM as described, PLP immunostaining is observed in cell bodies and processes in a normal pattern. These results show that under the right conditions jp OLs will exhibit normal phenotypic characteristics. Jp OLs, therefore, have the genetic capacity to produce myelin constituents even though this capacity is not normally expressed. CM from jp cultures did not interfere with normal OL development, suggesting that jp cells do not produce "oligodendrotoxic" factors which interfere with OL development. Our results suggest that normal brain cells provide a soluble factor, necessary for normal oligodendrocyte development, which is missing in jp cultures. Astrocytes are the most likely source of this factor since they constitute the vast majority of cells in conditioning cultures. This hypothesis is especially interesting in view of recent studies showing that astrocytes and/or factors produced by astrocytes are important in the expansion and differentiation of OL populations (eg. Noble and Murray, EMBO J., 3:2243, 1984; Bhat and Pfeiffer, J. Neurosci. Res., 15:19, 1986). Supported by NS 15338.

248.2 FURTHER OBSERVATIONS ON THE GLIAL CELL POPULATION OF THE OPTIC NERVE OF THE MYELIN DEFICIENT RAT. K.F. Jackson*, J.P. Hammang*, I.D. Duncan. (Spon: R.L. Sufit). Dept. of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706.

The myelin deficient (md) rat shows a failure of normal CNS myelination, caused by an x-linked recessive lethal gene. This study, the preliminary results of which have already been reported in part (Soc. Neurosci. Abstr. [1986] 427.10), was undertaken to investigate the kinetics of the glial cell population in the optic nerve of this mutant at a wide range of ages, and to determine the cell types involved in division, their morphology, and their ultimate fate. 4 md and 4 normal male littermate rats at 3,6,8,10,12,14,16,18,21,25 and 30 days of age were perfused with Karnovsky's aldehyde fixatives after pulse-labeling with tritiated thymidine. Both optic nerves were removed from each animal, the intracranial segments processed into epon blocks and ultrathin sections taken for EM, with adjacent 1µ sections taken for LM autoradiography. Labeling indices were determined as previously described, and it was found that at 14,16,18,21 and 25 days there were significantly more dividing cells ($p<0.05$) in the md rat optic nerves. At the other ages there was no significant difference between md and control rats. It was also observed that at 18 days there was a significantly higher total glial cell count in the md optic nerve, but this was reversed at 30 days. Counts of pyknotic cells in normal and md optic nerves revealed significantly more cell death ($p<0.025$) at 14 through 30 days in the md rat. EM examination of these cells suggested that many of them were oligodendroglial origin, their cytoplasm containing distended cisterns of RER as described in a previous study (Dentinger et al, 1985. Brain Res. 344:255-266). By comparing the LM autoradiographs with adjacent ultrathin sections examined by EM, dividing cells in the md and control rats at 14 through 25 days were classified according to morphological criteria. The majority of the dividing cell population in the md rat was found to consist of oligodendroglial cells, immature in appearance, and undifferentiated cells, the percentage of which apparently increased with age. At 14 days, 10% of dividing cells in the md rat were astrocytic in type, but this percentage declined to zero in the older mutants. There were more labeled microglial cells in the md rat than in the controls at all the ages studied, many with cytoplasm filled with phagocytosed material. In all the normal animals, the majority of labeled cells were oligodendroglia, with many undifferentiated cell types also taking up thymidine. There were very few labeled astrocytic or microglial cells in these animals. Thus it would appear that in the md rat there may be a defect in the oligodendroglial population, resulting in a protracted division of precursor and immature cell types, the production of morphologically abnormal oligodendrocytes and ultimately a high percentage of dying cells, with a concomitant microglial response and failure of normal myelin production.

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248.3 SOLUBLE FACTOR(S) REGULATE THE TIMING OF TYPE-2 ASTROCYTE DIFFERENTIATION IN VITRO. L.E. Lillien and M.C. Raff*. MRC Developmental Neurobiology Programme, Zoology Dept., University College London, London WC1E 6BT, UK.

Previous work on glial cell lineages in the rat optic nerve (O.N.) has demonstrated that oligodendrocytes and type-2 astrocytes develop from a common (O-2A) progenitor: while differentiation in vitro into oligodendrocytes appears to be constitutive, differentiation into type-2 astrocytes requires an inducing agent such as fetal calf serum.

In contrast to cultures of O.N., in cultures of embryonic rat brain we have found that type-2 astrocytes develop in the absence of exogenous inducing agents. In cultures prepared from 15 day embryonic rats (grown in 10% horse serum, selected for its lack of type-2-inducing activity), oligodendrocytes (identified by staining with anti-galactocerebroside [GC] antibody) first appeared after approximately 1 week in vitro, while type-2 astrocytes (defined by staining with A2B5 and GFAP antibodies) were first detected after 16-18 days in vitro (d.i.v.), a time equivalent to post-natal (PN) days 9-11, when type-2 astrocytes are first detectable in vivo. Supernatants were collected from these cultures at 3 day intervals and assayed for their ability to induce O-2A progenitor cells ($A2B5^+$, GC^-, $GFAP^-$ process-bearing cells) to express GFAP. Soluble inducing activity did not appear in the cultures until 16-18 d.i.v., at which time the supernatants induced 20-40% of O-2A progenitor cells from newborn brain or optic nerve to express GFAP.

The timing of the appearance of this activity does not simply reflect a threshold density of cells as the activity appears at the same relative time (equivalent to PN 9-11) over a range of initial plating densities and in cultures prepared from newborn rather than embryonic brain.

Studies of cultures of optic nerve, purified type-1 astrocytes, retina and co-cultures of these tissues suggest that the production of soluble GFAP-inducing activity depends on an interaction between O-2A lineage cells and type-1 astrocytes and does not require neurons.

248.4 CYCLIC AMP INDUCES EXPRESSION OF OLIGODENDROCYTE MARKERS DURING A CRITICAL PERIOD IN CULTURE. David W. Raible and F. Arthur McMorris. The Wistar Institute, Philadelphia, PA 19104.

We have previously shown that the oligodendrocyte enzyme 2',3'-cyclic 3'-phosphohydrolase (CNP) is induced by cyclic AMP analogs in primary cell cultures from 1 day old rat brain. Treatment of 1- to 2-week-old cultures with cyclic AMP analogs increased the amount of CNP protein per oligodendrocyte, not the number of oligodendrocytes per culture. We have now examined the effects of cyclic AMP analogs during the first week of culture, at which time there are few oligodendrocytes present. Cultures were grown on microscope slides, treated with 1 mM dibutyryl cyclic AMP (dbcAMP) or 8-bromo-cyclic AMP at various times, and fixed for immunocytochemistry. When cells were cultured with dbcAMP for three days, the percentage of CNP positive oligodendrocytes increased 3-4 fold. If treatment with dbcAMP was not started until day 7 of culture, no induction in oligodendrocyte number was seen. Similar results were obtained when cultures were stained for the oligodendrocyte markers myelin basic protein (MBP) and galactocerebroside (GC). Induction of oligodendrocyte number was similar in the presence or absence of serum, indicating that serum factors were not necessary for induction. When cultures treated with dbcAMP were grown in the presence of tritiated thymidine, no difference in DNA synthesis was seen in CNP positive cells as compared with control, indicating that dbcAMP does not induce proliferation of young oligodendrocytes. These observations are consistent with the hypothesis that cyclic AMP induces oligodendrocyte differentiation. Supported by NSF BNS 85-18023, NMSS RG1767-A-1 and NIH CA 09171 and NS 11036.

248.5 CONTROL OF SCHWANN CELL MYELIN FORMATION BY BASAL LAMINA. C.F. Eldridge*, R.P. Bunge and M.B. Bunge. Dept. Anatomy & Neurobiology, Washington University Sch. Med., St. Louis, MO 63110.

Observations on tissue culture preparations containing DRG neurons and Schwann cells (SCs), but no fibroblasts, have established that SCs interacting with axons are able to produce basal lamina and fibrous collagen components of endoneurial extracellular matrix and to myelinate axons. SCs grown with neurons in a serum-free defined medium do not assemble basal laminae or form myelin. Ascorbic acid stimulates both SC basal lamina assembly and myelin formation with similar dose-response relationships. This stimulation of SC differentiation by ascorbic acid, however, requires the presence of a non-dialyzable serum component(s); the serum component alone has essentially no detectable effects on SC differentiation. We have hypothesized that ascorbic acid has no direct effects on SC myelin formation, but that instead ascorbic acid acts directly to enable the SC to assemble a basal lamina, which is required for differentiation into a myelin-forming cell. This hypothesis is supported by the following evidence: (1) Oligodendrocytes, instead of SCs, can be grown with DRG neurons in this culture system; these central nervous system myelin-forming cells, which do not produce basal lamina, show no requirement for ascorbic acid (or serum) to form myelin in culture. This result indicates that ascorbic acid is not directly required for myelin biosynthesis or assembly. (2) Although there are few quantitative or qualitative differences in overall protein secretion between SCs cultured with neurons in defined medium or in the presence of ascorbic acid and serum, the formation of triple-helical (pepsin-resistant) type IV collagen is not detectable in the absence of ascorbic acid. The effect of ascorbic acid on SC basal lamina assembly may thus be mediated via effects on the biosynthesis of type IV collagen. (3) Exogenous basal lamina is able to completely substitute for ascorbic acid-stimulated endogenous SC basal lamina production. Thus, a gel-forming extract of EHS sarcoma basal lamina matrix (Matrigel) allows SCs to undertake myelination in the absence of ascorbic acid and serum; type I collagen gels have no similar effect. Surprisingly, purified laminin, but not type IV collagen or basal lamina heparan sulfate proteoglycan, can substitute for ascorbic acid; in the presence of serum, 50 ug/ml (50 nM) laminin promoted the formation of 261 myelin segments/mm^2 (SD=26, three experiments), whereas 50 ug/ml (280 uM) ascorbic acid promoted 293 myelin segments/mm^2 (SD=24). Laminin induces the formation of basal lamina-like structures on abaxonal SC surfaces; these structures contain not only laminin but also heparan sulfate proteoglycan and type IV collagen as assessed by immunofluorescence. The basal lamina may act to promote SC differentiation by influencing the organization of the cell's plasma membrane and cytoskeleton. (Supported by NIH grants NS09923 and NS07071.)

248.6 SCHWANN CELL DIFFERENTIAL EXPRESSION OF P_0 IN THE ABSENCE OF AXONAL INFLUENCE DURING POSTNATAL DEVELOPMENT. Kurt R. Brunden*, Anthony J. Windebank, Joseph F. Poduslo Departments of Neurology and Biochemistry, Mayo Clinic and Mayo Foundation, Rochester, MN 55905

Previous work from this laboratory (Poduslo, J.F. and Windebank, A.J., Proc. Natl. Acad. Sci., 82, 5987-5991, 1985) has revealed that Schwann cell (SC) cultures from permanently transected adult rat sciatic nerves are capable of continued expression of the major myelin glycoprotein, P_0, in the absence of both axons and myelin assembly. In contrast, SCs derived from actively myelinating, 4-day-old neonatal rat sciatic nerves lose the capacity to synthesize P_0 after culture, suggesting that the SCs are capable of reverting to a progenitor state. We now show that the ability of cultured SCs to suppress the expression of P_0 occurs over a short and finite time-span during development. If sciatic nerves of 12-day-old rats are explanted in culture for 5 days followed by [^{3}H]mannose precursor incorporation, active P_0 biosynthesis can be demonstrated. Pulse-chase analysis reveals that newly synthesized P_0 is degraded shortly after its formation. This post-translational catabolism has recently been documented in the permanently transected adult sciatic nerve and has been shown to be due to lysosomal targeting of the glycoprotein from a site at or after the medial Golgi (Brunden, K.R. and Poduslo, J.F., J. Cell Biol., 104, 661-669, 1987). Like P_0 of the transected nerve, the catabolism of the glycoprotein synthesized in the explant cultures from 12-day-old animals can be inhibited by the addition of the Golgi mannosidase II inhibitor, swainsonine (SW). This inhibition is presumably caused by the formation of an oligosaccharide structure that results in the inefficient recognition of P_0 by an undefined lysosomal transport system. P_0 synthesized in the SW-treated cultures is readily labeled with [^{3}H]fucose, indicating that the glycoprotein is routed for degradation sometime after reaching the medial Golgi. These data suggest that the neonatal SCs that are incapable of expressing P_0 after culture are only able to revert to a progenitor state for a finite period, after which they behave much like the permanently transected sciatic nerve. The post-translational degradation of P_0, and perhaps other myelin proteins, appears to be a common mechanism employed by SCs that are deprived of axonal influence. (Supported by NINCDS Grant NS-20551 and by the Borchard Fund).

248.7 DEVELOPMENT OF THE OLFACTORY NERVE FIBER LAYER IN THE OLFACTORY BULB OF MOUSE EMBRYOS. R. Doucette. Department of Anatomy, Univ. of Sask., Saskatoon, Saskatchewan S7N 0W0 Canada.

The olfactory epithelium is an interesting anomaly to the generally accepted pattern of mammalian neurogenesis in that its neurons continue to be produced throughout the entire life of the animal. To reach their target cells in the central nervous system (CNS) the axons of these newly formed neurons must grow through the olfactory nerve fiber layer (ONL), which is comprised of two morphologically distinct types of glial cell. One of these cell types is most definitely an astrocyte, but the other cell type, which is solely responsible for ensheathment of olfactory axons, is more difficult to classify. Doucette (Anat. Rec 210: 385, 1985), who referred to this latter cell type as an ensheathing cell, has hypothesized that it is a morphological variant of the typical astrocyte; this hypothesis implies that during development a common progenitor cell gives rise to both cell types. The aim of the present study was to examine the early development of the ONL. Pregnant Swiss mice were killed by cervical dislocation on days 10 to 16 of pregnancy; the day of the vaginal plug was designated embryonic day 1 (E1). The uterine horns were removed by cesarean section and the embryos were dissected free of the uterus. Following decapitation each head (E10-14) or dissected brain piece (E15-16) was fixed by immersion and embedded in Epon. The first olfactory axons penetrated the rostral wall of the cerebral vesicle (E13-14) prior to its evagination to form an olfactory bulb. From the earliest stage of vesicle evagination the olfactory axons and the cells that accompanied them (see below) formed quite a distinct ONL; even in E16 embryos the ONL was distinctly separate from the rest of the developing bulb, although small fascicles of axons did leave the layer to enter the deeper neuropil. The cells of the ONL were part of a morphologically homogeneous population, and were presumably glial precursor cells (not neurons) because they ensheathed the olfactory axons. Morphologically identical cells were found within the PNS portion of the olfactory nerve fascicles but not in the deeper neuropil of the developing bulb, suggesting that the glial precursor cells of the ONL were of peripheral origin. It was the cytoplasmic processes of these latter cells that gave the ONL such a distinct appearance by clearly separating it from the deeper layers of the bulb. In conclusion, the whole ONL of the E13-16 mouse olfactory bulb is of peripheral origin. If this interpretation proves to be correct, then the implication is that the two glial cell types in the ONL of adult mammals have separate developmental origins; the ensheathing cells arising from the PNS and the astrocytes from the CNS.

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248.8 DIFFERENTIATION OF TE671 HUMAN MEDULLOBLASTOMA CELLS: EFFECTS OF RETINOIC ACID, PHORBOL MYRYSTATE ACETATE AND HEXAMETHYLENE BIS-ACETAMIDE ON NEURONAL CHARACTER AND MORPHOLOGY.
Hal N. Siegel, Ph.D. and Ronald J. Lukas, Ph.D., Laboratories of Neurochemistry and Cell Culture, Barrow Neurological Institute, 350 West Thomas Road, Phoenix, Arizona 85013.

The TE671 human medulloblastoma cell line derives from a tumor of presumed neuroectodermal origin. Studies have since demonstrated that these cells possess particular characteristics of mature neurons, including neuron-specific intracellular enzymes and membrane-bound high affinity binding sites for certain neurotransmitters. Immunocytochemical staining demonstrated the expression of the neuron specific cell surface determinant neuron-specific enolase (NSE). In contrast, immunocytochemical analysis shows markers for other neuron-specific antigens, such as synaptophysin and neurofilament proteins, are not normally expressed. The absence of similarly localized glial-specific intermediate filament proteins (glial fibrillary acidic protein, GFAP), suggests this cell line is constituitively, nominally neuronal.

Treatment of low density, dividing cells with media supplemented with various differentiating agents demonstrates that the TE671 cells are capable of multiple differentiated states, initially defined by visually assessed morphology. Culture in micromolar phorbol myrystate acetate (PMA) induces a distinctively different morphology than does addition of millimolar concentrations of hexamethylene bis-acetamide (HMBA) or retinoic acid. Evidence from immunocytochemical determination of NSE, GFAP, synaptophysin, and the 68, 160 and 200 kilodalton neurofilament peptides demonstrates that the neuronal nature of the TE671 cell is magnified by these differentiating protocols, while no similar increase in glial quality occurs.

Thus, the TE671 cell undergoes only neuronal differentiation under the influence of these agents, which in other tissues demonstrate no such uniformity of action. Implications for the continued use of normal/differentiated TE671 cells as a model of central nervous system neurons will be discussed.

248.9 NEUROGENESIS IN MAMMALIAN CENTRAL NERVOUS SYSTEM: AN IN VITRO MODEL FOR OLFACTORY NEURON DIFFERENTIATION.
Brian L. Largent, Ronald G. Sosnowski*, Ernest Barbosa*, and Randall R. Reed*, Department of Molecular Biology and Genetics, Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The vertebrate olfactory neuronal system is unique among central neuronal systems in that it retains into adulthood the capacity to sustain a population of primary sensory neurons by steady state replacement of mature neurons. This process is accomplished by stem cells which differentiate and mature into primary olfactory neurons - replenishing neuronal cells that have degenerated. Indeed, this process mimics in microcosm many aspects of the developmental process of the nervous system itself - i.e., the maturation and commitment of neuroblasts to specific neuronal phenotypes as well as the death of neurons during the modeling of neuronal and synaptic organization.

We have taken a novel approach to the generation of clonal cell lines possessing the unique properties of primary olfactory neurons. To this end, we are immortalizing primary olfactory neurons by germline transformation of mice with a recombinant oncogene. Specifically, we have constructed a hybrid gene consisting of the early region of simian virus 40 (SV40) - encoding the oncogene product, large T-antigen - flanked 5' by the cell-specific regulatory elements (enhancers and promoters) of the rat gene for olfactory marker protein (OMP), a protein abundantly and uniquely expressed within primary olfactory neurons. Expression of SV40 large T-antigen (T-ag) within cells induces immortalization and/or transformation of those cells. The regulatory elements of the OMP gene should target expression of T-ag to primary olfactory neurons, thereby affecting those cells in a selective fashion within the transgenic mouse. The feasibility of such an approach in transforming and immortalizing specific cell types has been demonstrated in several paradigms (Hanahan, D. (1985) Nature 315:115-122O; Mahon et. al., (1987) Science 235:1622-1628).

Several interesting modifications of this approach utilize hybrid genes encoding various mutants of T-ag. One such variation of this paradigm, replacing wild type T-ag with a temperature-sensitive mutant, allows the regulation of oncogenic potential in cell culture by switching between permissive and non-permissive temperatures, thus, exerting some control over the effects of T-ag on the differentiation state of these cells. In obtaining immortalized neuronal cell lines with minimal transformed properties, two mutants of T-ag which immortalize but do not transform cells may prove to be optimal alternatives to wild type T-ag. We are currently generating transgenic mouse lines for each of these recombinant oncogene constructions. Olfactory neuroepithelium from these transgenic animals should be a source of primary olfactory neurons that will propagate indefinitely in culture. Such cell lines will facilitate a variety of molecular studies ascertaining the specific molecules which give primary olfactory neurons their unique attributes. This scheme for generating immortalized cell lines of defined origin and clonality is generally applicable for other neuronal phenotypes as well - given specific gene regulatory elements can be found to selectively drive expression of the trans-oncogene within a particular set of neurons.

248.10 BROMODEOXYURIDINE IMMUNOCYTOCHEMISTRY TO EXAMINE THE PROLIFERATION AND MIGRATION OF RODENT CORTICAL NEURONS. S.J. Muller, M.W. Miller, and R.S. Nowakowski (SPON: M.M. LaVail). Dept. of Anatomy, Sch. Osteopathic Med. and R.W. Johnson Med. Sch., UMDNJ, Piscataway, NJ 08854.

During development, cortical neuroblasts proliferate in one of two germinal zones, the ventricular or subventricular zone, from which they migrate to the cortical plate. [^{3}H]thymidine autoradiography has been used extensively to trace this early development. Recently, tumor biologists have developed an alternative method for studying cellular proliferation using the immunocytochemical localization of another thymidine analogue, 5-bromo-2'-deoxyuridine (BrdU). We are applying this new methodology to examine the development of the cerebral cortex. Pregnant rats or mice were injected simultaneously with 50 or 100 µg/g of BrdU and with 5.0 or 10.0 µCi/g of [^{3}H]thymidine. Animals were perfused with 70% ethanol 1 hr, 3 days, or 60 days after the injection. Brains were embedded in paraffin and cut at 3-6 µm. Tissue was reacted with a primary antibody against BrdU (Becton-Dickenson) using an avidin-biotin complex procedure (Vector). Immunostained tissue was then processed by standard autoradiographic techniques. In all experimental situations, the distribution of BrdU immunoreactive cells was identical to that of autoradiographically labeled cells. All autoradiographically labeled neurons were BrdU positive. Moreover, there was a high correlation in the intensity of label, i.e., densely-stained BrdU-immunoreactive neurons and heavily-labeled autoradiographically labeled cells were densely BrdU-immunostained. In animals that survived 1 hr after the injection, BrdU-positive neurons were distributed in the ventricular and the subventricular zones and the density of labeling of all cells was comparable. In tissue processed 3 days post-injection, the most densely stained BrdU-positive cells were distributed at the superficial edge of the cortical plate, and less densely stained cells were scattered through the deeper cortical plate, intermediate zone, subventricular zone, and ventricular zone. Following an injection of a rat on gestational day 17 densely-stained immunoreactive neurons were distributed in layers IV and V of the mature cortex, whereas less densely-stained immunoreactive cells were in the supragranular laminae. Thus, BrdU immunocytochemistry is suitable for developmental studies of the nervous system and provides an alternative to [^{3}H]thymidine autoradiography. In one important way, the immunocytochemical technique is superior to the autoradiography in that BrdU-positive cells are distributed throughout the depth of the tissue. Funded by AA 06916, DE 07734, a grant from the UMDNJ Foundation (M.W.M.), and by NS 23647 and a grant from the G.R.S. program of R.W. Johnson Med. Sch. (R.S.N.).

248.11 CELL LINEAGES AND MOVEMENTS IN THE FORMATION OF THE ZEBRAFISH EMBRYONIC AXIS. C. B. Kimmel and R. M. Warga*. Inst. of Neuroscience, Univ. of Oregon., Eugene, OR 97403

Cell lineage analyses during zebrafish development have revealed that clones developing from single gastrula cells are confined to single tissues. Moreover, cells in clones that populate the CNS are often distributed very regularly in the tissue (bilaterally, and periodically along the neuraxis). These restrictions may indicate that either the fates of the progenitor gastrula cells are already specified, or alternatively that the movements of uncommitted gastrula cells are developmentally programmed.

We have monitored the cell divisions, positions, movements, and eventual fates of gastrula cells in live developing embryos. Except for cells whose daughters remain confined to an outer enveloping epithelium, we found no evidence for programming of the plane or pattern of cell divisions. On the other hand, regular patterns of morphogenesis lead predictably to the formation of particular axial structures by cells originating at stereotyped positions in the early gastrula. Cell movements vary according to the germ layer of origin (ectoderm or mesoderm) of the structure that is formed, and these movements appear to more accurately predict cell fate than does cell lineage. The regular arrangements of clonally related neural cells arise as their progenitors successively intercalate with other cells in a manner that appears to be independent of cell lineage, and which may function to elongate the developing embryonic axis. We observed variability in neuron type and position within small clones that seems to argue against a strict role of cell lineage in determining cell fate. We suggest, alternatively, that observed clonal distributions are generated by developmental processes that are driven by morphogenesis - the formation of properly arranged and shaped systems of organs - in which cell lineage is unimportant. Thus it may be that decisions about cell fate are made only during or after the cell migrations that accompany gastrulation. (Supported by the NSF.)

248.12 REGULATION OF PRIMARY MOTONEURON AND ROHON-BEARD NEURON LINEAGES IN FROG EMBRYOS. S.A. Moody. Department of Anatomy and Cell Biology, University of Virginia School of Medicine, Charlottesville, VA 22908.

Fate maps of the number of primary motoneurons (PMN) and primary sensory neurons, the Rohon-Beard cells (RB), derived from each of the blastomeres of the 16-, 32-, and 64-cell stage of the South African clawed frog (Xenopus laevis) have been constructed. As cleavage proceeds, only certain daughter cells remain in the neuronal lineages, seeming as if parcelling of the mother cell cytoplasm also parcels neuronal fate. For example, when blastomere V2.2 divides, its anterior daughter cell (V2.2.2) produces 89% of the mother cell's RB progeny, and its posterior daughter cell (V2.2.1) produces only 11% of them. When blastomere V2.2.2 divides, both daughter cells produce approximately 50% of the mother cell's RB progeny. However, when blastomere V2.2.1 divides, only its anterior daughter cell (V2.2.1.2) continues to be a progenitor of RB neurons. In order to determine whether these lineages are specified at early cleavage stages or are capable of regulation, we have performed experiments in which single 32-cell stage blastomeres were removed microsurgically, and adjacent blastomeres were marked by the intracellular injection of a lineage tracer. The numbers of PMN and RBN produced by each lineage were counted and compared to those produced in an intact embryo.

Several different region-specific responses were observed. Blastomeres that lie on the boundaries of "neurogenic" regions no longer contributed to nervous system when a nervous-system producing neighbor blastomeres was ablated. Some blastomeres in equatorial regions overproduced PMN and others underproduced PMN and RB after neighbor cell ablation. Animal pole blastomeres made the normal numbers of both PMN and RB in ablated embryos. These results suggest that the potential to produce nervous system need not be intrinsically determined at early cleavage stages. Furthermore, certain regions of the blastula show regulative responses and other regions appear to require cell-cell interactions in order to fully express their normal neural lineages. Finally, non-neural branches of the lineages remained qualitatively similar to those in intact embryos except when blastomeres appeared to regulate. In cases in which there was an overproduction of PMN or RB, labeled cells were missing from organs that normally were populated by the labeled blastomere's lineage.

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248.13 **MULTIPOTENTIAL PRECURSOR CELLS IN XENOPUS OPTIC VESICLE: A CELL LINEAGE ANALYSIS.** **Richard Wetts and Scott E. Fraser. Dept of Physiol & Biophysics and the Developmental Biology Center, Univ Calif, Irvine, 92717.**

Cell lineage analysis is an important step in understanding when and how different cell types are specified during development. Intracellular injection of cell autonomous lineage tracers offer a means to approach this question at the single cell level. Here, we apply this technique to the study of the terminal lineages in the Xenopus neural retina. To perform a prospective lineage analysis, lysinated rhodamine dextran (LRD) was iontophoretically injected into a dividing cell in the optic vesicle (St. 22-24). After LRD injection, the animals were raised to free-swimming larvae (St. 40-49), fixed in 4% paraformaldehyde in phosphate buffer, embedded in methacrylate, and sectioned at 20-30 microns. Sections were examined with a 100x objective on an epifluorescence microscope equiped with a light-intensifying camera and an image processing system.

The vertebrate retina has 6 major types of glia and neurons located in 3 layers. In order to determine the potential of the injected cell, the goal is to identify the different types of cells which are labeled; identification of the layer is a first step towards this goal. Five of the 35 clones were located in only 1 layer, (mean size: 2.6 cells), 15 clones were distributed over 2 layers (mean: 11.9 cells), and 15 clones were distributed over all 3 layers (mean: 21.9 cells). Because many of the clones included cells of 3 different layers, there must be a precursor cell (in the optic vesicle) for at least 3 different types of cells in the retina. It is possible that this multipotential precursor can give rise to all types of retinal cells. Rods, cones, horizontal, bipolar, amacrine, and ganglion cells have all been identified within clones containing other cell types. Since the optic vesicle cells are often multipotent, developmental restrictions of cell phenotype must occur relatively late in the development of the frog neural retina.

While there is no data yet indicating this, it remains possible that there are restricted precursors (committed to producing only one cell type) as well as the multipotent cells in the optic vesicle. In fact, even those small clones located in 1 or 2 layers did not appear to consist of only one cell type. Furthermore, the same cell types were also seen in the larger clones. Thus, the labeled progenitor may have had the potential of forming many cell types, but actually produced only a limited subset due to the small clone size. This situation cannot be distinguished from a committed precursor: when a progenitor produces only a few cell types (regardless of clone size), the lineage must be challenged by experimental manipulations to determine if the progenitor was committed or not. Thus, the evidence to date supports the existence of multipotential precursor cells in the frog optic vesicle.

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248.14 COMMITTED NEURONAL AND GLIAL CELL PRECURSORS IDENTIFIED IN MOUSE NEUROEPITHELIUM. P.F. Bartlett*, K. Wycherley* and K.A. Bailey* (Spon: I. Darian-Smith). The Walter & Eliza Hall Institute of Medical Research, Melbourne 3050, Australia.

The neuronal and glial cells of the central nervous system are derived from a dividing population of neuroepithelial cells that form the neural tube. In order to study the early developmental pathways and the hierarchical arrangement of precursor populations in the CNS, the identification and subsequent isolation of pure populations of cells at various stages of differentiation are required. Furthermore, in vitro systems that support the subsequent differentiation of precursor cells are required to ascertain the full differentiation repertoire of each precursor population. In this report we have used a series of immunological markers combined with cell sorting procedures and in vitro assays to unequivocally identify neuroepithelial cells committed to either the neuronal or glial cell lineages. Our results indicate that as early as E10 progenitor cells in the neuroepithelium can be identified and purified by their surface phenotype and assigned to a specific cell lineage.

The neuronal progenitor cells are identified by their inability to express class I histocompatibility antigens ($H\text{-}2I^-$) in response to interferon-γ (IFN-γ), and they also express the surface ganglioside recognised by the A2B5 monoclonal antibody (Eisenbath et al., Proc.Nat.Acad.Sci, USA 76:4913, 1979). Type 1 astroglia progenitors are $H\text{-}2I^+$ and $A2B5^-$ and also express a surface marker recognised by the WEHY-NEP-6 monoclonal antibody. The NEP-6 marker is exclusively expressed on type 1 astroglial progenitors but is lost prior to glial maturation and the expression of GFAP. These two classes of progenitors have been isolated by flow cytometry, and with the use of high efficiency cell culture, which employs irradiated astroglia as an underlayer, have been shown to differentiate exclusively into cells belonging to either the neuronal or glial lineage. Thus, the neuroepithelium can be divided into distinct subpopulations based on surface phenotype and differentiation potential. This information has been used to construct a flow diagram of the differentiation pathways involved in early neural development. The fact that the vast majority of E9 neuroepithelial cells have a homogeneous surface phenotype ($H\text{-}2I^+$, $A2B5^-$, WEHY-NEP-6^-, $Keratin^+$) suggests that commitment to a particular cell lineage may be occurring during the E9-10 period.

248.15 IMMUNOCYTOCHEMICAL DEMONSTRATION OF THE ADRENERGIC NEUROTRANSMITTER PHENOTYPE IN THE HUMAN NEUROBLASTOMA CELL LINE LA-N-1 FOLLOWING DIFFERENTIATION. S. Sullivan and M.F.D. Notter. Dept. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642.

The human neuroblastoma cell line LA-N-1 was investigated for expression of adrenergic enzymes before and after differentiation to define the neuronal phenotype. Retinoic acid, a vitamin A analog, has previously been shown to induce morphologic differentiation in this cell line and therefore was employed in this study.

Cells were maintained in F12:MEM (1:1) medium supplimented with 15% heat inactivated fetal calf serum, and grown in a humidified incubator at 37°C with 5% CO_2: 95% air atmosphere. Two days after plating cells the culture medium was replaced with medium containing 10^{-5}M retinoic acid (RA) with subsequent refeeding with RA over the course of a week.

After four days, RA induced LA-N-1 cells to produce long neurites and to change from rounded to spindle shaped cells. After seven days in culture the cells were stained by immunocytochemistry using the PAP method for a number of adrenergic markers and specific neural proteins. Both the differentiated and undifferentiated cells stained positively for neurotransmitter enzymes tyrosine hydroxylase (TH), dopamine beta hydroxylase (DBH) and phenylethanolamine methyl-N-transferase (PMNT). After differentiation the level of staining for TH and DBH was significantly increased. There was no apparent difference in the staining of PMNT after differentiation.

A minority of the cells in these cultures were phenotypically different from the major cell population. They appeared as large flat cells that occasionally extended short processes. This sub-population did not stain for any of the adrenergic enzymes; nor did they stain with the astrocyte specific antibodies to glial fibrillary acidic protein or S100. However, these cells appeared to stain very faintly with neuron specific enolase. Therefore, the two different morphologies appear to be of the neuronal cell type.

Although the LA-N-1 cells stained for the adrenergic neurotransmitter enzymes they were not positive for catecholamine histofluorescence as determined by reaction with glyoxylic acid. Therefore, product may not be stored in these cells and a reuptake system for catecholamines is being examined.

The LA-N-1 cell line has been demonstrated to contain TH, DBH and PMNT suggesting that these cells have the capability of synthesizing catecholamines. If this is so LA-N-1 may provide a human derived supply of adrenergic cells for transplantation studies.

248.16 SECRETION OF TISSUE TYPE PLASMINOGEN ACTIVATOR DURING NEURONAL DIFFERENTIATION OF AN EMBRYONAL CARCINOMA CELL LINE, T. Whitford* and J.M. Levine, Program in Cellular and Developmental Biology and Dept.of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794

Developing neurons secrete plasminogen activators, enzymes which specifically cleave plasminogen to generate plasmin, a highly active, broad range protease. The ability of neurons to regulate the local level of extracellular proteolysis may be important in developmental processes such as cell division, cell migration, process outgrowth and neuron-glial interactions. To understand the role of the plasminogen activator (PA)/plasminogen (PMG) system in neuronal differentiation, we examined the secretion of PAs by an embryonal carcinoma cell line, P19S1801A1, which develops into neuron-like cells after aggregation with retinoic acid and growth as monolayers in a chemically defined, serum free medium.

The appearance of PAs in tissue culture supernatants was assayed in 3 ways: first, with a chromogenic assay in which PA activates PMG to cleave a synthetic substrate (H-D-Valyl-L-leucyl-L-lysine-P-nitroanilide dihydrochloride) generating a product detectable by its absorbance at 405 nm; second, with a PMG-casein-agar overlay of living cells and third, with a PMG containing SDS gel lytic assay described by Pittman (Dev. Biol. 110: 91-110, 1985). All 3 assays demonstrated that the neurally differentiated O1A1 cells secrete PAs. Secretion was maintained for up to 8 days in monolayer culture, at which time greater than 85% of the cells had developed neuritic processes and neurofilament-like immunoreactivity (J. Neurosci., 6:3374-3384, 1986). The appearance of PA activity correlated with the development of neuronal properties: neither undifferentiated O1A1 cells nor cells which were treated with DMSO and grown in serum-containing medium to induce mesodermal differentiation secreted PAs into the culture medium.

The PA secreted by neurally differentiated O1A1 cells was shown to be tissue-type PA (tPA) by 2 criteria. First, on PMG containing SDS gels, the PA activity electrophoresed as a single band of apparent MW of 65kd and it comigrated with authentic tPA. Second, antibodies against tPA but not those directed against urokinase inhibited the activation of PMG in the chromogenic assay.

These results demonstrate that the neuronal differentiation of O1A1 cells is accompanied by the secretion of tPA. Plasminogen activators provide a means by which cells interact with or modify the extracellular environment. Thus tPA may play a role in neurite outgrowth in this model system.

248.17 INDUCTION OF NEURONAL DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS BY DIFFERENT GROWTH MODULATORS. S.A.D. Sharma*, J.T. Hansen and M.F.D. Notter (SPON: L.G. Abood). Dept. of Neurobiol. and Anat., Univ. of Rochester, Rochester, NY 14642.

Embryonal carcinoma (EC) cells can be induced to differentiate into a number of different cell types, including neurons, depending on their treatment. We have previously reported that a population of neurons induced by retinoic acid (RA)-treatment of P19S18O1A1 (O1A1) mouse EC cells was adrenergic. Here we further characterize the RA-induced neurons and report on the ability of dibutyryl cyclic AMP (db cAMP) and nerve growth factor (NGF) to induce neuronal differentiation.

O1A1 cells were differentiated by growing for 2 days as monolayers followed by 3 days as aggregates in the presence of growth modulator(s): RA (500 nM); db cAMP (500 ug/ml); NGF (375 ng/ml) and db cAMP (500 ug/ml). The dissociated aggregates were then plated onto coverslips and maintained for an additional 6 days in the presence or absence of growth modulator(s). Cultures were therefore 11 days old when examined.

Scanning EM of RA-treated O1A1 cultures showed neurons growing on a background of glia and fibroblast-like cells. Many of the neurons appeared to be multipolar with long processes. The presence of bipolar as well as multipolar neurons was also seen at the light microscopic level. Transmission EM of RA-induced neurons showed neurotubules and neurofilaments typical of normal neurons. The neurons also had a high concentration of normal mitochondria, suggesting that they were metabolically very active. A small number of secretory granules could be seen. We have reported that the RA-induced adrenergic neurons can take up exogenous norepinephrine, and this would be expected to enhance the level of secretory granules. Electrophysiological recordings suggested that the neurons were electrically active. Therefore EM and electrophysiological data from these RA-induced neurons suggest that they have features similar to neurons in normal non-tumorous tissue.

In addition we have found that treatment of O1A1 cells with NGF and db cAMP together, or with db cAMP alone induced neuronal differentiation. Both treatments induced the development of cells with neuronal morphology. Positive immunocytochemical staining for neurofilament protein and neuron-specific enolase were obtained in db cAMP or NGF/db cAMP-treated cultures. The neuronal phenotype was expressed if these cultures were treated continually with db cAMP or NGF/db cAMP for 11 days, or if they were treated for 5 days with these modulators followed by 6 days without stimuli. Therefore the neuronal differentiation appeared to be irreversible. Preliminary observations suggest that NGF/db cAMP is more potent at inducing neuronal differentiation than db cAMP alone. However, further experiments are needed to determine the individual effects of NGF and db cAMP. Comparison of the neuronal properties induced by NGF, db cAMP and RA may provide insights into neuronal induction and development of different neuronal phenotypes in vivo.

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248.18 MONOCLONAL ANTIBODIES AGAINST DEVELOPMENTALLY REGULATED MEMBRANE ANTIGENS SELECTIVELY EXPRESSED BY ALL MATURE SLOW FIBRES IN CHICKEN SKELETAL MUSCLE. S. Shahin*, P.F. Bartlett* and J.A.P. Rostas. The Neuroscience Group, Faculty of Medicine, University of Newcastle, New South Wales, 2308, Australia and The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, 3050, Australia.

Using purified sarcolemma from adult chicken anterior latissimus dorsi (ALD) and posterior latissimus dorsi (PLD) as immunogen, two monoclonal antibodies have been prepared which have a selective reactivity with chicken skeletal muscle. The fibre specificity of the antibodies was determined on frozen sections of ALD, PLD, sartorius, and interior and exterior adductor muscles, by comparing the immunofluorescence staining with the myosin ATPase staining of adjacent sections. In mature muscle, both antibodies recognised slow twitch (type I) and slow tonic (type III) fibres but not fast twitch (type II) fibres. In early embryonic muscle all fibres appeared to express both antigens. Presumptive fast twitch (type II) fibres gradually lost their immunoreactivity during development until, by embryonic day 18, the selective adult staining pattern was established. The antigens recognised by both antibodies appear to be present in, but not confined to, membranes of the tubular system and sarcolemma. Between two weeks and adult the intensity of fluorescence observed on ALD with AA21 decreased with no apparent difference in distribution whereas the intensity of fluorescence with PA20 increased with the intracellular staining becoming more prominent. AA21 showed strong reactivity with heart, brain, pancreas, gizzard, small intestine and blood vessels but no reactivity with kidney, liver or sciatic nerve. By contrast the only other tissue with which PA20 shows strong reactivity was heart. As determined by western blotting of ALD sarcolemma, the major antigens recognised by AA21 and PA20 were proteins with apparent molecular weights of 95-100KDa and 125-140 KDa, respectively. Both antibodies, at high concentrations, detected minor antigens of different molecular weights. With both antibodies the antigens recognised in tissue homogenates were the same as those in ALD sarcolemma as determined by western blots.

248.19 POSITIONAL INFORMATION INFLUENCES EMBRYONIC MUSCLE FIBER-TYPE EXPRESSION. J. Butler* and E. Cosmos. Dept. Neurosci., McMaster Univ. H.S.C., Hamilton, Ontario, Canada, L8N 3Z5.

Recently, using as a marker the impaired wing motility characteristic of dystrophic chick embryos, we verified that thoracic somitic mesoderm transplanted heterotopically to replace brachial somites at 48-52 h in ovo is viable and capable of forming a functional wing musculature (Cauwenbergs et al., Neurosci. Letters 68: 149, 1986). Thus, during early embryogenesis premyogenic cells demonstrate a high degree of plasticity since their ultimate ability to form specific individual muscles is determined by positional signals. To determine whether or not such influences extend to the formation of distinct muscle fiber-types is the objective of the present study. This is an important issue in view of previous in vivo (Butler et al., J. Exp. Zool. 224:65, 1982) and in vitro (Miller and Stockdale, J. Cell Biol. 103:2197, 1986) studies which demonstrate the existence of fiber-type diversification among primary generation myogenic cells of the early chick embryo, a diversity which occurs independent of neural influences. If myogenic cells are endogenously programmed from the somite stage onward to express specific classes of myogenic cells, then brachial muscles derived from thoracic somitic mesoderm should form muscles whose fiber-type profiles reflect the heterogeneity characteristic of muscles formed from in situ thoracic somites (Butler et al., JEEM 95:147, 1986). If, instead, positional information influences fiber-type expression, then the experimental muscles should exhibit profiles characteristic of unoperated brachial muscles.

A total of 177 embryos were subjected to the unilateral replacement of brachial somites by thoracic somitic mesoderm and, to date, 14 surviving intraspecific chick/chick chimaeras between St 35 (8-9 d in ovo) to St 45 (19-20 d in ovo) have been analyzed. Results demonstrate that (1) transplanted thoracic somitic mesoderm forms, on schedule, a complete brachial musculature; (2) the fiber-type composition (myosin ATPase profile) of individual experimental muscles is equivalent to that expressed by either individual contralateral brachial muscles or brachial muscles formed from brachial somites transplanted to donor embryos. Thus, we conclude that positional information influences embryonic fiber-type expression. Furthermore, such an influence is operative during a temporally limited period since limb bud transplants (Laing and Lamb, JEEM 78:67, 1983) performed approximately 20 h later than the present experiments demonstrate that the fiber-type composition of wing muscles transplanted to the leg region is determined by the site of origin, not by the site of transplantation.

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ADRENERGIC RECEPTORS

249.1 **PHARMACOLOGY AND BIOLOGICAL ACTIVITY OF BIOTIN-LABELED ALPHA$_1$-ADRENERGIC RECEPTOR LIGANDS.** D. Lorton, C.L. Hix, M. Caron*, and J.N. Davis. V.A. Medical Center and Depts. of Medicine (Neurology) and Pharmacology, Duke University, Durham, NC 27705.

Biotin-labeled alpha$_1$-adrenergic receptor ligands were synthesized and their pharmacology and biological activity characterized. A55453 is an analogue of prazosin and shares its high selectivity and affinity for alpha$_1$-adrenergic receptors. A55453 was modified by biotinylation via amino group substitution using either a short hydrocarbon chain (N-hydroxy succinimidyl biotin, A55453-SC-B) or a long hydrocarbon chain (sulfo succinimidyl 6-(biotin amido) hexanoate, A55453-LC-B) biotin derivative under conditions which produced a 90 - 95% yield.

Biotinylated A55453 derivatives were compared with A55453 for their abilities to compete with [^{3}H]-prazosin for binding to alpha$_1$-adrenergic receptors in rat cortical membranes. The ability of A55453-SC-B and A55453-LC-B to displace [^{3}H]-prazosin from alpha$_1$-adrenergic receptors was similar to A55453 (K_d's, 8×10^{-10}, 1.5×10^{-8}, 1.8×10^{-9} M, respectively). A55453-SC-B was almost as effective as prazosin in inhibiting epinephrine-stimulated K^+ release from dispersed parotid acinar cells, demonstrating the biological activity of A55453-SC-B.

The biotin components of both derivatives retained their ability to bind to avidin following conjugation to the ligand. Conjugation of avidin to A55453-SC-B reduced the affinity of the ligand for the alpha$_1$-adrenergic receptor by 1000 fold. By contrast, conjugation of avidin to A55453-LC-B reduced the affinity of this ligand by only 4 fold. Avidin-conjugated A55453-SC-B did not compete as well for receptor binding as the avidin-conjugated A55453-LC-B, perhaps because of steric hinderance.

Although in vitro receptor autoradiography has allowed localization of many brain neurotransmitter receptors at the light microscopic level, the relationship of receptors to pre- and post-synaptic elements can only be studied with electron microscopy. The data presented here suggests that biotinylated receptor ligand derivatives may provide powerful tools for the localization of membrane bound receptors at the light and electron microscopic levels.

(Supported by the V.A. and NS 06233)

249.2 **QUANTITATIVE AUTORADIOGRAPHY OF [125I]BE 2254 BINDING IN THE RAT BRAIN: LABELLING OF SITES IN ADDITION TO α_1-ADRENERGIC RECEPTORS IN STRIATUM.** D.M. BURNETT* and N.R. Zahniser (SPON: R.S. Lasher) Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

BE 2254 is a potent and selective α_1-adrenergic receptor (α_1-AR) antagonist. In the iodinated form, [125I]-BE 2254 (IBE) has been shown by several investigators to be a very useful radioligand for labelling both peripheral and central α_1-AR's (Kd value = 50pM). We have used IBE along with quantitative autoradiography (QAR) to localize and study α_1-AR's in the rat brain. Image analysis of saturation binding of IBE revealed a distinctly large component of nonspecific binding within the striatum. At a Kd concentration of the radioligand, competition experiments with prazosin (an α_1-AR antagonist with unusually high selectivity) showed that only 35% of the total binding was associated with α_1-AR's within the striatum. In contrast to this and in good agreement with other reports, prazosin displaced IBE binding by 80-90% in other areas of the brain such as thalamus and cerebral cortex. We have attempted to identify this nonspecific binding site(s) in the striatum which is labelled by IBE.

Competition curves against a Kd concentration of IBE were constructed in striatal homogenates for several pharmacologically selective receptor antagonists. In some experiments a saturating concentration of prazosin (1μM) was included to prevent radioligand binding to the α_1-AR's. Compounds examined included: mazindol for competition at the dopamine uptake site, SCH-23390 for the D_1 dopamine receptor, (S)-sulpiride for the D_2 dopamine receptor, pyrilamine and cimetidine for the H_1 and H_2 histamine receptors, respectively, and ketanserin and pirenperone for competition at the $5HT_2$ receptor. Of the compounds tested, only the $5HT_2$ receptor antagonists had activity against IBE binding in the striatum. In the absence of prazosin, both ketanserin and pirenperone displaced IBE with IC_{50} values of 20nM, which correspond to Ki values of 10nM for the α_1-AR. These data agree well with previous reports that ketanserin and pirenperone inhibit, with high affinity, the binding of α_1-AR ligands. When binding to α_1-AR's was prevented by the presence of 1μM prazosin, ketanserin displaced IBE binding further by 40% with an IC_{50} value of 10nM. Pirenperone likewise displaced IBE binding beyond that defined with 1μM prazosin, but its activity was much less potent (IC_{50} value = 0.4μM). These data suggest that IBE may label $5HT_2$ receptors in the striatum. However, additional experiments will be performed to verify this finding. In addition, the use of prazosin to define the nonspecific binding of IBE in the rat brain provides an accurate measurement of specific binding to α_1-AR's for conventional filtration assays as well as for assays using QAR.

This work was supported by USPHS AG-04418.

249.3 CHARACTERIZATION OF ALPHA-2 ADRENERGIC RECEPTORS IN CHICKEN PINEAL GLAND USING [^{3}H]RAUWOLSCINE. D.B. Bylund, L.J. Petterborg*, C. Ray-Prenger* and P.K. Rudeen. Departments of Pharmacology and Anatomy, University of Missouri Medical School, Columbia, MO 65212.

The avian pineal gland has an endogenous circadian rhythm of melatonin production which can be entrained by either neural or photic inputs. Pharmacologic studies in the intact chicken indicate that norepinephrine may decrease pineal melatonin by the stimulation of alpha-2 adrenergic receptors. Using the antagonist radioligand [^{3}H]rauwolscine, we have identified alpha-2 adrenergic receptor binding sites in membrane preparations of chicken pineal glands. The binding was rapid, reversible, saturable, of high affinity and had the pharmacologic characteristics of other alpha-2 adrenergic receptors systems. Preliminary experiments indicate no marked differences in binding between male and female birds or between glands taken just before lights off and those just after lights on. However, there was an increase in receptor density (B_{max}) with age, but no change in affinity (K_D).

Age	B_{max}, fmol/mg protein	K_D, nM
3 week	150	0.31
4 week	250	0.39
5 week	300	0.36
adult	600	0.49

These results confirm the presence of alpha-2 adrenergic receptors in the chicken pineal gland. The chicken pineal gland should be useful both in understanding pineal function and as a model system for studying alpha-2 adrenergic receptors. Supported by NIH Grant 32931.

249.4 CHARACTERIZATION OF α_2-ADRENOCEPTORS WITH THE AGONIST ^{3}H-UK 14304 IN POSTMORTEM HUMAN BRAIN. J.A. García-Sevilla*, J.J. Meana* and F. Barturen* (SPON: M. Lafarga). Dept. of Pharmacology, Medical School, Univ. of the Basque Country, 48940 Leioa, Bizkaia, Spain.

The full agonist ^{3}H-UK 14304 (5-bromo-6[2-imidazolin 2-ylamino]-quinoxaline) has been used to characterize α_2-adrenoceptors in postmortem human brain following the method described for the rat brain (Loftus et al., Life Sci., 35: 61, 1984) with minor modifications. In cortical membranes the binding at 25°C was rapid (t1/2 of association: 4.6 min) and reversible (t1/2 of dissociation: 14.1 min) and the equilibrium dissociation constant (K_D) determined from these kinetic studies was 0.48 nM. The binding sites for ^{3}H-UK 14304 showed the specificity required for an α_2-adrenoceptor. The rank order of potency of inhibitors of ^{3}H-UK 14304 binding was (-)epinephrine ≈ UK 14304 > clonidine > oxymetazoline > idazoxan > yohimbine >> phenylephrine >>> prazosin > (-) propranolol. Competition curves for most adrenergic drugs were shallow (n_H<1) indicating heterogenous inter actions with ^{3}H-UK 14304 binding sites. The nucleotide GTP also inhibited (K_i=5x10^{-6}M) the specific binding of ^{3}H-UK 14304, suggesting that the radioligand preferentially labelled the high affinity state of the α_2-adrenoceptor. The inhibition constants (K_i) of adrenergic drugs in competing with ^{3}H-UK 14304 were correlated with the K_i of these drugs in competing with ^{3}H-clonidine (r=0.96; P<0.001) which suggested that both radioligands labelled the same α_2-adrenoceptor in postmortem human brain. In the frontal cortex, linear (Scatchard) and non-linear analyses of ^{3}H-UK 14304 binding (10^{-11} - $4x10^{-9}$M) indicated the existence of a single population of non-interacting sites (K_D= 0.23± 0.01 nM; Bmax= 80±12 fmol/mg protein; n= 7). In other brain regions (hypothalamus, hippocampus, amygdala, brainstem, caudate nucleus and cerebellum) the density of α_2-adrenoceptors (Bmax) ranged from 68±10 to 25±6 fmol/mg protein. In these brain regions K_D values for ^{3}H-UK 14304 ranged from 0.32±0.03 to 0.56±0.09 nM. The binding of the full agonist ^{3}H-UK 14304 to post-mortem human brain membranes might be a useful tool for the study of dysfunctions related to the high affinity state of the α_2-adrenoceptor (e.g. endogenous depression) Supported by CAICYT Grant 1244/84 and by a grant from the Departamento de Educación, Universidades e Investigación (Gobierno Vasco).

249.5 COMPARISON OF HIGH AFFINITY BINDING FOR TWO ALPHA$_2$-RECEPTOR AGONISTS, GUANABENZ AND CLONIDINE, IN SHEEP AND RAT CEREBRAL CORTEX. R.K. Zoltoski* and C.E. Dunlap III (SPON: D.J. Goode). Bowman Gray Sch. of Med. of Wake Forest University, Winston-Salem, NC 27103.

Previous studies have demonstrated (^{3}H)clonidine binding with K_d and B_{max} values of 5 nM and 14 pmol/g tissue in rat brain (U'Prichard et al., Mol Pharmacol, 13:454-473, 1977) and 2.37 nM and 5.39 pmol/g tissue in rat cerebral cortex (Fluck et al., Drug Dev Res, 3:91-99, 1983), respectively. The latter investigators also reported guanabenz to inhibit (^{3}H)clonidine binding with a K_i of 0.96 nM, in agreement with the value of 0.9 nM reported by Jarrott et al. (Biochem Pharmacol, 27:141-144, 1979).

We have undertaken a series of experiments employing tritiated guanabenz in our laboratory, which have included radioligand binding studies of (^{3}H)guanabenz in fetal and maternal sheep and rat cerebral cortex homogenates. (^{3}H)GBZ binding in maternal ovine cortex was first-order, of high affinity, and saturable, with a K_d of 6.45 ± 0.95 nM and B_{max} of 415 ± 111 fmol/mg protein. Binding in cortex from fetal sheep (124 - 129 days gestation) displayed a K_d of 5.68 ± 0.97 nM and B_{max} of 195 ± 44 fmol/mg protein. While there appeared a trend toward fewer binding sites in fetal compared to maternal cortex, the values were not statistically different for the sample sizes used.

Interestingly, competition curves for clonidine vs. (^{3}H)GBZ binding produced a K_i value of 400.8 ± 95.0 nM, suggesting different binding sites for (^{3}H)GBZ and (^{3}H)clonidine. To test this observation further, parallel binding studies with both radioligands were conducted in rat cerebral cortex homogenate preparations. (^{3}H)Clonidine binding in rat cortex had K_d and B_{max} values of 1.27 nM and 86.4 fmol/mg protein (6.1 pmol/g tissue) respectively, in good agreement with values obtained by Fluck et al. Competition curves for clonidine displacement of (^{3}H)GBZ binding yielded a K_i value of 313 nM, while guanabenz displacement of (^{3}H)clonidine binding had a K_i value of 1.95 nM, providing further evidence for separate high affinity binding sites for the two drugs.

This research was supported by NIH grant HL34460 and by a research grant from Wyeth Laboratories.

249.6 **AUTORADIOGRAPHIC EVIDENCE FOR ASSOCIATION OF α_2-ADRENERGIC RECEPTORS WITH CENTRAL EPINEPHRINE PATHWAYS USING TWO INBRED RAT STRAINS.** J.R. Unnerstall, M.J. Connors*, J.M. Stolk and D.C. U'Prichard. Dept. Neurology and The Alzheimer Center, Case Western Reserve Univ. Sch/Med, Cleveland, OH 44106; Dept. Psychiatry, Univ. Maryland Sch/Med, Baltimore, MD 21228; and, Stuart Pharmaceuticals, Wilmington, DE 19897.

Two inbred rat strains, Fischer 344 (F344) and Buffalo (Buf), which differ markedly in peripheral and central levels of PNMT activity and brain epinephrine (EPI) content, have been shown to differ phenotypically with respect to a major structural gene locus coding for PNMT. Buf rats exhibit 3-10 fold less PNMT activity than F344 rats in adrenals, brain and other tissues, and also exhibit 3-10 fold lower EPI levels in hypothalamus and brain stem. Homogenate binding experiments have shown a selective increase in a-adrenergic binding sites in the medulla and brainstem of Buf rats as compared to F344 rats. In this report, we have used high-resolution quantitative receptor autoradiographic techniques (QAR) to demonstrate the selective increase of α_2-adrenergic binding sites in Buf rats in brain regions innervated by EPI-utilizing brainstem neurons.

Four Buf and four F344 rats (approximately 6 weeks old) which were bred and raised at the Maryland Psychiatric Research Center (JMS) were utilized in these experiments. The animals were sacrificed by perfusion with 5% sucrose in phosphate-buffered saline and the brains were rapidly removed and frozen over dry ice. Serial cryostat sections (10 μm) were incubated with 1.0 nM [^{3}H]para-aminoclonidine ([^{3}H]PAC) or 2.0 nM [^{3}H]rauwolscine ([^{3}H]RW) in 170 mM Tris-HCL (pH7.4) in the absence or presence of 10 μM phentolamine to define non-specific binding. Under these conditions [^{3}H]PAC or [^{3}H]RW labels approximately 50% of their respective high-affinity binding sites with minimal occupancy at their low-affinity site.

Selective large increases (>100%) in α_2 binding were seen in several forebrain and brainstem regions in the Buf rats that normally are innervated by medullary EPI-utilizing neurons. These include the bed nucleus of the stria terminalis, central and medial nuclei of the amygdala, paraventricular nucleus of the hypothalamus, dorsal medial thalamus, nucleus of the solitary tract, dorsal motor nucleus of the vagus and ventral lateral medulla. Smaller (>50%) but still significant increases were seen in other areas such as the lateral septum, medial preoptic area, anterior hypothalamus, nucleus reuniens of the thalamus, dorsal raphe, dorsal parabrachial nucleus, locus coeruleus and hypoglossal nucleus. Small insignificant changes were observed in areas such as the cortex and hippocampus.

These data confirm and extend the homogenate binding studies and more selectively emphasize the presence of an inverse correlation between regional EPI utilization and α_2 receptor density.

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249.7 BRIGHT ARTIFICIAL LIGHT SUBSENSITIZES THE PRESYNAPTIC α_2-ADRENOCEPTOR. S.C. Dilsaver* and M.J. Majchrzak (SPON: N.E. Alessi). Dept. of Psychiatry, Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109.

Supersensitivity of presynaptic α_2-autoreceptors could be involved in the biology of depression. The authors used a thermoregulation paradigm to test the hypothesis that chronic treatment with bright artificial light (at an intensity of 11,500 lux) produces subsensitivity to the hypothermic effects of an α_2 agonist, clonidine. Core temperature was measured using a telemetric thermosensor, the Model VM Mini-Mitter (Mini-Mitter Corp., Sunriver, OR). Full spectrum bright artificial light was emitted from a bank of eight 122 cm long Vita Light tubes suspended 50 cm above the animals. This light unit (Duro Test Corp., Bergen, NJ, Model 5599) is used to treat patients with seasonal affective disorder (SAD). Mini-Mitters were implanted into the peritoneal cavities of 10 adult, male rats. Experiment I involved measurement of hypothermic response to clonidine, 0.1 mg/kg ip, prior to exposure to light, following a week of constant light exposure and one week after light was withdrawn. Core temperature was measured prior to the injection of clonidine and every 10 minutes thereafter for 120 minutes. A second study involved measurement of the thermic response to clonidine, 0.4 mg/kg ip, at baseline and after one and two weeks of treatment in 7 animals. The objective was to demonstrate that multiple injections of clonidine do not alter thermic responsiveness to itself. Nine of 10 animals exhibited blunting of the hypothermic response to clonidine at $\alpha < 0.04$. The mean thermic response of this sample, was -1.22 ± 0.15 (X ± SEM) prior to light treatment and -0.03 ± 0.1°C after chronic treatment ($p < 0.00009$, t=6.74, df=9). A week after the discontinuation of light the mean thermic response of the sample was -1.16 ± 0.14°C. This differed from the response during treatment ($p < 0.0003$, t=5.8 df=9) but did not differ from the baseline response ($p > 0.8$, t=0.2, df=9). Multiple injections of clonidine did not alter thermic responsiveness to itself. Conclusion: Chronic treatment with bright artifical light subsensitizes the α_2-autoreceptor. This is consistent with the reports that other forms of antidepressant treatment, tricyclic antidepressants, and monoamine oxidase inhibitors, ECT and lithium carbonate have the same effect.

249.8 ALPHA-2 ADRENERGIC RECEPTOR INHIBITION OF MELATONIN SYNTHESIS IN CHICK PINEAL CELLS: BLOCKADE BY PERTUSSIS TOXIN AND ACTIVATORS OF THE CYCLIC AMP PATHWAY. B.L. Pratt and J.S. Takahashi. Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois 60201.

Postjunctional alpha-2 adrenergic receptors mediate the regulation of melatonin release by norepinephrine in chick pineal cells (Pratt and Takahashi, J. Neurosci., in press). We have begun to test whether an inhibition of cAMP, caused by norepinephrine-induced alpha-2 receptor activation, is involved in the regulation of melatonin release in avian pineal cell cultures.

The experimental protocol utilized was the same as that described previously to identify the adrenergic receptor subtype pharmacologically (Pratt and Takahashi, Soc. Neurosci. Abst. 12, 384, 1986). We have found that increasing cAMP levels by introducing cAMP analogues, activating adenylate cyclase with forskolin or inhibiting phosphodiesterase with IBMX, all block alpha-2 receptor-mediated inhibition of melatonin release in a dose dependent manner. In all cases, 9 doses of each drug were used to antagonize the inhibition of melatonin release caused by 1 X 10^{-7}M norepinephrine. The relative potencies of cyclic nucleotide analogues were as follows (EC_{50}, mM): 8-bromo-cAMP (0.125), monobutyryl-cAMP (1.0) and 8-bromo-cGMP (>2.0). Cyclic AMP, 8-bromo-5'AMP and 8-bromo-5'ATP were without effect at doses up to 2.0mM. The EC_{50} values for forskolin and IBMX were 0.3uM and 125uM, respectively.

In most systems, alpha-2 adrenergic receptor activation appears to act through an inhibition of adenylate cyclase. This inhibition is mediated by a guanine nucleotide regulatory protein known as Gi or Ni. The function of Gi can be inactivated by pertussis toxin. If norepinephrine decreases cAMP by inhibiting adenylate cyclase, then toxin pretreatment should block the inhibition of melatonin release caused by norepinephrine in chick pineal cells. Pertussis toxin treatment blocked the effect of norepinephrine on melatonin release in a noncompetitive manner. Pertussis toxin-induced blockade was partial at 1 and 10 ng/ml doses and was complete at a 100 ng/ml dose. Although pertussis toxin is known to inactivate several G proteins, the data cited above, showing that activators of the cAMP pathway antagonize the effect of norepinephrine, suggest that pertussis toxin may be blocking the inhibition of melatonin release by inactivating Gi. Further data, measuring the effects of norepinephrine on cAMP levels and identifying the presence of pertussis toxin-sensitive G proteins in avian pineal cell cultures should aid in further understanding of the events initiated by alpha-2 adrenergic receptor activation.

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249.9 ALPHA$_2$- ADRENERGIC RECEPTOR-MEDIATED REGULATION OF PHOSPHATIDYL INOSITOL METABOLISM IN HUMAN PLATELETS. H. Mori*, M. Mikuni, T. Koyama* and I. Yamashita*. Dept. of Psychiatry, Hokkaido Univ. Schl. of Med., Sapporo, Hokkaido 060 Japan and Division of Mental Disorders Research, National Institute of Neuroscience, National Center for Neurology and Psychiatry, Kodaira, Tokyo, 187 Japan.

The Metabolism of inositol phospholipids in response to epinephrine was investigated in human platelets using sensitive radioisotopic method of Berridge(1983). In platelets prelabelled with [^{3}H]-myo-inositol, in Ca^{++} free HEPES buffer containing 10mM LiCl which blocks the enzyme inositol-1-phospatase, epinephrine caused a dose-dependent accumulation of inositol-1-phosphate (IP_1) over a concentration range of 1-100uM during the 15 min incubation. The EC_{50} value of epinephrine was 5 uM.

Yohimbine, a selective alpha$_2$-adrenergic receptor antagonist, inhibited ephinephrine-stimulated IP_1 accumulation with a Ki value of 42 nM. Mianserin(1uM), a potent blocker of alpha$_2$ adrenergic receptors, also inhibited 70% of the epinephrine(10uM) response. Prazosin(1uM), a selective alpha$_1$-adrenergic receptor antagonist, failed to inhibit the epinephrine(10uM) response. Ketanserin(1uM), a selective 5HT$_2$ receptor antagonist, failed to inhibit the epinephrine(10uM) response. (-)Sulpiride(1uM), a selective D_2 receptor antagonist, also failed to the epinephrine(10uM) response. Mezerein, a potent protein kinase C activator, inhibited 70% of the epinephrine(10uM) response at a concentration of 100 uM.

The only direct biochemical consequence of alpha$_2$-adrenergic receptor activation thus far identified in the platelet is inhibition of adenylate cyclase. However, our results indicate that epinephrine also stimulates phosphatidylinositol turnover by activating receptors of the alpha$_2$ type in human platelets.

249.10 Electrophysiological Effects of Locally-Applied Adrenergic Agonists at Cerebellar Purkinje Neurons: Receptor Specificity. K.D. PARFITT*, R. FREEDMAN, and P.C. BICKFORD-WIMER. Depts. of Pharmacology and Psychiatry, UCHSC, Denver, CO 80262.

Older literature indicates that when norepinephrine (NE) is applied locally by microiontophoresis to cerebellar Purkinje cells a beta receptor-mediated inhibition of cell firing rate occurs in situ. Using perfusion of NE on an in vitro cerebellar slice or on an in oculo cerebellar transplant, an alpha receptor-mediated inhibition of spontaneous activity was demonstrated. Because of this discrepancy, we investigated the receptor subtype mediating the inhibition of cerebellar spontaneous firing rate using local application of adrenergic agonists and antagonists, in situ, via pressure micro-ejection. Extracellular action potentials of cerebellar Purkinje neurons were recorded from anesthetized Fischer 344 rats. Timolol, a beta receptor antagonist, did not affect NE-induced inhibition in 9 of 12 cells studied. Phentolamine, an alpha receptor antagonist, blocked the effect of NE in 8 of 11 cells. To determine the subtype of alpha receptor involved, the effects of the alpha$_1$ antagonist prazosin and alpha$_2$ antagonists idazoxan and yohimbine were examined. While prazosin had no effect on NE-mediated inhibition, both idazoxan and yohimbine blocked NE-mediated inhibition.

The inhibitory action of NE upon Purkinje cell firing rate was mimicked by the selective alpha$_2$ agonist clonidine; this inhibitory action of clonidine was blocked by idazoxan but not by timolol or prazosin. In addition, the alpha$_1$ adrenergic agonist phenylephrine and the beta adrenergic agonist isoproterenol inhibited Purkinje cell firing rate. Inhibition by phenylephrine was blocked by prazosin but not by timolol or idazoxan. Isoproterenol-induced inhibition was blocked by timolol but not phentolamine. These studies with adrenergic agonists and antagonists indicate that both alpha and beta receptors alter Purkinje cell firing rate; the inhibitory action of locally-applied NE, however, seems to be mediated predominantly via an alpha$_2$ adrenergic receptor. This work was supported by the VAMC and USPHS grant AG04418.

249.11 ELECTROPHYSIOLOGICAL CHARACTERIZATION OF ADRENOCEPTORS MEDIATING THE EFFECT ON ENDOGENOUS NOREPINEPHRINE ON RAT HIPPOCAMPAL PYRAMIDAL NEURONS IN VIVO. O. Curet* and C. de Montigny (spon: L. Vachon). Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Canada H3C 3J7.

The suppression of firing activity of rat hippocampal pyramidal neurons by microiontophoretic application of norepinephrine (NE) is mediated by an α_2 type of adrenoceptor (Curet, O. and de Montigny, C., Neurosci. Abstr., 12: 349.9, 1986). The present study was undertaken to characterize the nature of the adrenoceptor mediating the effect of endogenous NE, released by the electrical stimulation of the locus coeruleus (LC), on dorsal hippocampus pyramidal neurons and to evaluate the modulatory role of terminal α_2 adrenergic autoreceptors on NE neurotransmission.

Male Sprague-Dawley rats (260-300 g) were anesthetized with chloral hydrate (400 mg/kg, i.p.). CA_3 hippocampal pyramidal neurons were recorded extracellularly with five-barrelled micropipettes. The central barrel was filled with 2M NaCl saturated with Fast Green and side barrels with NE (0.1M in 0.2M NaCl; pH 4), acetylcholine (ACh) (0.02M in 0.2M NaCl; pH4) and 2M NaCl for automatic current balancing. ACh was used to activate pyramidal neurons to a physiological range (10-14 Hz). A bipolar concentric electrode (NE 100, DKI) was positioned in the LC. 150 square pulses of 0.5 ms were delivered at 1 Hz with an intensity of 800 µA. The degree of suppression of hippocampal pyramidal neuron firing activity produced by the LC stimulation was quantified from computer-generated peristimulus time histograms.

Prazosin (150 µg/kg, i.v.) reduced the effect of LC stimulation by 86% without altering the response of these same neurons to the microiontophoretic application of NE. The subsequent i.v. injection of idazoxan restored the effectiveness of the stimulation and decreased the effect of microiontophoretic application of NE by 50%.

Increasing the frequency of the stimulation from 1 to 5 Hz reduced its effectiveness by 80%. This reduction of the efficacy of the stimulation by increasing the frequency was abolished by the α_2-adrenoceptor antagonist idazoxan (500 µg/kg, i.v.). Furthermore, clonidine (10 µg/kg, i.v.), an α_2-adrenoceptor agonist, and desipramine (500 µg/kg, i.v.), a selective NE reuptake blocker, reduced the efficacy of LC stimulation by 83% and 84%, respectively. These effects were also reversed by the subsequent i.v. injection of idazoxan.

In conclusion, these data demonstrate that the effect of endogenously released NE on dorsal hippocampus pyramidal neurons is mediated by α_1 adrenoceptors. Since the effect of microiontophoretically-applied NE on the same neurons is mediated by α_2 adrenoceptors, these data provide evidence that, in the CNS, as appears to be the case in the periphery, postsynaptic α_2 adrenoceptors are extrasynaptic, whereas α_1 adrenoceptors are intrasynaptic. Furthermore the present results provide evidence for the potent regulatory role of terminal NE autoreceptors in controlling the release of NE into the synaptic cleft.

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249.12 EFFECTS OF CHRONIC COCAINE TREATMENT ON SPONTANEOUSLY FIRING NORADRENERGIC LOCUS COERULEUS NEURONS IN THE RAT. D. K. Pitts and J. Marwah. Dept of Path., Univ. of Med. & Dent. of New Jersey - S.O.M., Camden, NJ 08103.

Previous acute studies using standard extracellular electrophysiological techniques have established that intravenous (iv) cocaine (coc) invariably and reversibly inhibits single identified spontaneously discharging locus coeruleus (LC) neurons in urethane anesthetized rats (Pitts, D.K. & Marwah, J., Life Sci. 38:1229, 1986). The inhibition of LC neurons by iv coc appears to be mediated at least in part by an augmented action of endogenous catecholamines on alpha-2 adrenergic autoreceptors. The following evidence has been previously reported (Pitts, D.K. & Marwah, J., JPET 240:345, 1987) in support of this hypothesis: selective alpha-2-adrenoceptor antagonists (piperoxane & yohimbine) can reverse, or with pretreatment, significantly attenuate the inhibitory effects of coc on LC; reserpine pretreatment significantly reduces inhibition of LC neurons by coc; and finally the local anesthetics procaine and mepivacaine do not significantly affect LC neuron firing rate or action potential waveform in doses greater than those which elicit LC inhibition by coc. Recent studies also suggest that the inhibition of LC neuron activity by iv coc cannot be attributed to cardiovascular effects or anesthetic condition. Pretreatment with the polar alpha-adrenoceptor antagonist, phentolamine (3 mg/kg i.v.), blocks the increase in mean arterial pressure from iv coc administration (1 mg/Kg), but not central LC neuron inhibition. The inhibition of LC neurons by cocaine was also still observed in gallamine paralyzed and artificially ventillated conscious rats. In chronic studies, cocaine (10 mg/Kg i.p.) or vehicle (saline) was administered to rats daily over a three week period. This i.p. dose elicited a pronounced inhibition of LC neurons acutely in urethane anesthetized rats. On testing days one coc and one saline treated rat were surgically prepared for LC recording in the morning and afternoon in an alternating fashion. Once a stable LC neuron was located with the micropipette a challenge dose of cocaine (1 mg/Kg i.v.) was administered. After a minimum of one hour had elapsed, a second LC neuron was located in the same animal and challenged with clonidine (clon, 10 µg/Kg i.v.). The results are summarized below:

Chronic Treatment (n):	SALINE (15)		COCAINE (17)	
Drug Challenge:	COC	CLON	COC	CLON
Baseline Firing Rate:	2.7 ±0.4Hz	2.4 ±0.3	3.4 ±0.4	3.3 ±0.2
Percent Inhibition:	72.9 ±5.3	76.4 ±5.5	57.6 ±6.5	49.6 ±7.8*

*P<0.02 relative to Saline Treated/Clon Challenge (2-tail t-test).

These results suggest that chronic cocaine administration may down regulate alpha-2-adrenoceptors on LC neurons. Further studies employing different dosage regimens are currently being conducted to confirm these preliminary findings. Supported: NIDA DA-04158.

249.13 PHARMACOLOGIC EVALUATION OF CENTRAL ADRENERGIC INVOLVEMENT IN CHLORDECONE-INDUCED HYPOTHERMIA. L.L. Cook,* F.W. Edens* and H.A. Tilson. (SPON: R.D. Myers). Neurotoxicology Div., EPA and Laboratory of Behavioral and Neurological Toxicology, NIEHS, Research Triangle Park, N.C. 27711, and Toxicology Program and Poultry Science Dept., NC State University, Raleigh, NC. 27695.

Chlordecone(CLD) is a neurotoxic chlorinated hydrocarbon insecticide which produces thermoregulatory alterations in addition to tremor and hyperexcitability in rodents following acute, systemic administration. Previous work in our laboratory has shown that CLD-induced hypothermia may be the result of cutaneous vasodilation and that adrenergic receptors, possibly located in the vasomotor control center within the medulla, may be involved (Cook et al., Toxicol. Appl. Pharm., In Press; Cook et al., The Toxicologist, 7:48, 1986). The receptor population within the region of the medulla comprising the vasomotor control center has been shown to be primarily of the α-adrenergic type (Unnerstall et al., Brain Res. Rev. 7:69-101, 1984).

Adrenergic involvement in CLD-induced hypothermia was evaluated in the rat using central pretreatment with 6-hydroxydopamine (6-OHDA) and the α-adrenergic receptor antagonists, phenoxybenzamine and phentolamine, prior to systemic CLD exposure. The effect of IP 75 mg/kg CLD administration on colonic temperature (T-col) in male Fischer-344 rats was measured 7 days after 6-OHDA and 30 min following pretreatment with antagonists by intracisternal (IC) injection. Prior depletion of brain catecholamines with IC 250 µg 6-OHDA administration attenuated CLD-induced hypothermia without affecting basal T-col. Phenoxybenzamine (10 or 20 µg) and phentolamine (5 or 10 µg) also reduced the hypothermic response to CLD. The β-adrenergic receptor antagonists propranolol (50 or 100 µg) and atenolol (10 or 20 µg) did not attenuate CLD-induced hypothermia. These data suggest that CLD-induced hypothermia is a result of alterations involving central α-adrenergic mechanisms in the sympathetic control of vasomotor tone.

249.14 OXYMETAZOLINE INHIBITS THE ADENYLATE CYCLASE OF OK CELLS THROUGH A RECEPTOR DISTINCT FROM THE ALPHA-2 ADRENERGIC RECEPTOR. T.J. Murphy* and D.B. Bylund (SPON: J. Lewis). Dept. of Pharmacology, Univ. of Missouri Medical School, Columbia, MO 65212.

We have recently reported the characterization of alpha-2 adrenergic receptors (A2AR) in the opossum kidney (OK) renal epithelial cell line (Fed. Proc. 46:1445). UK-14,304 (UK), epinephrine (EPI), norepinephrine (NE) and oxymetazoline (OXY) each inhibited parathyroid hormone (PTH) stimulated cAMP production in a dose-dependent manner. Although the effect of UK, EPI and NE could be blocked by A2AR antagonists, the attenuation of cAMP production elicited by OXY was not. In radioligand binding studies using [^{3}H]yohimbine, inhibition binding curves for UK, EPI and NE were shallow and best fit a two-site binding model whereas the curve for OXY was steeper and was consistently better fit by a one-site model. The effect of GTP in these studies was to induce a rightward shift in the UK, EPI and NE inhibition curves whereas GTP was without significant effect on the OXY curve. Thus, OXY behaved as an A2AR antagonist in the binding studies, yet the K_i for binding was quite similar to its EC_{50} value for the inhibition of cAMP production. We have explored the hypothesis that OXY is an agonist at a receptor negatively coupled to adenylate cyclase distinct from the A2AR. We have discounted several alternative explanations for the effect of OXY on cAMP production in OK cells. 1) OXY does not activate a phosphodiesterase (PDE) since its inhibitory effect can be elicited in the presence of PDE inhibitors. 2) OXY is not a PTH antagonist since OXY attenuates forskolin-stimulated cAMP production in the OK cell with a potency similar to its effect on PTH. 3) OXY does not bind irreversibly to A2AR since pretreatment of OK cell monolayers with A2AR antagonists is without effect on the OXY responsiveness and since [^{3}H]rauwolscine saturation binding curves in the presence of increasing OXY concentrations result in a decreased affinity of the A2AR for the radioligand with no change in the B_{max}. The effect of OXY does appear to be mediated through a guanine nucleotide inhibitory protein (G_i) since pretreatment of OK cells with pertussis toxin prevents attenuation of PTH-stimulated cAMP production by both A2AR agonists and OXY. Finally, we have been able to desensitize the A2AR-mediated attenuation of cAMP production by prior treatment of OK cells with EPI. This desensitization appears to be homologous for the A2AR since OXY, but not A2AR agonists, can attenuate cAMP production following EPI pretreatment. We conclude that the effects of OXY are mediated by an inhibitory receptor which is not an A2AR. Supported by NIH Grant 32931.

249.15 SPECIFIC, REVERSIBLE AND STEREOSELECTIVE BINDING OF ^{3}H-L-TYROSINE TO RAT FOREBRAIN (P_2) MEMBRANES. S.P. Arnerić, P. Ernsberger, A.M. May*, P-Y. Cheng* and D.J. Reis. Dept. Pharmacology, Southern IL Univ. Sch. of Medicine, Springfield, IL 62708 and Lab of Neurobiology, Cornell Univ. Medical College, New York, NY 10021

Activation of various peptide receptors present in rat brain slice and synaptosomal-P_2 preparations increase release of endogenous tyrosine (Tyr) through a Ca^{2+}-dependent, regionally selective mechanism (Arnerić et al., J. Neurochem. 48:1581, 1987). This study sought to determine whether Tyr, like classical transmitters, has specific binding sites on neuronal membranes. Membrane fractions (P_2) were prepared from rat forebrain and binding experiments were performed using modified procedures of Sharif and Roberts (J. Neurochem. 34:779, 1980). Microscopic examination of the pellets indicated classical synaptosomal elements with no microvascular contaminants. Membrane fractions were resuspended in 50 mM Tris-HCl buffer (pH=7.2); disrupted with a polytron; then sequentially centrifuged and washed (20 vol. buffer, 3x). Membranes were resuspended in 50 mM Tris-HCl buffer at concentrations ranging from 0.1-0.5 mg protein/tube. Binding was performed in polypropylene microcentrifuge tubes (1.5 ml capacity; 1 ml final vol.) using 100 nM ^{3}H-L-Tyr as ligand and 1 mM L-Tyr to define nonspecific binding. Tubes were centrifuged (16,000 x g; 10 min) to separate bound and free ligand. The supernatants were aspirated; then pellets were rapidly washed and solubilized with protosol for quantification of radioactivity. The specific binding of ^{3}H-L-Tyr was saturable and Scatchard analysis (LIGAND) of the data indicated two populations of binding sites with dissociation constants of high (K_{DH}=3.2±1.4 μM) and low (K_{DL}=252±150 μM) affinities; and density of binding sites B_{Hmax}=598±390 pmol/mg tissue and B_{Lmax}=15,861±12,157 pmol/mg tissue, respectively (N=4). Equilibrium binding of ^{3}H-L-Tyr at 4°C was rapid (maximum: 60-90 min); dissociation following addition of 1 mM L-Tyr was rapid, and complete within 10 min. Heat denaturation (100°C for 5 min) abolished specific binding. Specific binding represented 40-60% of the total binding and was highly dependent on pH (optimum: 7.2-7.5), as well as total protein concentration. Importantly, specific binding of ^{3}H-L-Tyr was stereoselective, since D-Tyr was without effect using concentrations up to 1 mM (122% of control; $p > 0.05$, N=5). Catecholamine and amino acid neurotransmitters such as dopamine, norepinephrine, glycine and GABA did not significantly affect specific ^{3}H-L-Tyr binding (up to 10^{-4}M; $p > 0.05$, N=4-5). A specific inhibitor of Tyr hydroxylase, alpha-methyl-p-tyrosine, was also without effect ($p > 0.05$, N=3). CONCLUSION: These data suggest that specific, low affinity, high capacity, saturable, reversible, stereoselective binding sites for L-Tyr exist on brain (P_2) membranes which are not associated with Tyr hydroxylase. (Supported by SIU School of Medicine).

249.16 BINDING OF [^{3}H]DIHYDROALPRENOLOL TO BETA-ADRENERGIC RECEPTORS IN BRAIN: DIFFERENCES IN BINDING ACCORDING TO CHOICE OF LIGAND FOR MEASUREMENT OF NONSPECIFIC BINDING. S. Dhawan and K.J. Kellar, Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

Binding to beta adrenergic receptors in brain have been measured using several different radioactive ligands. One of the most frequently used ligands is [^{3}H]dihydroalprenolol ([^{3}H]DHA). Typically, specific binding of [^{3}H]DHA to beta receptors is defined as the difference between binding in the absence and presence of a high concentration (1-20 uM) of the beta adrenergic receptor antagonist propranolol. This definition of specific binding relies heavily on the presumed selectivity of [^{3}H]DHA and propranolol for beta-adrenergic receptors; however, in fact, the selectivity of these and other beta antagonists has been questioned (Middlemiss et al., Nature 267:289, 1977; Pazos et al., Brain Res. 343:403, 1985; Hoyer et al., Eur. J. Pharmacol. 118:1, 1985). We have compared the binding of [^{3}H]DHA in membranes from rat cerebral cortex and hippocampus using either 1 uM d,l-propranolol, or 40-200 uM l-isoproterenol to define nonspecific binding. In addition, we measured binding of [^{3}H]DHA in the presence or absence of 1 uM serotonin (5-HT).

When propranolol was used to define specific binding, saturation isotherms of [^{3}H]DHA binding were consistent with a 2-site model in both brain areas. The high affinity component had a K_d of 1-3 nM in both areas and represented 50-70% of the sites in the cortex and 30-50% of the sites in the hippocampus. The low affinity component had a K_d of approximately 10-20 nM in both areas. In contrast, when isoproterenol was used to define specific binding of [^{3}H]DHA to beta receptors the data were consistent with a 1-site model with a K_d of 1-2 nM in the cortex and 2-4 nM in the hippocampus. When 1 uM 5-HT was included in the assay, specific binding of [^{3}H]DHA appeared to fit a 1-site even when propranolol was used to define nonspecific binding.

These results indicate that when propranolol is used in the definition of specific binding of [^{3}H]DHA to beta-adrenergic receptors in some brain tissues, a second component of apparent specific binding exists, part of which appears to consist of a serotonin receptor. And further, isoproterenol (40-200 uM) appears to provide a safer measurement of nonspecific binding than does binding in the presence of propanolol.

249.17 EVIDENCE THAT THE REGULATION OF PLASMA TYROSINE AND BRAIN TRYPTOPHAN CONCENTRATIONS BY ANTIDEPRESSANTS IS MEDIATED BY β-ADRENOCEPTORS. D.J. Edwards and D.A. Sorisio*. Dept. of Pharmacology-Physiology, Univ. of Pittsburgh Sch. of Dent. Med., Pittsburgh, PA 15261.

Previous studies have indicated that the antidepressant drug desipramine lowers brain and plasma levels of tyrosine (Tagliamonte et al., J.P.E.T. 177, 475 (1971)). Since agonists and antagonists of peripheral adrenoceptors are known to influence plasma tyrosine and tryptophan levels (Eriksson and Carlsson, Life Sci. 30, 1465 (1982)), we undertook studies to inquire whether effects of antidepressants on these aromatic amino acids also are mediated by α- or β-adrenergic receptors. In the first experiment, we found that 1.25 mg/kg i.p. of norepinephrine (NE) caused a 72% rise in plasma tyrosine after 40 min. This effect was totally prevented by an injection 50 min before NE of the α-blocker phenoxybenzamine (10 mg/kg) but not by imipramine (IMI; 20 mg/kg). Brain tyrosine concentrations were unaffected by both phenoxybenzamine and NE administration, although the latter blocked the ability of IMI to lower brain tyrosine. These data argue against IMI's α-blocking property as being responsible for alterations in brain and plasma tyrosine levels. A second experiment was conducted to test the possibility that IMI's effects on tyrosine or tryptophan are due to stimulation of β-adrenoceptors. Rats were injected with either saline, the β-blocker propranolol (15 mg/kg) or phenoxybenzamine. Twenty min later and 90 min before sacrifice, the rats received an injection of either IMI or saline. Whereas IMI alone reduced plasma tyrosine to 75% of control, this effect was prevented by propranolol but not by phenoxybenzamine pretreatement. On the other hand, both phenoxybenzamine and propranolol failed to block the decrease in plasma tryptophan induced by IMI. In contrast, IMI caused an elevation in brain tryptophan that was completely abolished by pretreatment with propranolol but not with phenoxybenzamine. In another experiment, we injected rats with salbutamol (10 mg/kg), a β_2 agonist that can enter the brain. This drug decreased plasma tyrosine and raised brain tryptophan concentrations. In conclusion, our results suggest that the ability of IMI to lower plasma tyrosine and raise brain tryptophan is mediated by the stimulation of β-adrenoceptors. Supported by NIMH grant #MH 28340.

249PO ROLE OF CORTICOSTERONE IN STRESS-INDUCED DESENSITIZATION OF CORTICAL ALPHA-1 RECEPTORS. E.A. Stone, B.M. McEwen and A.S. Herrera*, Dept. Psychiatry, New York Univ. Sch. Med., New York, NY 10016 and Lab. Neuroendocrinology, The Rockefeller Univ., New York, NY 10021.

Repeated stress is known to reduce the cAMP response to catecholamines in rat brain slices. This effect has been shown to be due to a desensitization of alpha-1 receptors that modulate the cAMP response to beta adrenergic and other receptor stimulation. The physiological factor that mediates this desensitization is not presently established. There is evidence however that corticosterone may be involved since the latter hormone is secreted during stress and has been shown to produce a similar alpha-1 receptor desensitization when administered exogenously. In the present study therefore we have tested the role of corticosterone by examining the effect of adrenalectomy (ADX) on the ability of stress to desensitize these receptors. We have also investigated the actions of acute administration of exogenous corticosterone to determine if the hormone produces an acute sensitization effect on these receptors.

Rats were subjected to bilateral ADX or sham operation. The ADX rats were maintained on a mixture of 0.9% saline and a low dose of corticosterone (10 μg/ml) in their drinking water. Plasma levels of corticosterone in tail vein blood were assayed by RIA. Repeated stress was administered by restraining the rats for 2 h/d for 7-9 days. The ADX rats were found to tolerate the stress well with no fatalities or apparent ill-effects on their general condition. Corticosterone was administered subcutaneously in sesame oil at 50 mg/kg. The cAMP response to beta and alpha adrenergic receptor stimulation was assayed in cerebral cortical slices by previously published methods using norepinephrine in combination with timolol or phentolamine to activate the beta and alpha responses.

Preliminary results indicate that chronic stress reduces the functional response to alpha stimulation (potentiation of the beta cAMP response) in the sham operated but not ADX animals. These findings therefore support a role of corticosterone in the stress-induced desensitization of central alpha receptors. Preliminary results also indicate that acute administration of corticosterone increases the magnitude of the alpha response suggesting that the desensitization to chronic hormone treatment may be linked to an initial sensitization. (Supported in part by grant MH22768).

249PO FUNCTIONAL EVIDENCE FOR DISTINCT ALPHA-2 ADRENERGIC RECEPTOR SUBTYPES IN THE NON-HUMAN PRIMATE A.F.T. Arnsten, J.X.Cai and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale Medical School, 333 Cedar St., New Haven, Ct 06510.

The present study defines more precisely the alpha-2 receptor mechanisms underlying clonidine's ability to improve memory in aged monkeys (Science 230: 1273, 1985). We wished to determine whether clonidine's beneficial mnemonic effects might result from stimulation of one or both of two newly proposed alpha-2 receptor subtypes (Boyajian et al. Soc. Neuro. Abstracts, 1043, 1985), one labeled by the alpha-2 antagonist, idazoxan (IDA), and the other by both rauwolscine (RAU) and IDA. This was accomplished by assessing the hypotensive, sedative and memory-enhancing properties of alpha-2 agonists with widely varying affinities for the proposed receptor subtypes.

Drug responsivity was tested in 10 aged monkeys that had previously been shown to exhibit improved memory after the administration of the alpha-2 agonist, clonidine (Science 230:1273,1985). Memory was measured using the delayed response task as previously described (ibid); sedation was scored by a 5 point rating scale. A subgroup of aged monkeys was adapted to chair restraint for blood pressure monitoring.

Two agonists with opposing affinities for the proposed receptor subtypes were examined: BHT-920, which resembles clonidine in having a higher affinity for the RAU-labeled site, and guanfacine, which has more than 14 times higher affinity for the IDA-labeled site (Boyajian et al., 1985). The BHT-920 response profile was similar to that described for clonidine. Low doses substantially decreased systolic blood pressure while higher doses produced marked improvements in memory; at the highest doses tested sedative effects interfered with performance. A completely opposite profile was seen with guanfacine: memory was improved by the lowest doses tested, while blood pressure was lowered in the higher dose range. Little sedation was seen in this dose range. The finding that the behaviorial and cardiovascular effects of alpha-2 agonists can be dissociated according to their affinity for the proposed subtypes supports the the existance of two distinct types of alpha-2 receptors: an IDA-labeled receptor subserving memory enhancement, and a RAU-labeled site involved with blood pressure regulation. The ability of guanfacine to improve memory at doses that have little hypotensive or sedative effects recommends the agonist as a superior candidate for treating age-related memory disorders.

249PO STUDY OF ONTOGENY OF RECEPTORS FOR CATECHOLAMINES AND OTHER NEUROTRANSMITTERS IN CHICK EMBRYO. Stephen Zamenhof. Dept. of Microbiology & Immunology, and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The present work is concerned with a method for the study in vivo of the time of appearance of receptors (or receptors-effectors) for catecholamines and other neurotransmitters, in chick embryo. The substances studied were norepinephrine, dopamine, epinephrine and its antagonist propranolol, carbachol and GABA. They were singly introduced into eggs (albumen) in doses 0.1 to 0.5 μM per egg. Serving as solvent (and as control) was 0.05 ml of Ringer solution for chicks. Solvent alone and GABA introduced at any time (before 13 days of incubation [E13]) did not produce any mortality, suggesting that the procedures used were in themselves non-lethal. In contrast, norepinephrine, dopamine, epinephrine, propranolol and carbachol did not produce any mortality only if introduced before day 8 (E8), but produced sharply increased mortality (60-100%) when introduced on days E8 to E13, with maxima on days E9 to E11, depending on substance used. At the same concentration, dopamine was less effective than norepinephrine. These results are interpreted as indicating the appearance of receptors (or receptors-effectors) for these substances around day E8-E9. In general, these ages agree with single data reported in the literature and obtained by much more laborious in vitro studies. The above natural substances (except propranolol) became lethal to the embryo because they are in excess of the amounts produced and needed at that time by the embryo for optimal development. The receptors themselves may be produced in excess of those needed for reacting with natural amounts of ligands at that time; thus, receptors can accomodate this extraneous excess of neurotransmitters, with lethal effect. Extraneous propranolol gives here the same result as extraneous epinephrine because it lethally blocks the receptors for epinephrine naturally produced by the embryo (same receptors and same time of their appearance). The organ(s) affected which ultimately resulted in lethality is unknown. For those embryos which survived, the weights of embryos, cerebral hemispheres, optic lobes and cerebella did not differ significantly from the controls.

CYCLIC NUCLEOTIDES I

250.1 DIFFERENTIAL EFFECTS OF BENZYL ALCOHOL ON ADENYLATE CYCLASE ACTIVITY IN RAT STRIATAL AND CEREBELLAR MEMBRANES. A. Malnoë (SPON: K. Ornstein). Nestle Research Centre, Nestec Ltd., Vers-chez-les-Blanc; CH 1000 Lausanne 26 (Switzerland).

The lipid fluidizing agent benzyl alcohol (BA) was used to probe the effect of membrane fluidity on adenylate cyclase (AC) activity in synaptosomal membranes from rat striatum and cerebellum. AC activities in these two regions differ in their sensitivity to forskolin (FOR) (40-fold activation in the striatum vs 8-fold in the cerebellum) as well as to Ca^{2+}.calmodulin (2-fold activation in striatum vs 4-fold in cerebellum). Moreover, 5'-guanylyl imidodiphosphate (Gpp(NH)p) is stimulatory in the striatum whereas it has a biphasic effect in the cerebellum.

BA (up to 50 mM) increased basal AC activity in both regions followed by inhibition at 70 mM. Stimulation of AC activity by Gpp(NH)p (10 μM) was strongly enhanced in a dose-dependent fashion by BA in the striatum but not in the cerebellum. In addition, BA increased the stimulatory response to dopamine in the striatum but had no effect on the response to isoproterenol in the cerebellum. The stimulatory effect of BA on the Gpp(NH)p-dependent AC activity was lost upon solubilization of the striatal enzyme in Lubrol PX suggesting that this effect is linked to membrane fluidity. The Ca^{2+}.calmodulin-dependent activation of AC was inhibited at high concentrations of BA (70 mM) in both striatum and cerebellum.

BA was a potent inhibitor of AC activation by FOR in both regions. 50 mM BA decreased the FOR response in the striatum and cerebellum by 50% and 70%, respectively. BA decreased the Vmax with no apparent effect on the affinity of FOR for AC in the cerebellum. The inhibitory effect was also present with the Lubrol-solubilized enzyme indicating that BA is not acting solely through changes in membrane fluidity but that it may also act directly on the enzyme possibly by competing for sites previously occupied by lipids or detergent.

The results demonstrate that striatal and cerebellar AC activities are modulated differently by the lipid fluidizing agent BA suggesting there are differences in the structural organization and/or lipid environment of the enzyme in these two regions. The data further suggest that protein/lipid interactions are involved in the mode of activation of AC by FOR.

250.2 EFFECTS OF DIMETHYLSULPHOXIDE ON CYCLIC AMP PRODUCTION AND INOSITOL PHOSPHOLIPID BREAKDOWN IN GUINEA PIG BRAIN SLICES. D.A. Kendall* and S.J. Hill* (SPON: European Neuroscience Association). Dept. of Physiol. & Pharmacol., Univ of Nottm. Med. Sch., Nottingham NG7 2UH, U.K.

Dimethyl sulphoxide (DMSO) is widely used in biomedical research as a relatively inert vehicle for water insoluble compounds. We have recently used DMSO as a solvent for a number of adenosine receptor antagonists in studies of the effects of adenosine on cyclic AMP (cAMP) production and inositol phospholipid hydrolysis in brain slices. We now report some complex effects of DMSO on second messenger formation which might influence its selection as a suitable solvent for neurochemical procedures.

Inositol phospholipid hydrolysis was monitored by meaurement of total ^{3}H-inositol phosphates (^{3}H-IP) accumulation in the presence of LiCl in brain slices prelabelled with ^{3}H-inositol (Brown et al, J.Neurochem. 42:1379, 1984). ^{3}H-cAMP formation was measured using the ^{3}H-adenine prelabelling method of Shimizu et al (J.Neurochem. 16:1609, 1969).

In guinea-pig cerebral cortical slices adenosine, despite having no effect alone, selectively enhanced the accumulation of ^{3}H-IP due to histamine. DMSO had no effect on the response to histamine alone but it antagonized the potentiation due to adenosine analogues in a concentration dependent manner. The dose response curve to 2-chloroadenosine (2-CA) was shifted 5-fold to the right in the presence of 1.5% DMSO and the potentiation was abolished by 6%. DMSO caused a small increase in ^{3}H-cAMP formation which was possibly due to adenosine release since it was abolished after incubation of slices with adenosine deaminase. The cAMP dose response curve for 2-CA was shifted 3-fold to the right in the presence of 6% DMSO. In contrast cAMP formation due to histamine was enhanced 2.5-fold by 6% DMSO. This effect was on the maximum response to histamine with no change in the EC_{50} value. The effect of DMSO was apparently on the histamine H_2 receptor since the reduced response to histamine seen in the presence of the H_1 receptor antagonist mepyramine was still enhanced by DMSO. These effects on cAMP formation were not specific for histamine or adenosine responses since 6% DMSO enhanced the effect of a depolarizing concentration of KCl (31 mM) by 5.9-fold and reduced that of forskolin (100 μM) by 76%.

Although the mechanism of action of DMSO is, as yet, unclear these data suggest caution in using this agent as an 'inert' vehicle.

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250.3 CALMODULIN ACTIVATION OF ANTERIOR PITUITARY ADENYLATE CYCLASE. S. Okada* and D.V. Greenlee. Dept. of Zoological and Biomedical Sciences and the College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

Rat anterior pituitary membranes were depleted of endogenous calmodulin (crude membranes = 1.22 ug CaM/mg protein; washed membranes = 0.33 ug CaM/mg protein) by washing with EGTA-containing buffers in order to observe calmodulin activation of pituitary adenylate cyclase. Calmodulin (CaM) was assayed using the soluble adenylate cyclase from Bordetella pertussis. The distribution of CaM in the extract was 80% cytosolic/ 20% particulate, which differed from the equal distribution previously reported for CaM in GH_3 pituitary tumor cell membranes. Activation of pituitary adenylate cyclase by CaM in the presence of 200 uM EGTA was Ca^{2+} dependent and occurred in a biphasic manner, with activation at lower and inhibition at higher Ca^{2+} concentrations. Although some activation was observed at 0.5-5 uM calculated free Ca^{2+}, maximal activation by 7 uM CaM occurred at 225 uM added $CaCl_2$, suggesting that maximal activation by micromolar CaM levels requires a high concentration of free Ca^{2+}. In the presence of CaM, Mn^{2+} activated adenylate cyclase at lower concentrations than required in the absence of CaM, indicating that Mn^{2+} could substitute for Ca^{2+} in elliciting CaM activation of adenylate cyclase. In the presence of 200 uM EGTA and 225 uM $CaCl_2$, calmodulin activated adenylate cyclase in a dose-dependent manner up to 25 uM, with "apparent" inhibition occurring at higher CaM concentrations. In contrast to brain adenylate cyclase, micromolar levels of CaM were required to activate pituitary adenylate cyclase, while minimal stimulation occurred at 100 nM calmodulin. The apparent inhibition by higher CaM levels was due to insufficient Ca^{2+}, since addition of optimal Ca^{2+} resulted in further stimulation of the enzyme. Using 2 uM CaM, calmidazolium inhibited CaM stimulation of adenylate cyclase with an $IC_{50}^{!}$ = 4 uM. Higher calmidazolium concentrations were required to inhibit stimulation by 5 uM and 10 uM CaM. These data indicate that (1) anterior pituitary adenylate cyclase is at least 50 fold less sensitive to CaM than is brain adenylate cyclase, (2) anterior pituitary membranes appear to have a lower affinity for calmodulin than GH_3 cell membranes, and (3) CaM can activate pituitary adenylate cyclase in a specific manner, although high Ca^{2+} concentrations are required for maximal activation.

250.4 PHOSPHOLIPASE A2 INHIBITORS ATTENUATE THE MUSCARINIC INHIBITION OF CYCLIC AMP ACCUMULATION IN N18TG2 NEUROBLASTOMA CELLS. K.A. O'Donnell* and A.C. Howlett. Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

Muscarinic agonists such as carbachol decreased secretin-stimulated cyclic AMP accumulation in intact N18TG2 neuroblastoma cells by 70% to 90%. However, adenylate cyclase in plasma membranes derived from these cells was inhibited only 20% to 30% by muscarinic agonists. This observation led to speculation that multiple effector mechanisms may operate in intact cells to decrease cyclic AMP accumulation in response to muscarinic receptor stimulation. This study tested the hypothesis that arachidonic acid release via phospholipase A2 may be involved in muscarinic regulation of cyclic AMP accumulation in neuroblastoma cells.

Bromophenacylbromide (BPB) and quinacrine were used as two chemically unrelated phospholipase A2 inhibitors. Both BPB and quinacrine attenuated the inhibition of cyclic AMP production by the muscarinic agonist carbachol. Neither compound had any effect on the basal cell content of cyclic AMP or on the stimulation of cyclic AMP production by secretin. The effects of both phospholipase A2 inhibitors were dose-dependent over a range of 1 µM to 100 µM. It was necessary that the phospholipase A2 inhibitor be present during the incubation in order to attenuate the muscarinic inhibition of cyclic AMP production. Preincubation of the cells with either BPB or quinacrine followed by washing the cells and testing for muscarinic inhibition of cyclic AMP accumulation yielded results that were not different from controls. The diglyceride lipase inhibitor, RHC-80267, which does not alter phospholipase A2 or C activities, had no effect on muscarinic inhibition of cyclic AMP accumulation. In order to see if protein kinase C could modify the response, cells were incubated with an active phorbol ester, phorbol 12-myristate 13-acetate (PMA) for 20 min prior to determination of cyclic AMP accumulation. PMA had no effect on basal, secretin-stimulation or muscarinic-inhibited cyclic AMP accumulation. Furthermore, PMA did not alter the ability of quinacrine to diminish the muscarinic inhibition of cyclic AMP accumulation. In summary, the activity of phospholipase A2 may be intrinsic to the muscarinic response in neuroblastoma cells.

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250.5 SYNAPTIC MEMBRANE TUBULIN MAY INHIBIT ADENYLATE CYCLASE VIA THE EXCHANGE OF GUANOSINE TRIPHOSPHATE ANALOGS AMONG GTP BINDING PROTEINS. M.M. Rasenick, C.A. Moore* and N. Wang*. Department of Physiology and Biophysics and the Committee on Neuroscience, University of Illinois College of Medicine. Chicago, IL 60680.

GTP-binding proteins which mediate neuronal signal transduction have considerable structural and functional homology. In addition to the binding and hydrolysis of GTP, these proteins interact with cell surface receptor molecules and/or enzymes which generate cellular second messengers. This laboratory has demonstrated, recently (Proc. Nat. Acad. Sci., USA 83: 5439-5443, 1986), that the GTP binding proteins (GN) which inhibit (GNi) or stimulate (GNs) rat cerebral cortex adenylate cyclase, appear to interact directly in the exchange of nucleotide. Such observations raise the possibility that GN proteins couple not only with receptor and effector proteins, but with other GN proteins as well. Microtubules have also been implicated as modulators of the adenylate cyclase system and tubulin, the primary constituent of microtubules, is a GTP-binding protein with structural similarities to other GN proteins. Tubulin binds two moles of GTP/mol dimer and one of the GTP binding sites is exchangeable. Certain hydrolysis-resistant GTP analogs support the polymerization of tubulin, and in doing so, occupy the exchangeable GTP-binding site. Incubation of rat cerebral cortex synaptic membranes with tubulin dimers which have GppNHp bound (tubulin-GppNHp) followed by washing of those membranes results in a stable inhibition of adenylate cyclase. Under identical conditions, incubation of membranes with a GTP binding protein from the rod outer segment (transducin), with GppNHp bound, did not cause inhibition of adenylate cyclase. Tubulin (regardless of the nucleotide bound to it) did not alter the activity of the adenylate cyclase catalytic unit directly. When tubulin was polymerized with the hydrolysis-resistant photoaffinity GTP analog, (^{32}P) AAGTP, AAGTP-tubulin, added to synaptic membranes under conditions which resulted in persistent inhibition of adenylate cyclase, appeared capable of transferring nucleotide to the inhibitory GTP-binding protein, GNi. Transfer of AAGTP from tubulin to GNi, was blocked by prior incubation of the membranes with GppNHp or U.V. irradiation of tubulin-AAGTP (inducing covalent binding of AAGTP to tubulin) prior to exposure of tubulin-AAGTP to membranes. Incubation of membranes with GppNHp subsequent to incubation with AAGTP-tubulin results in a decrease in AAGTP bound to GNi and a compensatory increase in AAGTP bound to the stimulatory GTP-binding protein, GNs. This parallels the reversal of persistent inhibition of adenylate cyclase by addition of GppNHp to the assay. Whereas, GppNHp promotes persistent inhibition of synaptic membrane adenylate cyclase after incubation on ice, tubulin (with AAGTP or GppNHp bound) requires 30 seconds incubation at 23°C to effect adenylate cyclase inhibition. Photoaffinity experiments yield parallel results. These data are consistent with synaptic membrane tubulin regulating neuronal adenylate cyclase by transferring nucleotide directly to GNi and, subsequently, to GNs.

250.6 GDPβS PARTIALLY ACTIVATES ADENYLATE CYCLASE IN NEURAL CELLS. N. Wang*, J.M. Hughes* and M.M. Rasenick (spon. C.L. Melchior). Department of Physiology and Biophysics and the Committee on Neuroscience. University of Illinois College of Medicine, Chicago, IL 60680.

The stable GDP analog, Guanosine 5'O-(2 thiodiphosphate) [GDPβS] has been used for some time as an inhibitor of GTP-dependent processes. GDPβS has been thought to inactivate the GTP-binding proteins [GN], which stimulate [GNs] or inhibit [GNi] adenylate cyclase [AC] and mediate other cellular processes such as ion channels [GNi, GNo, GNk] and phospholipid metabolism [GN?]. Pressure injection or iontophoresis of GDPβS has been utilized by several investigators, often with the assumption that GDPβS interfered with the AC system by preventing AC activation through GNs. Whereas GDPβS is capable of blocking hormone or neurotransmitter-induced AC stimulation (or inhibition); hormone independent effects of this compound have not been studied in neural tissue. We find that GDPβS, when incubated with rat cerebral cortex synaptic membranes, activates adenylate cyclase with an apparent EC50 of 2.4×10^{-7}M (compared to 1.9×10^{-8}M for GTPγS or 3.6×10^{-7}M for GppNHp) but with a Vmax only 50% of the latter compounds. GDPβS displays "partial agonist" kinetics in the activation of adenylate cyclase but is ineffective in the GTP-dependent inhibition of that enzyme, and is capable of mitiating that process by 25-30%.

Whereas, hydrolysis-resistant GTP analogs reverse a persistent AC inhibition (induced by the GTP a photoaffinity analog, azidoanilido GTP [AAGTP]), GDPβS is ineffective in this process or the concomitant nucleotide exchange (Proc. Nat. Acad. Sci. USA 83:5439, 1986) reaction. Thin layer chromatography reveals a minor amount of GTPβS (<5%) contamination, however, this compound is not likely to account for these results as GTP neither activates nor inhibits AC in this preparation.

Permeabilized C6 glioma cells provide a paradigm for GDPβS pressure injection studies in that they provide a method for measuring AC in cells which are largely intact (FEBS Lett. 207: 296, 1986). Unlike the cerebral cortex synaptic membrane preparation, permeabilized C6 cells display AC which is dependent upon β adrenergic agonists for AC activation. GDPβS blocks isoproterenol (IPR) induced AC stimulation, whereas GppNHp or GTPγS promote and accentuate that process. In the absence of IPR, GDPβS elevates permeabilized C6 AC by about 50% at 10^{-4}M. Similar results are obtained in pertussis-toxin treated cells, indicating that GDPβS effects may be independent of GNi, despite the ability of both GN and GNs to bind that nucleotide (see abstract by J.S. Gordon et.al.). We conclude that high doses of GDPβS result in a receptor-independent activation of GNs and that GDPβS may be partial agonist for the GNs protein in certain neural cells and tissues.

250.7 SODIUM FLUORIDE INDUCES CYCLIC GMP FORMATION IN MOUSE NEUROBLASTOMA N1E-115 CELLS. W.S. Lai and E.E. El-Fakahany. Department of Pharmacology and Toxicology, Univ. of Maryland School of Pharmacy, Baltimore, MD 21201.

Sodium fluoride (NaF) has been shown to induce phosphoinositide hydrolysis in a variety of cells (Blackmore et al., J. Biol. Chem. 260: 14477, 1985; Taylor et al., Biochem. Biophys. Res. Commun. 136: 362, 1986; Guillon et al., FEBS Lett. 204: 183, 1986; Paris and Pouyssegur, J. Biol. Chem. 262: 1970, 1987) probably by activating phospholipase C. When mouse neuroblastoma cells (clone N1E-115) were incubated with 20 mM NaF for 5 or 10 min at 37°C in the presence of 10 mM LiCl, there was a significant increase in the accumulation of inositol phosphates. This response was significantly higher than that induced by the muscarinic receptor agonist carbamylcholine (1 mM for 30 min). Many of the calcium-mobilizing receptors that induce increases in phosphoinositide hydrolysis in a variety of tissues elevate the cellular level of cyclic GMP as well. This has also been demonstrated in mouse neuroblastoma N1E-115 cells (Kanba and Richelson, Biochem. Pharmacol. 36: 869, 1987). Therefore, the hydrolysis of phosphoinositides and the formation of cyclic GMP may be interrelated (Berridge, Biochem. J. 220: 345, 1984). Thus, it was interesting to test the effects of NaF on cyclic GMP formation in N1E-115 cells. NaF (5-20 mM) induced cyclic GMP formation in intact N1E-115 cells in both a time- and a concentration-dependent manner. The time-to-peak response was inversely proportional to the concentration of NaF. Again, this cyclic GMP response to 20 mM NaF was much more pronounced than the response elicited by 1 mM carbamylcholine. Such increases in NaF-induced cyclic GMP production were not significantly blocked by the muscarinic receptor antagonist atropine or the histamine H_1 receptor blocker pyrilamine at concentrations that totally inhibited carbamylcholine-and histamine-induced cyclic GMP synthesis in these cells, suggesting that the effect of NaF on cyclic GMP formation was not due to the activation of muscarinic receptors or histamine receptors. Although NaF induced cyclic GMP accumulation in intact N1E-115 cells, it inhibited guanylate cyclase activity in cell homogenates. Phorbol 12-myristate 13-acetate (PMA) has been shown to inhibit carbamylcholine- and histamine-mediated cyclic GMP formation in these cells. However, preincubation of cells with 100 nM PMA for 60 min at 37°C did not inhibit NaF-mediated cyclic GMP synthesis. Thus, our present data demonstrate a novel effect of NaF in inducing cyclic GMP synthesis in neuronal tissue.

250.8 INCREASES IN PHOSPHOINOSITIDE HYDROLYSIS, INTRACELLULAR CALCIUM AND CYCLIC GMP FORMATION BY PHOSPHOLIPASE C. W. Surichamorn, L. Noronha-Blob* and E.E. El-Fakahany. Dept. of Pharmacology and Toxicology, Univ. of Maryland School of Pharmacy, Baltimore, MD 21201 and NOVA Pharmaceutical Corp., Baltimore, MD. 21224.

Activation of some neurotransmitter receptors in mouse neuroblastoma cells (clone N1E-115) results in an increase in phosphoinositide (PI) hydrolysis and a transient elevation in cyclic GMP (cGMP) formation (Kanba and Richelson, Biochem. Pharmacol. 36, 869, 1987). Since the breakdown of PI in several tissues has been demonstrated to be through a specific PI phosphodiesterase (Phospholipase C; PLC) and thereby causes the release of diacylglycerol (DAG) and inositol trisphosphate which act as intracellular second messengers, we decided to investigate the effects of exogenous PLC in mouse neuroblastoma N1E-115 cells. These cells were treated with PLC from B. Cereus and [^{3}H]cGMP and [^{3}H]inositol phosphates ([^{3}H]IP) were determined. PLC increased the accumulation of the total [^{3}H]IP, determined in the presence of 10 mM LiCl, in a time- and a concentration-dependent manner. PLC (5 U/ml) caused a linear increase in the accumulation of [^{3}H]IP up to 15 min . At 10 min, PLC in concentrations of 5, 10, 20, and 30 U/ml stimulated the release of [^{3}H]IP by 5, 7, 9, and 12 fold compared to basal, respectively. This response was completely blocked by neomycin (10 mM). PLC also increased cGMP levels in these cells. The stimulation of [^{3}H]cGMP formation by PLC (5 U/ml) showed a peak effect at 1-3 min which was maintained up to 10 min, then declined gradually. At 5 min, PLC stimulated the [^{3}H]cGMP synthesis with an EC_{50} of 5 U/ml and a maximum effect at 20 U/ml. Also, neomycin (10 mM) completely inhibited this response. At 5, 10 and 20 U/ml, PLC did not directly stimulate guanylate cyclase. It has been suggested that arachidonic acid release might be linked to cGMP formation (Snider et al., Proc. Natl. Acad. Sci. USA. 81, 3905, 1984). The cGMP response to 5 U/ml PLC was inhibited by 45% by the phospholipase A_2 inhibitor quinacrine (300 μM). The lipoxygenase inhibitors eicosatetrayonic acid (50 μM) and nordihydroguaiaretic acid (50 μM) also attenuated the response by 40% and 14%, respectively, whereas the DAG lipase inhibitor monooleoyl glycerol (200 μM) antagonized the response only slightly. In addition, we studied the effects of PLC on Ca^{2+} mobilization using quin-2 fluorescence. PLC (0.01-1.0 U/ml) caused a rapid increase in intracellular Ca^{2+}, while higher concentrations (1.0-20 U/ml) induced a more marked increase. In summary, our findings indicate that exogenous PLC increases PI hydrolysis, intracellular Ca^{2+} and cGMP formation in mouse neuroblastoma cells. Although the latter response is not mediated by a direct activation of guanylate cyclase, it might involve, only in part, the release of arachidonic acid.

250.9 REGULATION BY cAMP AND VASOACTIVE INTESTINAL PEPTIDE OF THE PHOSPHORYLATION OF THREE LOW MOLECULAR WEIGHT PROTEINS IN STRIATAL CELLS IN CULTURE I.Shalaby, J.-A.Girault*, N.Rosen and P.Greengard, The Rockefeller University New York, NY 10021

Three proteins that are substrates for cAMP-dependent protein kinase have recently been purified from bovine striatum. These proteins have Mr values of 16 kDa, 19 kDa (accompanying abstract by Horiuchi et al.) and 21 kDa (Hemmings et al., Soc. for Neurosci. Abst. 12, 281.10). The 16 kDa and 21 kDa proteins are particularly enriched in the striatum whereas the 19 kDa protein is present in all brain regions as well as in certain peripheral organs. These three proteins have been referred to as ARRP-16, ARPP-19 and ARPP-21 for cAMP regulated phosphoproteins with Mr values of 16, 19 and 21 kDa respectively (accompanying abstract by Girault et al.). We have now investigated the effects of cAMP and of agents known to raise intracellular cAMP concentration, on the state of phosphorylation of these 3 proteins in cultured cells from mouse striatum. Reaggregate cultures were prepared from striata of fetal mice at embryonic day 14. Pharmacological studies were performed after 3 weeks of culture. The agents tested were added to the incubation medium for 10 min. The proteins were extracted and phosphorylated with ^{32}P-ATP in the presence of cAMP-dependent protein kinase catalytic subunit. The 3 proteins studied were immunoprecipitated with specific antibodies, separated by SDS-PAGE and the radioactivity incorporated in each protein was counted. Under these conditions of "back-phosphorylation" an increase in the level of phosphorylation in vivo is detected as a decrease in the incorporation of radioactive phosphate into the protein in vitro.

Addition of 8 bromo-cAMP (4mM) increased the phosphorylation of the 3 proteins (decrease in the dephosphorylated form : 64±4% for ARPP-21, 40±6% for ARPP-19, 26±6% for ARPP-16). Forskolin, a potent activator of adenylate cyclase, induced a dose-dependent increase in the phosphorylation of the 3 proteins (maximal decrease in the dephosphorylated form 70% with an apparent EC50 of 0.5-1 micromolar). Vasoactive intestinal peptide (VIP), which is reported to stimulate adenylate cyclase in striatal cells in culture, increased the phosphorylation of the 3 proteins (maximal decrease in the dephosphorylated forms of 40% for ARPP-19 and ARPP-21). Effects of VIP on ARPP-19 and ARPP-21 were observed at concentrations as low as 10-100 pM. In conclusion we have observed that the phosphorylation state of ARPP-16, ARPP-19 and ARPP-21 is regulated by cAMP in cultured striatal cells. In addition, VIP stimulates the phosphorylation of these 3 proteins suggesting that they may play a role in the physiological actions of this neuropeptide in the striatum.

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250.10 BASAL GANGLIA-ENRICHED LOW MOLECULAR WEIGHT PHOSPHOPROTEINS : REGULATION BY cAMP AND REGIONAL DISTRIBUTION IN THE RAT BRAIN J.-A.Girault*, A.Horiuchi*, H.C.Hemmings Jr., A.C.Nairn, and P.Greengard, Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021

Three protein substrates for cAMP-dependent protein kinase, with Mr values of 16kDa, 19kDa and 21kDa respectively, have recently been purified from bovine striatum and antibodies have been prepared against them. The 16 kDa and 19 kDa proteins show a high degree of immunological cross-reactivity suggesting a high degree of structural homology (see accompanying abstract by Horiuchi et al.). In the present study we have investigated the in vivo regulation of the phosphorylation of these proteins by cAMP in striatal slices and their distribution in the rat brain.

Slices from rat striatum were preincubated for 120 min. and the compounds studied were added to the incubation medium for 10 min. The proteins were then extracted, phosphorylated with ^{32}P-ATP in the presence of cAMP-dependent protein kinase catalytic subunit, immunoprecipitated with the specific antibodies, separated by SDS-PAGE and the radioactivity incorporated in each protein of interest was counted. 8 bromo-cAMP (4mM) stimulated the phosphorylation of all 3 proteins (the decrease in the dephosphorylated form was : 50±5 % for the 21 kDa protein, 48±5 % for the 19 kDa and 29±6 % for the 16 kDa). Forskolin, a compound that activates adenylate cyclase, induced, in the presence of 0.1mM IBMX, a dose-dependent stimulation of the phosphorylation of all 3 proteins with an apparent EC 50 around 1 micromolar.

The amount of the proteins of interest in each region was determined by phosphorylation/immunoprecipitation as described above and by immunoblot. Both methods gave similar results. The highest concentrations of 21 kDa and 16 kDa proteins were found in the striatum. These proteins were also enriched, but to different extents in the substantia nigra, the globus pallidus, the olfactory tubercle, the amygdala and the cerebral cortex. In contrast the 19 kDa protein was present at similar levels in all brain regions studied including the white matter. This latter protein was also detected in several peripheral organs (e.g. pituitary gland, thymus, pancreas).

In conclusion, in striatal slices the phosphorylation of all 3 proteins is regulated by cAMP. Therefore, these proteins can be referred to as ARPP-16, ARPP-19 and ARPP-21 (for cAMP regulated phosphoproteins of Mr values of 16, 19 and 21 kDa respectively). ARPP-16 and ARPP-21 appear to be enriched in certain types of neurons from the basal ganglia and the cerebral cortex, whereas ARPP-19 is present in all brain regions, possibly both in neurons and in glial cells.

250.11 PURIFICATION AND BIOCHEMICAL STUDIES OF A 90 kDa BASAL GANGLIA-ENRICHED PHOSPHOPROTEIN. Steven Cala* and Paul Greengard (SPON: S. Halpain) Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021

A set of phosphoproteins which are substrates for cyclic AMP-dependent protein kinase (cAMP-PK) have previously been shown to be enriched in the rat basal ganglia (Walaas et al., J. Neuroscience, 3, 291-311, 1983) Like DARRP-32, these substrates are believed to be involved in signal transduction within the basal ganglia. One of these phosphoproteins appears on sodium dodecyl sulphate (SDS)-polyacrylamide gels as a protein doublet with an apparent molecular weight of approximately 90 kDa. The 90 kDa phosphoprotein has been purified from the supernatant of bovine caudate nucleus using chromatography on DEAE-Sephacel, heparin-agarose, and Mono Q (FPLC), ammonium sulphate precipitation, and preparative SDS-polyacrylamide gel electrophoresis.

Gel filtration on Superose 12 (FPLC) revealed two peaks of the 90 kDa doublet with Stokes radii of 38 and 62 A. These values correspond to globular proteins of molecular weights approximately 85 and 240 kDa. These data indicate that the 90 kDa protein exists in its native form as both a single polypeptide and a higher molecular-weight complex.

Analysis of the two 90 kDa proteins using the two-dimensional (2D) gel system of O'Farrell demonstrated that the two forms have slightly different isoelectric points. The 240 kDa form is rapidly phosphorylated upon addition of the catalytic subunit of cAMP-PK and 5uM [^{32}P]-ATP. In contrast, upon incubation with the catalytic subunit of cAMP-PK and 1mM ATP for much longer times, both forms of the 90 kDa are phosphorylated and their patterns on 2D gels become superimposable.

In summary, the 90 kDa basal ganglia-enriched phosphoprotein can be isolated as both a single polypeptide and a more complex form. These two forms appear to differ in their isoelectric point, and this difference is eliminated by phosphorylation.

250.12 PURIFICATION, CHARACTERIZATION AND IMMUNOHISTOCHEMICAL LOCALIZATION OF ARPP-16, A SUBSTRATE FOR cAMP-DEPENDENT PROTEIN KINASE, ENRICHED IN THE BASAL GANGLIA. A. Horiuchi *, E. Gustafson, A.C. Nairn and P.Greengard, Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021

A phosphoprotein substrate for cAMP-dependent protein kinase, which has an apparent molecular weight of 16,000 measured by SDS-polyacrylamide gel electrophoresis, has been found to be enriched in the basal ganglia. The protein, termed ARPP-16, was purified to homogeneity from the supernatant of bovine caudate nucleus following ammonium sulphate precipitation, and chromatography on CM-cellulose, Mono S (FPLC) and C_{18} reverse phase (HPLC). Two-dimensional non-equilibrium pH gradient electrophoresis indicated that the protein is basic with a pI of ~10.6; amino acid analysis indicated the protein contains high levels of lysine. The protein was phosphorylated, in the presence of the catalytic subunit of cAMP-dependent protein kinase and ^{32}P-ATP with a stoichiometry of 1 mol/mol. Two-dimensional tryptic mapping revealed one major phosphopeptide.

Rabbit serum antibodies were prepared against purified ARPP-16. An additional substrate for cAMP-dependent protein kinase, with an apparent molecular weight of 19,000, as measured by SDS-polyacrylamide gel electrophoresis, was found to cross-react with the antibodies prepared against ARPP-16. The protein, termed ARPP-19, was purified to homogeneity by the same techniques used for purification of ARPP-16. ARPP-19 had a two-dimensional tryptic map similar to that of ARPP-16 and had a more neutral pI (8-8.5). Phosphorylation/immunoprecipitation and immunoblot indicated that ARPP-16 was enriched in the basal ganglia. In contrast, these two techniques indicated that ARPP-19 was present in similar levels in all brain regions studied and was also present in non-neuronal tissues (see abstract by Girault et al.)

Immunohistochemical localization of ARPP-16 was studied using the avidin-biotin complex procedure. Under the antibody dilution conditions used, strong immunoreactivity appeared to reflect the levels of ARPP-16. Neurons strongly immunostained for ARPP-16 were concentrated in the caudate-putamen, presumably in medium spiny neurons, and in the olfactory tubercule, claustrum, and layers II, III, and VI of neocortex. The substantia nigra pars compacta contained a plexus of ARPP-16 immunoreactive fibers which appeared to be organized in patches throughout the nigra. Weakly immunoreactive neurons were found in nucleus accumbens and a few immunoreactive axons were found in the globus pallidus.

Supported by USPHS Grant MH-40899.

250.13 ADENYLATE CYCLASE AND G-PROTEINS IN DISCRETE BRAIN NUCLEI: CHARACTERIZATION AND REGIONAL VARIATIONS. R.S. Duman, J. Erdos*, R. Terwilliger*, E. Nestler and J.F. Tallman. Dept. of Psychiatry, Yale University School of Medicine, Abraham Ribicoff Research Facilities and Connecticut Mental Health Center, 34 Park Street, New Haven, CT 06508.

Recent studies have indicated regional variations in G-proteins and the catalytic component of adenylate cyclase in brain (Worley et al., PNAS 83: 4561, 1986). However, these studies were qualitative and did not demonstrate the functional significance of the regional differences observed. In this study discrete brain nuclei were isolated for analysis of adenylate cyclase and G-proteins. Male Sprague-Dawley rats (150g) were sacrificed, the brains rapidly removed, and gross dissection of frontal cortex (FC), hippocampus (Hp) and cerebellum (Cb) performed on ice. Brain nuclei, including the locus coeruleus (LC), dorsal raphe (DR), ventral tegmentum (VT) substantia nigra (SN) and neostriatum caudate putamen (NS), were isolated by taking 15 gauge punches from 0.5-1.0mm coronal cross sections of brain. Adenylate cyclase assays and ADP-ribosylation and immunoblotting of G-proteins were performed using standard procedures.

GTP-stimulated adenylate cyclase activity was highest in the LC, DR and VT (400% basal), intermediate in SN, FC and Hp (150-200% basal) and lowest in the NS and Cb (150% basal). GTP-dose response curves were monophasic in all regions except NS, where inhibition of adenylate cyclase was observed at higher GTP concentrations. GTPγS- (a nonhydrolyzable analogue of GTP)-stimulated adenylate cyclase activity was highest in LC, DR, VT, FC (700% basal), intermediate in SN and Hp (500%), and lowest in NS and Cb (250 and 150% basal). Presumably, adenylate cyclase activity reflects the sum of the influence of Gs and Gi on the enzyme. Therefore, it is possible that the regional differences observed in GTP-regulation of adenylate cyclase activity reflect regional variations in the levels of these G-proteins. This interpretation is supported by the finding that Gi/Go, determined by immunoblotting and pertussis toxin-mediated ADP-ribosylation, is highest in NS and SN. Finally, forskolin (5μm) activation of adenylate cyclase was greatest in NS and SN (1200-1500% basal) intermediate in LC, DR, VT, FC, Hp (700-900% basal), and lowest in Cb (500% basal), suggesting possible regional differences in the amount of adenylate cyclase catalytic subunits as well.

The results demonstrate that analysis of adenylate cyclase and G-proteins can be performed in discrete brain nuclei, and that the brain shows striking regional differences in levels and functional activities of these proteins. In related studies (Erdos et al., Nestler et al., this volume) we have also found regional differences in the regulation of adenylate cyclase and G-proteins by chronic morphine.

250.14 DETECTION OF PHOSPHORYLATING PROTEINS IN THE COCHLEAR NUCLEUS: EFFECTS OF 8-BROMO CYCLIC AMP AND CALCIUM/CALMODULIN. L. Winsky, J.A. Harvey and D.M. Jacobowitz, Dept. of Psychology and Pharmacology, Univ. Iowa, Iowa City, IA 52240 and Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

There has been considerable interest and progress aimed at determining the importance of intracellular second messengers such as cAMP and calcium in the regulation of neuronal function via the phosphorylation of proteins. However, relatively little is known regarding the effects of these molecular signals within sensory nuclei. In this study, we employed two-dimensional gel electrophoresis and computerized optical densitometry to examine the phosphorylation of proteins in the cochlear nucleus, the first order sensory nucleus of the auditory system. Samples of dorsal and ventral cochlear nuclei of rabbits, obtained by micropunch and containing approximately 40 μg protein, were sonicated in Tris buffer containing EGTA and PMSF. Equal aliquots were added to tubes containing Tris and $MgCl_2$ (basal condition) in addition to 8-bromo cyclic AMP or $CaCl_2$ and calmodulin for examining cAMP and calcium/calmodulin stimulated phosphorylation, respectively. Samples were then incubated for 60 sec with 2.5 μCi of ^{32}P-ATP followed by the addition of sample detergent to stop the reaction. Proteins were separated by two-dimensional gel electrophoresis and silver stained. Gels were then dried and placed on autoradiographic film. Inspection of these autoradiograms indicated a number of proteins which were phosphorylated under the basal condition. The addition of calcium and calmodulin to the incubation medium resulted in an increase in the phosphorylation of several acidic proteins (pI < 6) and at least one basic protein (pI > 7.0). In contrast, the addition of 8-bromo cAMP did not produce any apparent increases in phosphorylation but instead resulted in a decrease in the phosphorylation (from basal) of three proteins. Similar patterns of protein phosphorylation and effects of 8-bromo cAMP and calcium/calmodulin were seen in both the dorsal and ventral divisions of the cochlear nucleus. Inspection of silver stained gels revealed one protein (30 kDa, pI 5.2) previously not observed in several other brain areas examined which may be specifically concentrated in the cochlear nucleus or more generally in first order sensory nuclei. In conclusion, these results demonstrate that the cochlear nucleus is an active site of protein phosphorylation. [Supported by USPHS Grant MH16841].

250.15 SPATIAL AND TEMPORAL EXPRESSION OF mRNA ENCODING THE α SUBUNIT OF G_s: MAPPING IN RAT BRAIN BY IN SITU HYBRIDIZATION. R.R. Reed*, B.L. Largent, D.T. Jones*, C. Pearson*, P.F. Worley, and S.H. Snyder. (SPON: K. Braas) Dept. of Molecular Biology and Genetics, Howard Hughes Medical Institute, and Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The G-proteins represent a family of proteins that bind guanine nucleotides and actively participate in many signal transduction processes. Members of this G-protein family are highly conserved at the protein and nucleotide level. To distinguish in our studies among various members of this family, we generated specific oligonucleotide probes to unique 3' untranslated regions of each G-protein cDNA. These probes were used to detect mRNA abundance and distribution in rat brain. The oligonucleotide probes were labeled using a novel technique involving the synthesis of a 58mer oligonucleotide containing 46 nucleotides of target-specific sequence followed by an additional 12 nucleotides (CAR-common annealing region). A second oligonucleotide (22mer), consisting of T_{10} followed by the sequence complementary to the CAR region, was annealed to the 58mer oligo and extended with α-^{35}S-dATP and DNA polymerase. These probes of defined specific activity and uniform length were then used for in situ hybridization studies.

G_s, the stimulatory G-protein, is associated with the receptor-mediated activation of adenylyl cyclase, an enzyme abundant in neural tissues and essential for many types of neurotransmission. Mapping of mRNA encoding the α subunit of G_s reveals abundant hybridization and heterogenous distribution throughout the brain. Control studies confirm the hybridization specificity. G_s mRNA is prevalent throughout the brain, being particularly evident in large neurons such as pyramidal cells of the piriform cortex and hippocampus, and neurons of motor nuclei and reticular formation. Interestingly, the general pattern of hybridization is quite similar to the immunocytochemical distribution of adenosine (K. Braas, et al, J. Neurosci., 6:1952-1961, 1986). Localization of G_s message does not parallel [^{3}H]forskolin binding (a marker for G_s-coupled adenylyl cyclase) in a fashion that might be expected. Notably, cells of the caudate nucleus, whose neurons contain the highest levels of adenylyl cyclase in the brain, demonstrate the lowest abundance of G_s mRNA. This mismatch may suggest that adenylyl cyclase does not exclusively couple through G_s. Alternatively, G_s in other areas of the brain may effect transduction through other second messenger enzymes. Presently, we are examining the modulation of G_s message levels after intracranial injection of the bacterial toxins cholera and pertussis, known to covalently modify Gα subunits. Additionally, the ontogeny of G_s mRNA in embryonic and neonatal brain is being assessed.

250.16 BINDING OF GUANINE NUCLEOTIDE ANALOGUES TO ADENYLATE CYCLASE REGULATORY PROTEINS IN NEURAL CELL MEMBRANES. J.H. Gordon, M.M. Marcus* and M.M. Rasenick. The Chicago Med Schl, N. Chicago, IL 60064 and Dept Physiol and Biophys, U. of Illinois College of Med, Chicago, IL 60680.

Neural cell membranes, when exposed to hydrolysis-resistant analogues of GTP, show a dose-dependent stimulation or inhibition (depending upon assay conditions) of adenylate cyclase (AC). The activation or inhibition of AC persist subsequent to washing of membranes and is independent of neurotransmitter(s). Although the binding of GTP analogues to detergent-solubilized and purified guanine nucleotide binding regulatory proteins (GN) has been studied, the kinetics of guanine nucleotide binding to individual GN in membranes has not been characterized.

Rat cerebral cortex membranes were incubated with either varying concentrations of the hydrolysis-resistant, photoaffinity GTP analog, azidoanilido GTP (^{32}P-AAGTP) or with a constant amount of ^{32}P-AAGTP and varying concentrations of cold GTP analogues. Membranes were incubated for 3 min at 23°C, centrifuged, resuspended in fresh buffer and exposed to UV irradiation for 5 min. Following irradiation the suspension was centrifuged. The resulting pellet was dissolved in sample buffer, subjected to SDS-PAGE and radioautography. Radioactive bands corresponding to AC stimulatory GN (52 and 42 KDa bands; GN_{SH} and GN_S), inhibitory GN (40 KDa doublet composed of GN_i and GN_O) and a novel neural GN (32 KDa band; GN_{32}) were excised from the dried gels and the amount of radioactivity quantitated.

Isotherm analysis revealed that the $GN_{i/O}$ band had the highest affinity for ^{32}P-AAGTP, K_D= 2.12±0.50 μM (mean ± SE), followed by GN_{32} (3.59±0.59), GN_S (4.87±0.64) and GN_{SH} (5.70±1.25). Separation of the $GN_{i/O}$ doublet into its components (i.e. GN_i and GN_O) indicated that these two GN were not significantly different in their binding affinity for nucleotide. Analysis of competition assays indicated that all of the guanine nucleotides studied were similar to AAGTP as they all displayed the highest affinity for the $GN_{i/O}$ band and the lowest affinity for the GN_{SH}. Of the GTP analogues studied GPPNHP displayed the highest affinity for all GN followed by GDPβS>GTPγS>AAGTP>GTP. GppNHp and AAGTP displayed a significantly higher affinity for $GN_{i/O}$ than GN_S; whereas GTPγS GDPβS and GTP (which is rapidly hydrolyzed to GDP) displayed nearly equal affinity for both proteins. Whether these observations are a result of assay conditions or innate affinities is not known at this time. These data indicate that components of synaptic membranes may alter apparent affinity of GN for nucleotide and that the activation/inhibition of AC is a dynamic process with multiple sites of regulation.

250.17 ISOLATION AND CHARACTERIZATION OF THE INOSITOL 1,4,5 TRISPHOSPHATE (IP_3) BINDING SITE. S. Supattapone, P. Worley, J. Baraban and S.H. Snyder. Department of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

IP_3 appears to be the second messenger responsible for mobilizing calcium from internal stores (Berridge, M.J., and Irvine, R.F. Nature, 312:315, 1984). Therefore, it would be of interest to characterize the binding site for IP_3 in the cell. Previous reports (Worley, P.L., et al., Nature, 325:159, 1987) have demonstrated that the rat cerebellum is a very abundant source of a high affinity IP_3 binding site.

In this study, we have solublized and purified the IP_3 binding site to apparent homogeneity from rat cereblum. The purified receptor is globular and has a Stokes' radius of 10 nm. Therefore, its molecular weight is on the order of one million daltons. While IP_3 binding is reversibly inhibited by 300 nM calcium in crude homogenates and solubilized membranes, the purified binding site is not inhibited by calcium concentrations up to 1.5 mM. Inhibition by calcium could be reconstituted by addition of crude solubilized cerebellar membranes, but not by the cytosolic fraction of cerebellum.

250.18 IDENTIFICATION AND PURIFICATION OF BRAIN TYPE II PHOSPHODIESTERASE: A DISTINCT cGMP RECEPTOR PROTEIN IN MAMMALIAN BRAIN MEMBRANES. M.E. Whalin*, W.J. Thompson*, and S.J. Strada (SPON:H.E.Longenecker). University of South Alabama, Dept. of Pharmacology, College of Medicine, Mobile, AL 36688.

Type II (cGMP stimulated) cyclic nucleotide phosphodiesterase(PDE) as purified from heart, liver, and adrenal tissues shows a preference for cGMP as substrate and displays enhanced cAMP hydrolysis by low, physiological concentrations of cGMP. Our studies of brain Type II PDE indicate it to be the majority of the hydrolytic activity found in membrane fractions. It is not released by either hypotonic or high ionic strength buffers. Detergent solubilization of the Type II PDE does not preserve its regulation by cGMP. However, if released by limited proteolysis using TPCK-trypsin, full cGMP regulation is retained. The solubilized enzyme was purified to apparent homogeneity, utilizing DEAE-cellulose anion exchange, cGMP epoxy-sepharose 6B, and hydroxylapatite chromatography. A 3000 fold increase in specific activity was observed. Its Mr is 240 kD by gel filtration. The subunit Mr of the enzyme determined by SDS-PAGE analysis (7.5%) shows a major protein band at 103-105 kD. Maximum velocities are 157 U/mg and 159 U/mg for cAMP and cGMP respectively. S 0.5 are 28 uM for cAMP and 16 uM for cGMP. The Kact for cGMP stimulation of cAMP hydrolysis at 5 uM substrate is 0.33 uM and maximum stimulation (5 fold) is acheived at 2 uM cGMP. The purified enzyme is phosphorylated by the catalytic subunit of cAMP dependent protein kinase and retains the same subunit Mr. Phosphorylation does not appear to affect cAMP hydrolysis at 5 uM substrate in the absence or presence of 2 uM cGMP, but does reduce cGMP hydrolysis measured at 40 uM substrate by 30%. Monoclonal antibodies produced against purified Type II PDE immunoprecipitate enzyme activity (>90%) with the immunoprecipitate retaining full regulation by cGMP. Immunocytochemical studies are being pursued to define the regional distribution and localization of the Type II PDE observed by activity analysis in related studies (Garrett et al., this volume). This enzyme may constitute a major cGMP receptor and may serve an important regulatory role in controlling the level of cyclic nucleotides during neuronal function.

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250.19 **EFFECTS OF SELECTED PHOSPHODIESTERASE (PDE) INHIBITORS ON CALCIUM-INDEPENDENT PDE ACTIVITY AND ROLIPRAM BINDING SITES OF CEREBRAL CORTEX.** L. L. Russo*, L. A. Lebel* and B. K. Koe. Central Research Division, Pfizer Inc., Groton, CT 06340.

Rolipram, Ro 20-1724 and ICI 63197 are selective PDE inhibitors that are markedly more active on the calcium-independent (cyclic AMP) enzyme (IPDE) than on the calcium-dependent (cyclic GMP) enzyme (DPDE). Recently, membranes, as well as soluble extracts, of various rat brain regions have been reported to contain stereospecific, high affinity binding sites for [3H]rolipram. These binding sites display a linear Scatchard plot and apparently consist of several types characterized by very rapid, moderately fast or very slow dissociation of the radioligand (Schneider *et al., Eur. J. Pharmacol.* 127: 105, 1986). In the present study, we confirmed the presence of high affinity binding sites for [3H]rolipram in mouse and rat brain preparations and detected these binding sites in several regions of marmoset brain. In addition, we found that the IPDE of rat cerebral cortex (Craig, *Biochim. Biophys. Acta* 797: 354, 1984) contains high affinity binding sites for [3H]rolipram. Another selective IPDE inhibitor, nitraquazone (TVX 2706; Glaser and Traber, *Agents Action* 15: 341, 1984), was found also to inhibit [3H]rolipram binding. High affinity binding to similar mouse brain preparations and IPDE was found for [3H]nitraquazone, which appears to label the same binding sites as [3H]rolipram. A 2-min association of mouse cortical membranes with either radioligand resulted in binding that was only slowly dissociated by 10 μM rolipram (22 hr).

Since rolipram is a potent inhibitor of IPDE, it was of interest to ascertain if inhibition of IPDE activity correlated with inhibition of binding to rolipram binding sites. For this purpose, we compared the effects of selected PDE inhibitors on hydrolysis of cyclic AMP by rat cortical IPDE and [3H]rolipram (and [3H]nitraquazone) binding to membranes of mouse cerebral cortex. The rank order of inhibitory potency (IC_{50} in nM) on [3H]rolipram or [3H]nitraquazone binding did not parallel the rank order of inhibitory potency on IPDE (IC_{50} in μM) (Table). These results suggest that inhibition of IPDE may be independent of binding to rolipram binding sites.

Compound	IPDE IC_{50} μM	[3H]Rolipram IC_{50} nM	[3H]Nitraquazone IC_{50} nM
Rolipram	0.49	2.6	4.5
(-)Rolipram	0.59	1.6	2.6
GYKI 13380	0.87	15	36
Nitraquazone	1.9	15	18
(+)Rolipram	2.2	5.0	15
Papaverine	4.3	>10000(42%)	>10000(25%)
Proquazone	4.4	7200	10000
Ro 20-1724	6.0	23	50
ICI 63197	6.8	69	160
IBMX	27	780	1500

250.20 **REGIONAL VARIATION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE ISOENZYMES IN DISCRETE BRAIN NUCLEI.** R. L. Garrett, Jr*, W. J. Thompson* and S. J. Strada, University of South Alabama, Department of Pharmacology, College of Medicine, Mobile, AL 36688, and R. S. Duman, E. J. Nestler, and J. F. Tallman, Yale University, Department of Psychiatry, New Haven, CT 06508.

Previous studies have shown considerable variations in cyclic AMP and cyclic GMP phosphodiesterase (PDE) hydrolytic activities among different brain regions (Soc. Neurosci. 4: 386, 1974). To further extend these observations, we analyzed the activities of three distinct types of (PDE) isoenzymes in homogenates of eight brain regions, including five distinct nuclei. These areas included cerebellum (CB), dorsal raphé (DR), hippocampus (HC), locus coeruleus (LC), neocortex (NC), neostriatum (NS), substantia nigra (SN), and ventral tegmentum (VT). Discrete nuclei were excised as 1mm punches from coronal brain sections prepared from 150 g, male, Sprague-Dawley rats. Samples were homogenized with buffer conditions designed to minimize proteolysis (Adv. Cyclic Nucleotide Res. 10: 69-92, 1979).

SN showed the highest Type I cGMP hydrolytic activity (specific activity; 82 nmol/min/mg), measured at 25μM cGMP, and Type IV (high affinity) cAMP PDE activity (8.5 nmol/min/mg), assayed at 0.25μM substrate. These activities were 10-20 fold higher in SN than those measured in either DR or LC. The ratio of cGMP to cAMP hydrolytic activity (G/A ratio) was highest in HP and NC and lowest in SN and NS. Type II (cGMP stimulated) PDE activity, assayed at 5μM cAMP in the absence and presence of 2μM cGMP, showed the greatest cGMP stimulation of cAMP hydrolysis in VT and HP (3.3-3.6 fold), and the least in SN and CB (1.4 fold). Consistent with earlier results, the CB contained the lowest specific activities for each form of PDE. The variations in PDE isoenzyme profiles may have important implications with respect to functional differences in cyclic nucleotide mechanisms among discrete brain nuclei. These studies were supported by a grant from the USPHS (GM 33538) and a contract from the United States Air Force (49620-85-K-0014).

BIOGENIC AMINES: TOXINS

251.1 HISTOCHEMICAL LOCALIZATION OF MPTP OXIDATION BY MAO-B IN SEROTONIN AND HISTAMINE NEURONS IN THE MOUSE BRAIN. S.R. Vincent, Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

Conversion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to 1-methyl-4-phenylpyridinium (MPP+) appears to be a necessary step for the neurotoxic actions of this drug on the nigrostriatal dopamine system. This conversion is mediated by monoamine oxidase (MAO) of the B type. In the present study, MPTP has been used as a substrate for the histochemical localization of MAO activity in the brain of C57 black mice. The localization of MPTP oxidation by MAO in the brain was compared with the distribution of various monoaminergic neurons determined using immunohistochemistry.

Adult male C57 black mice were anesthetized and perfused with buffered aldehyde fixative. MAO activity was demonstrated on 50 μm thick vibratome sections by incubating the sections in 50 mM Tris-Cl buffer (pH 8.5) containing 0.9% MPTP hydrochloride, 0.1% horseradish peroxidase, 0.005% diaminobenzidine, 0.6% nickel ammonium sulfate and 0.9% sodium azide. Sections incubated without MPTP showed no positive reaction. For the immunohistochemical localization of catecholamine neurons sections were incubated with antisera against tyrosine hydroxylase, dopamine-B-hydroxylase and phenylethanolamine-N-methyltransferase. Serotonin and histamine neurons were localized with antibodies to serotonin and histidine decarboxylase.

The distributions of monoamine cell groups observed in the mouse brain were similar to those found in the rat. Tyrosine hydroxylase immunohistochemistry demonstrated that the major dopaminergic cell group in the mouse was in the substantia nigra pars compacta and adjacent ventral tegmental area. These dopamine neurons did not display MAO activity when MPTP was employed as a substrate. Instead, MAO activity capable of oxidizing MPTP was found in other discrete groups of neurons. These included the serotonin and noradrenaline neurons of the brainstem, and the histamine neurons of the caudal hypothalamus. Preincubation of sections with the MAO-A inhibitor clorgyline blocked the MAO staining in noradrenergic neurons, but not in the serotonin or histamine neurons. The activity in these cell groups was inhibitied by the MAO-B inhibitor deprenyl.

These results indicate that MPTP can be converted to the Parkinsonism-inducing toxin MPP+ by MAO-B in serotonin and histamine neurons which innervate the striatum and substantia nigra.

Supported by the British Columbia Health Care Research Foundation.

251.2 **MPTP EFFECTS ARE REGIONALLY SPECIFIC IN MICE - A NEUROCHEMICAL STUDY.** M. Gupta, S.Y. Felten, and D.L. Felten, Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

MPTP causes degeneration of the nigrostriatal dopamine system in humans, non-human primates, and rodents. Although degeneration of the nigrostriatal dopaminergic neurons is the most prominent abnormality in human Parkinsonism, additional monoamine cell groups also are known to be affected by this disease. We previously have shown that MPTP treatment in young adult mice also leads to decreased dopamine levels in the nucleus accumbens and olfactory tubercle(dopamine terminal projection sites from neurons of the ventral tegmental area) in a dose-dependent manner, in addition to decreased dopamine levels in the caudate-putamen (Gupta et.al., in: MPTP-A neurotoxin producing a Parkinsonian syndrome, eds. Markey et.al., 1985). In the present study, we investigated whether MPTP treatment in mice produces changes in other monoamines in addition to its already established changes in dopamine levels. Young adult male Swiss-Webster mice were injected intraperitoneally with 3, 30, or 60mg MPTP/kg body weight. Control animals received vehicle injections. Treated and control animals were sacrificed by decapitation 21 days following the last injection. Brains were removed quickly and samples from various regions of the brain, both nuclei and terminal sites, were microdissected, placed in 100mM perchloric acid, frozen and stored in liquid nitrogen. Levels of monoamines were determined using high performance liquid chromatography with electrochemical detection. MPTP treatment increases norepinephrine (NE) levels in the ventral tegmental area and decreases NE levels in substantia nigra, whereas no changes were seen in locus coeruleus, mediobasal hypothalamus, dorsal raphe, medullary raphe, and nucleus tractus solitarius. Furthermore, serotonin levels appeared to be decreased in substantia nigra in a dose-dependent manner, but remained unaltered in dorsal raphe, medullary raphe, mediobasal hypothalamus, and nucleus tractus solitarius. We conclude that MPTP affects only specific monoaminergic regions of the brain while leaving other regions unaltered.

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251.3 GABA-TRANSAMINASE INHIBITOR PROTECTS AGAINST METHYLENEDIOXY-METHAMPHETAMINE (MDMA)-INDUCED NEUROTOXICITY. D.M. Stone*, G.R. Hanson and J.W. Gibb (SPON: W. Stevens). Dept. of Pharmacol. and Toxicol., University of Utah, Salt Lake City, UT 84112.

The ring-substituted amphetamine analog, 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"), has been previously implicated as a selective serotonergic neurotoxin in rats, due to its ability to induce immediate (1 h) and prolonged (2 weeks after a single 10 mg/kg dose, unpublished observations) decreases in both the central concentrations of 5-hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA), and the central activity of the serotonin biosynthetic enzyme, tryptophan hydroxylase (TPH). While the mechanism(s) underlying these toxic changes has not been determined, prolonged effects have been assumed to result from drug-induced damage to serotonergic axon terminals. We have previously attempted to characterize the neurochemical mechanisms of MDMA toxicity by examining the effects on MDMA-induced serotonergic changes of a variety of centrally-acting agents which preferentially influence specific neurotransmitter systems. Because inhibitors of GABA-transaminase, the major enzyme catalyzing GABA degradation, prevent the toxic effects of multiple doses of methamphetamine (Hotchkiss and Gibb, *J. Pharmacol. Exp. Ther.* 214: 257, 1980), the ability of such an agent to prevent the toxic effects of MDMA was examined. In male Sprague-Dawley rats, a single injection of amino-oxyacetic acid (AOAA; 30 mg/kg, i.p.), a GABA-transaminase inhibitor which causes a marked elevation of brain GABA concentrations (Walters et al., *J. Neurochem.* 30: 759, 1978), 30 min prior to MDMA (15 mg/kg, s.c.) significantly attenuated the immediate decrease in neostriatal and hippocampal TPH activity which normally occurs 1 h after MDMA administration (neostriatal TPH was decreased to only 77% of control vs. 63% of control after MDMA alone; $p < 0.005$). Similarly, at the time when maximal serotonergic effects of acute MDMA are normally observed (6 h post-injection), neostriatal TPH activity, while significantly reduced from control, was decreased to only 69% of control, vs. 32% of control after MDMA alone ($p < 0.02$). Finally, the prolonged "toxic" effects of acute MDMA were completely prevented by prior AOAA administration: 1 week after MDMA no residual serotonergic effects remained in AOAA pretreated animals, whereas those rats treated with MDMA alone exhibited significant decreases in both TPH activity and 5-hydroxyindole concentrations. Administration of AOAA (30 mg/kg; 3 doses, 1 every 6 h) beginning 2 h after MDMA did not prevent the toxic MDMA-induced serotonergic changes (those effects persisting 1 week after treatment). These results suggest that increased levels of GABA in the brain may protect central serotonin axons from MDMA-induced damage. While the mechanism of this protective effect remains unknown, the ability of both methamphetamine and MDMA to cause central dopamine release, and the possible involvement of dopamine in the neurotoxic effects of these compounds (Schmidt et al., *J. Pharmacol. Exp. Ther.* 233: 539, 1985; Stone et al., *Neurosci. Abstracts* 12: 608, 1986) suggest a role for central dopamine-GABA interactions in the serotonergic effects of MDMA. (Supported by USPHS grants DA 00869 and DA 04222).

251.4 MECHANISTIC STUDIES ON THE ACUTE DEPLETION OF RAT BRAIN SEROTONIN BY 3,4-METHYLENEDIOXYMETHAMPHETAMINE. C.J. Schmidt* and V.L. Taylor* (SPON: F.P. MILLER). Merrell Dow Research Institute, Cincinnati, OH 45215.

A single injection of the psychedelic amphetamine analogue, methylenedioxymethamphetamine (MDMA), produced a rapid decline in both striatal and cortical concentrations of 5-HT and in the activity of the rate-limiting enzyme for 5-HT synthesis, tryptophan hydroxylase (TPH). In the cortex, 5-hydroxyindoleacetic acid (5-HIAA) concentrations first increased 30 min following MDMA treatment and then declined in parallel with 5-HT. In the same experiment the fall in 5-HT concentrations and TPH activity first reached statistical significance at 60 min indicating transmitter release preceded the loss of enzyme activity and transmitter depletion. Both stereoisomers of MDMA were found to produce a similar loss of TPH activity at 3 hr indicating no stereochemical requirement for this effect of the drug. Blocking N-demethylation of MDMA to methylenedioxyamphetamine by coadministration of piperonyl butoxide had no effect on the loss of TPH activity. Kinetic analysis 3 hr after drug-treatment revealed that MDMA administration caused a 50 percent reduction in the V_{max} of cortical TPH with no significant change in the enzyme's K_m for either tryptophan or the semisynthetic cofactor, $6MPH_4$. Incubation of cortical P2 synaptosomes for 2 hr with 100 µM MDMA had no consistent effect on TPH activity, although synaptosomal levels of both 5-HT and 5-HIAA declined. Similarly, TPH activity was not altered in striatal slices superfused with concentrations of MDMA up to 250 µM for 2 hr. Intracerebroventricular MDMA (400 µg) failed to alter either cortical or hippocampal TPH activity at 3 hr nor were striatal monoamine concentrations affected. Direct stereotactic injections of 300 µg of MDMA into the dorsal raphe area or substantia nigra of metophane anesthetized rats failed to alter cortical or striatal 5-HT concentrations, although striatal concentration of dopamine and its metabolites were elevated after intranigral MDMA. The results suggest that the acute effects of MDMA on serotonergic neurons are the consequence of a relatively complex mechanism possibly requiring MDMA metabolism in vivo.

251.5 ACUTE ADMINISTRATION OF MDMA (ECSTASY): NEUROCHEMICAL CHANGES PERSIST UP TO 120 DAYS IN RAT BRAIN. S.F. ALI, A.C. SCALLET, R.R. HOLSON, G.D. NEWPORT AND W. SLIKKER, JR. Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, Arkansas 72079.

MDMA (methylenedioxymethamphetamine) is a hallucinogenic stimulant and has been used by some health professionals in the field of psychotherapy and by the general public as a recreational drug. Several investigators have reported that acute sc administration of MDMA reduced serotonin (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) content for up to 7 days. Recently we have also shown that in male rats, orally administered MDMA (40 and 80 mg/kg) reduced 5-HT and 5-HIAA concentration in different regions of the brain for up to 4 weeks. The present study was designed to determine if MDMA produced the same kind of effects in the female and the duration of effect. Adult female Sprague Dawley rats were dosed by gavage with either 40 mg/kg of MDMA or saline vehicle once every 12 hr for 4 days. These animals were sacrificed 120 days after the last dose of MDMA. Brains were dissected into different regions including the caudate nucleus, frontal cortex, hippocampus, hypothalamus, amygdala nucleus, cerebellum and brain stem and frozen on dry ice. An HPLC/electrochemical system was used to determine tissue concentration of dopamine (DA), dihydroxyphenyl acetic acid (DOPAC), 5-HT and 5-HIAA. During the dosing and up to 2 weeks after the last dose, we noted fighting behavior in MDMA treated rats. In the frontal cortex, we found a significant ($p < 0.05$) reduction of 5-HT and 5-HIAA. In the hippocampus, significant reductions were observed in 5-HT concentration and 5-HIAA showed the same trend. Other regions did not show any significant changes, although the 5-HT and 5-HIAA reduction pattern was observed. DA and DOPAC did not show any significant changes in any brain region studies. These data suggest that multiple oral doses of MDMA produced significant changes in serotonin metabolism in several regions of the brain for up to 4 weeks (Slikker et al., 1986) and that these changes persist in frontal cortex and hippocampus for up to 120 days.

251.6 NEUROHISTOLOGICAL EFFECTS 120 DAYS AFTER ORAL ECSTASY (MDMA): MULTIPLE ANTIGEN IMMUNOHISTOCHEMISTRY AND SILVER DEGENERATION STAINING. A.C. Scallet, S.F. Ali, R.R. Holson, G.W. Lipe, and W. Slikker, Jr. Division of Reproductive and Developmental Toxicology, NCTR, Jefferson, AR 72079.

MDMA (methylenedioxymethamphetamine) is a hallucinogenic stimulant that has been used by the general public as a recreational drug and by some health professionals as an adjunct to psychotherapy. Several laboratories have described the same pattern of acute MDMA neurochemical effects in rats: rapid and large reductions in serotonin content of terminal regions with smaller decreases in the cell-body-rich brainstem. It is important to determine if these effects are irreversible and whether they are accompanied by neurohistological alterations. Ali et al., (this meeting) reports that serotonin content remains lowered 120 days after 8 doses of 40 mg/kg MDMA over 4 days, primarily in hippocampus. Nerve terminal damage to the caudate nucleus has been shown by silver-degeneration (Fink-Heimer) procedures for the related stimulant MDA (methylenedioxyamphetamine, Ricaurte et al., Science 229:986, 1985). We have applied these procedures together with immunohistochemical labeling of serotonin (5-HT), tyrosine hydroxylase (TH), and dopamine B-hydroxylase (DBH) to determine if similar neuropathology can be identified in MDMA-treated rats and if alterations are detectable long after dosing. Acute treatment with single doses of either 40 or 80 mg/kg doubled the density of silver-impregnated (degenerating) terminals in the caudate nucleus compared to vehicle controls when rats were sacrificed 17 hours after treatment. Eight repeated doses of 80 mg/kg produced a smaller (67%) increase in caudate silver-impregnated terminals when rats were sacrificed 14 days after the initial dose, perhaps because of removal of some of the damaged terminals by repair processes during this time. Additional rats sacrificed 120 days after an 8-dose regimen of 40 mg/kg were pre-treated with tryptophan to enhance serotonin immunohistochemical staining. Despite a normal-appearing population of 5-HT labelled dorsal raphe cell bodies and no change in their numerical density compared to controls, these rats demonstrated a 43% reduction in the density of 5-HT fibers in the stratum lacunosum region of the hippocampus. Lesser changes were evident in other regions or with staining for TH or DBH. These results suggest that some aspects of MDMA neurotoxicity are irreversible at least up to 120 days post-treatment and that there may be regional differences in recovery with the major residual effect on serotonin terminals of the hippocampus.

251.7 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) SUPPRESSES SEROTONERGIC DORSAL RAPHE NEURONAL ACTIVITY IN FREELY MOVING CATS AND IN MIDBRAIN SLICES IN VITRO. T.J. Trulson and M.E. Trulson. Dept. Anat., Coll. Med., Texas A&M Univ., College Station, TX 77843.

The synthetic amphetamine analog, 3,4-methylene-dioxymethamphetamine (MDMA) has attracted a great deal of attention recently due to its widespread abuse. This substituted phenylethylamine is structurally related to a large variety of other synthetic and naturally occurring compounds including amphetamine, mescaline, and DOM. Although a close structural analog of certain hallucinogenic drugs, MDMA is neither a true hallucinogen nor a potent stimulant. Rather, MDMA produces a unique state of enhanced emotional and sensory awareness. Since previous studies have shown that certain substituted phenylethylamines have potent actions on serotonin (5HT)-containing neurons, we investigated the effects of MDMA on the activity of 5HT-containing dorsal raphe (RD) neurons in freely moving cats and in midbrain slices in vitro. Cats were implanted for macro- and micro-electrodes as previously described (Brain Res. 163, 1979, 135). After recovery from surgery the cats were placed in recording chambers and allowed to adapt to the novel setting. They then received i.p. doses of dl-MDMA (0.25 - 5.0 mg/kg) and unit activity which was monitored for several hours. MDMA produced a dose-dependent decrease in RD unit activity from approximately 10% at the lowest dose tested to a nearly total inhibition of unit activity at the highest dose ($P<0.01$, ANOVA). An additional group of cats was pretreated with P-chlorolphenylalanine (PCPA), 150 mg/kg/day for 3 consecutive days) and then administered a high dose of MDMA (5 mg/kg). Pretreatment with PCPA greatly attenuated the suppressant action of MDMA on RD neurons. In order to ascertain whether the effects of MDMA on RD neurons are mediated by a local effect on RD cells or due to an indirect action, we examined the effects of MDMA on the activity of 5HT-containing RD neurons recorded from mouse brain slices in vitro. Brain slices were prepared using a standard procedure as previously described (Brain Res. Bull., 18, 1987, 179). MDMA was administered to the incubation bath using a nonpulsating exchange pump. MDMA produced a dose-dependent decrease in the activity of 5HT-containing RD neurons recorded in vitro. An additional group of mice was pretreated with PCPA (400 mg/kg/day for 3 consecutive days) prior to recording. The suppressant effects of the MDMA on RD unit activity was greatly attenuated by prior depletion of 5HT with PCPA. The effects of MDMA on RD neurons appear to be due to the release of 5HT onto autoreceptors since the suppression of unit activity is blocked by depletion of 5HT with PCPA. Thus, the effects of MDMA on the central 5HT system seem to be very similar to those of P-chloroamphetamine.

251.8 (±)METHYLENEDIOXYMETHAMPHETAMINE (MDMA) EXERTS TOXIC EFFECTS ON CENTRAL SEROTONERGIC NEURONS IN PRIMATES. G.A. Ricaurte*, L.S. Forno, M.A. Wilson*, L.E. DeLanney, I. Irwin, M.E. Molliver and J.W. Langston (SPON: J. Hotson) Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305; Dept. of Pathology, Veterans Adm. Med. Ctr., Palo Alto, CA 94304; Depts. of Neuroscience and Neurology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; Inst. for Med. Res., San Jose, CA 95128.

MDMA is a popular recreational drug that has been proposed as a useful adjunct to psychotherapy. Recent reports indicate that MDMA is toxic to central serotonergic nerve terminals in rats. Since studies in rodents do not necessarily predict the toxicity of a drug in primates, this study was undertaken to evaluate the toxic potential of MDMA on serotonergic neurons in the brain of non-human primates.

Eleven squirrel monkeys (Saimiri sciureus) and six cynomolgus monkeys (Macaca fascicularis) were used. MDMA was administered subcutaneously twice daily at approximately 0800 and 1700 hours for 4 days. The following doses were tested: 2.5, 3.75 and 5 mg/kg. Two weeks later, monkeys were killed under deep ether anesthesia and the brain was removed for combined chemical and anatomical analysis.

MDMA produced a selective, long-lasting, dose-related depletion of serotonin and 5-hydroxyindoleacetic acid (5HIAA) in all of the brain regions analyzed (cerebral cortex, caudate nucleus, hippocampus and hypothalamus). The most severely affected area was the cerebral cortex where serotonin was reduced by 90%. Since long-term depletions of serotonin and 5HIAA appear to be reliable predictors of serotonergic nerve fiber degeneration, these results suggest that MDMA damages serotonergic fibers in the primate brain. This was confirmed morphologically by means of immunohistochemical studies which showed that MDMA produced a marked reduction in the number and density of serotonin-immunoreactive axons throughout the cerebral cortex.

The effect of MDMA on serotonergic cell bodies was also examined. MDMA produced no obvious cell loss in either the dorsal or median raphe nuclei. However, in the dorsal raphe nucleus striking cytological changes were found. Numerous shrunken nerve cells were observed which contained brownish-red spherical cytoplasmic inclusions. These inclusions often displaced the nucleus to the periphery of the cell. Whether these changes reflect direct damage to cell bodies or reaction to extensive axonal injury remains to be determined. The eventual fate of these cell bodies needs to be ascertained.

The present results indicate that central serotonergic neurons in non-human primates are very sensitive to the toxic effect of MDMA. Given the increasingly widespread use of MDMA, these findings may have important public health implications.

251.9 A COMPARISON OF THE EFFECTS OF REPEATED DOSES OF MDMA ("ESCTACY") ON BIOGENIC AMINE LEVELS IN ADULT AND NEONATE RATS. D. J. Mokler, S.E. Robinson and J.A. Rosecrans Dept. of Pharmacol. and Toxicol., Va. Commonwealth Univ., Richmond, VA 23298

(±)MDMA was administered to adult rats as a single 40 mg/kg injection or 40 mg/kg (s.c.) every other day for 4 injections. Sixteen days after the last injection rats were killed rapidly and brain area biogenic amine and metabolite levels determined using HPLC techniques. MDMA produced significant depletions of 5-HT and its metabolite, 5-HIAA, in the hippocampus (Hp) and frontal cortex (FC). 5-HT was depleted to 30% of control values in the Hp following single doses. 5-HT levels were unaffected by MDMA in the hypothalamous suggesting a differential effect on 5-HT containing neurons. DA levels were significantly increased in the hypothalamous while frontal cortex NE levels were decreased to 73% of control values following 4 doses of MDMA. These data suggest that MDMA is neurotoxic to 5-HT neurons in the rat.

In a separate study neonate rats were administered MDMA in doses 10,20 and 40 mg/kg (s.c.) on days 4,6,8 and 10. An analysis of biogenic amine turnover (metabolite/-amine ratios) indicated that DA turnover was significantly increased 18 days after the last 4 doses of either 10 or 20 mg/ks (+18-27%), while 40 mg/kg of MDMA, induced a decrease in turnover. A similar increase in 5-HT turnover was observed at lower doses (10 and 20 mg/kg); 5-HT levels were also reduced by at least 25% in all MDMA treated rats. In addition there appeared to a dose-dependent decline in 5-HIAA levels suggestive of an MDMA-neurotoxic effect similar to that observed in the adult.

EFFECTS OF MDMA ON DOPAC AND 5-HIAA LEVELS

DOSE	DOPAC (ng/g)	5-HIAA (ng/g)
0	70.6	499
10	85.6 (+21%)	399 (-21%)
20	85.5 (+21%)	361 (-28%)
40	58.9 (-17%)	324 (-36%)

(D.M.J. was supported a NRSA Fellowship; 1F32DA 05260-01A1)

251.10 THE EFFECT OF MDA AND MDMA ("ECSTACY") ISOMERS IN COMBINATION WITH PIRENPERONE ON OPERANT RESPONDING IN MICE J. A. Rosecrans and R.A. Glennon*, Depts. of Pharmacol. and Toxicol. and Med. Chem., Va. Commonwealth Univ., Richmond, VA 23298.

The optical isomers of MDA and MDMA were evaluated as to their ability to disrupt operant behavior (FR-20) in the mouse. All agonists were administered (i.p.) 15 min prior to behavioral testing; animals received daily injections of saline except on test days which were conducted every 3rd day. Results were expressed as % of vehicle rates of operant responding; vehicle response rates from the day prior to the test session served as control. These compounds as well as S(+)-amphetamine (AMPH) and DOM disrupted behavior in a dose-related manner. ED-50 values indicated the order of potency amongst isomers to be as follows: S(+)-MDA >R(-)MDA>S-(+)MDMA>R(-)MDMA. AMPH was several times more potent than these isomers and disrupted behavior at <1 mg/kg. The preadministration (PRE) of the 5-HT-2 receptor antagonist, pirenperone (PIR), significantly antagonized DOM responding (0.1 mg/kg, s.c. 60 min. prior to 3 mg/kg DOM) supporting previous data obtained in rat drug discrimination studies. In the present studies, pirenperone was observed to antagonize only R(-)-MDA in the mouse operant. The results of this study are in accord with other research conducted in these laboratories. That is, the 5-HT-2 antagonist, PIR, is able to antagonize the effects of R(-)-MDA at a dose comparable to that which blocks the disruptive effects of DOM. On the other hand, this dose of PIR was unable to antagonize the effects of S(+)-MDA or of either optical isomer of MDMA. Apparently, the disruption of behavior produced by the isomers of MDA is via a different mechanism. In addition, the disruptive effects produced by the R(-)-isomer of MDMA appear to be via a mechanism that differs from that implicated for R(-)-MDA (i.e., a 5-HT-2 mechanism).

% DISRUPTION OF OPERANT BEHAVIOR IN PRESENCE OF PIR

APPROX. ED-50 DOSE	(MG/KG)	SALINE PRE	PIR PRE
DOM	3.0	38.6	90.8*
(+)MDA	2.5	53.2	58.2
(-)MDA	3.0	53.0	82.1*
(+)MDMA	3.1	57.9	57.8
(-)MDMA	11.2	48.0	49.5

(This was supported by a NIDA grant, DA-01642)

251.11 NEUROTOXICITY OF METABOLITES OF MDA AND MDMA (ECSTASY) IN THE RAT. S. Y. Yeh* and Fu-Lian Hsu* (SPON: N. Khazan). Neuropharm. Lab., Addiction Res. Ctr., NIDA, Baltimore, MD 21224 and Chem. Res. Devel. & Eng. Ctr., U. S. Army, Aberdeen Proving Ground, MD 21010-5423.

3,4-Methylenedioxyamphetamine (MDA) and 3,4-methylenedioxymethamphetamine (MDMA) destroyed 5-HT axon terminals, and decreased 5-HT and 5-HIAA content in the frontal cortex, hippocampus and hypothamus when administered s.c. to rats (O'Hearn, E. et al.; Yeh, S.Y. et al., Soc. Neurosci. Abstr., 12:1233, 1234). In another study, MDA and MDMA, however, did not destroy 5-HT axon terminals when administered intracerebrally (Molliver, M.E. et al., Soc. Neurosci. Abstr., 12:1234). It was postulated that the neurotoxicity of MDA and MDMA may be due to their metabolites. Alpha-methyldopamine and 4-hydroxy-3-methoxyamphetamine have been identified as metabolites of MDA in the urine of dogs and monkeys. MDA has been found in the urine of human subjects who received MDMA. We postulated that, through beta-hydroxylation of alpha-methyldopamine, alpha-methylnorepinephrine and alpha-methylepinephrine may be metabolites of MDA and MDMA, respectively. Our studies were designed to compare the effects of MDA, MDMA and their metabolites on the motor activity and regional concentration of various brain monoamines and their metabolites.

We either synthesized metabolites of MDA and MDMA or obtained them from Sterling-Winthrop and Merck Sharp & Dohme. Male Sprague-Dawley rats were injected s.c. with either saline (2 ml/kg), or MDA, 4-hydroxy-3-methoxy-amphetamine, alpha-dopamine, alpha-methylnorepinephrine, MDMA, and alpha-methylepinephrine (10 mg/kg) twice daily for five consecutive doses. Motor activity was measured after the first dose. The rats were killed 24 hr following the last injection. Brain concentrations of monoamines and their metabolites were measured using HPLC-EC.

MDA and MDMA dramatically decreased the 5-HT and 5-HIAA levels in the frontal cortex, while the metabolites of MDA and MDMA showed only mild effects. MDA, MDMA, 4-hydroxy-3-methoxy-amphetamine, alpha-methyldopamine and alpha-methylepinephrine decreased 5-HT (85%, 57%, 35%, 16% and 0% of control, respectively) and 5-HIAA contents (78%, 55%, 5%, 7%, and 0%), and generally increased NE levels (48%, -49%, 42% 76% and 7%), respectively. Total horizontal activity induced by MDA, MDMA, 4-hydroxy-3-methoxy-amphetamine, alpha-methyldopamine, alpha-methylnorepinephrine, alpha-methylepinephrine and saline were 16823, 26614, 2335, 2520, 3042 and 1217 counts, respectively. After 3 and 4 doses of alpha-methylnorepinephrine, 3 and 4 out of five rats died, respectively. It was concluded that the neurotoxicity of the metabolites of MDA and MDMA administered s.c. was less than that of the parent compound.

251.12 **PSYCHOTROPIC AMPHETAMINES HAVE DIFFERENT SITES OF ACTION AT SEROTONERGIC (5-HT) SYNAPSES: A COMPARISON OF p-CHLOROAMPHETAMINE (PCA) AND 3,4-METHYLENEDIOXYAMPHETAMINE (MDA) WITH 2,5-DIMETHOXY-4-METHYLAMPHETAMINE (DOM).** U. Berger*, G. Hung*, M.E. Molliver and R. Grzanna. Dept. of Neuroscience, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Several of the psychotropic amphetamines that are widely used drugs of abuse have been shown to act at central 5-HT synapses and to cause degeneration of 5-HT axons. In an attempt to characterize the relationship between drug action and neurotoxicity, we studied the acute effects of the amphetamine derivatives PCA, MDA and DOM on central 5-HT axon terminals using immunocytochemistry (ICC) and neurochemical assays.

Rats (12/group) received a single i.p. injection of PCA (10mg/kg), MDA (20 mg/kg), fluoxetine (9 mg/kg), MDA (20mg/kg) plus fluoxetine (9mg/kg), DOM (2 mg/kg or 23.5 mg/kg), or saline (controls). Animals were sacrificed 4 hours later. For morphologic analysis, rats were perfused and brain sections processed for 5-HT ICC. For assays of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), tissue blocks from frontal and parietal cortex, hippocampus and striatum were assayed by HPLC with electrochemical detection. ICC revealed a dramatic reduction in the number of 5-HT-containing axons in cortex, hippocampus and striatum 4 hours after administration of PCA and MDA. Assays of transmitter levels confirmed the marked depletion (>70%) of 5-HT and 5-HIAA in each of these brain regions. The 5-HT uptake inhibitor fluoxetine has no effect on 5-HT staining or concentration when given alone; depletion of 5-HT is prevented when fluoxetine is administered along with PCA and MDA. In rats treated with DOM, there is no reduction in staining of 5-HT axons and no effect on 5-HT and 5-HIAA levels in frontal cortex and hippocampus; DOM produced only a slight decrease of 5-HT markers in parietal cortex and striatum. No degeneration is seen after DOM at long survivals.

The drug induced decrease in transmitter levels and in staining of axons reflects profound depletion of 5-HT in axon terminals. The rapidity and magnitude of this depletion indicate that PCA (as previously shown) and MDA cause marked release of 5-HT from forebrain axon terminals. Those drugs which cause release also produce degeneration of axons. We suggest that psychotropic amphetamines may be divided into two classes with different sites of action: one type (DOM) acts postsynaptically at 5-HT_2 receptors (Glennon & Titeler, Life Sci. '84), while the other (PCA & MDA) acts presynaptically to release 5-HT from axon terminals. Since PCA and MDA but not DOM produce degeneration of 5-HT axons in long-term experiments, the ability of psychotropic amphetamines to cause massive release of 5-HT appears to be related to the potential neurotoxicity of these compounds. [Support: USPHS #MH41977 and NS15199]

251.13 **ADMINISTRATION OF p-CHLOROAMPHETAMINE (PCA) IN NEWBORN RAT: SELECTIVE ABLATION OF FINE SEROTONERGIC AXONS IN NEOCORTEX.** M.E. Blue and M.E. Molliver. Dept. of Neuroscience, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Recent studies in adult rats have shown that the substituted amphetamines, MDA, MDMA and PCA are neurotoxic to serotonergic (5-HT) axons in forebrain. Fine fibers with small, pleomorphic varicosities are selectively vulnerable to the effects of these neurotoxins, whereas coarse, beaded fibers are resistant. Previous studies report that, unlike the effects observed in the adult, PCA does not reduce brain levels of serotonin when administered to neonates. The aim of the present study was to characterize the neurotoxic effects upon 5-HT axons after administration of PCA to newborn rats.

Neonatal rats were given two injections of PCA (10 mg/kg) or saline at postnatal days 3-4 and sacrificed at either one week or one month after treatment. Forebrain sections were processed for serotonin immunocytochemistry. One week after neonatal PCA treatment, the density of 5-HT axons in dorsal neocortex is markedly reduced, with partial recovery seen at one month. Within neocortex, fine 5-HT axons are selectively diminished by the neurotoxic effects of PCA, whereas the density and the distribution of coarse, beaded fibers does not differ from controls. While neocortex is severely denervated, the density of 5-HT axons remains essentially unchanged in striatum and hippocampus, where both fiber types are spared.

The present results demonstrate that PCA is highly neurotoxic to one class of cortical 5-HT axon terminals when administered to neonates. Fine 5-HT axons are selectively ablated by PCA treatment, as reported in adult rats; beaded axons are spared at all ages. While there is evidence for partial reinnervation of neocortex at one month, the initial loss of fine fibers does not induce compensatory sprouting of coarse axons. There are striking regional differences in neurotoxicity after neonatal administration of PCA, which differ from those seen after adult PCA treatment. The effect of PCA on fine 5-HT axons is restricted to neocortex in the newborn. Fine 5-HT axons in striatum and hippocampus survive neonatal administration of PCA, in contrast to the adult rat where PCA denervates these regions of fine axons. These results suggest that the neurotoxicity of PCA is more regionally selective in the neonate. The observed regional differences in neurotoxicity may explain why earlier studies did not detect reduced brain levels of serotonin after neonatal PCA treatment. The sparing of fine 5-HT axons in hippocampus and striatum following neonatal PCA administration indicates that there are age-related differences in the vulnerability of 5-HT axons to PCA. The mechanisms for these age-dependent changes in neurotoxicity are not known. [Support: USPHS grant HD-19920].

251.14 **THE PSYCHOTROPIC DRUG 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) DESTROYS SEROTONERGIC AXONS IN PRIMATE FOREBRAIN: REGIONAL AND LAMINAR DIFFERENCES IN VULNERABILITY.** M.A. Wilson, G.A. Ricaurte, and M.E. Molliver. Dept. of Neuroscience, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205, and Dept. of Neurology, Stanford Univ. School of Medicine, Stanford, CA 94305.

MDMA is a psychotropic drug that is widely used for recreational purposes by humans. Biochemical and anatomic evidence indicates that MDMA selectively ablates serotonergic (5HT) terminals in the rat brain. The present study was undertaken to characterize the neurotoxicity of MDMA in the primate. Adult macaque monkeys received MDMA (5 mg/kg, s.c.) every 12 hrs. for 4 days. Two weeks after completion of drug treatment, treated monkeys or untreated controls were sacrificed, and the morphology and distribution of 5HT fibers were examined, utilizing 5HT immunocytochemistry.

MDMA produces a striking loss of 5HT terminals throughout the cerebral cortex of monkeys. The degree of denervation is greater than that observed in rats, although the dose of MDMA utilized in this study is lower than that used in rats. Characteristic regional differences are found in the magnitude of denervation produced by MDMA. With some exceptions, the distribution of spared 5HT axons in the primate is similar to that in the rat. Relatively few 5HT fibers survive in frontal and parietal cortex, whereas there is consistent sparing of some of the fibers in other regions, such as striate cortex, the hippocampus, dentate gyrus and amygdala. There are also differences in the laminar distribution of spared fibers among different areas of neocortex. In most neocortical areas, spared fibers are found predominantly in layer I and outer layer II, with scattered fibers in deeper layers. A different pattern is found in striate cortex, where spared fibers are found predominantly in layers IVC alpha and VA; very few fibers are spared in layers I and II. Although the morphology of 5HT terminals in the primate is somewhat more heterogeneous than in the rat, MDMA appears to ablate preferentially the fine 5HT fibers in the monkey, as in the rat (O'Hearn et al., NS Abs 12:1233). In all areas examined, there was sparing of fibers having large spherical varicosities, and loss of fibers having small pleomorphic varicosities. The distribution of spared fibers observed in MDMA-treated animals corresponds to the distribution of fibers having large round varicosities in control animals. These results lead us to propose that in the primate, as in the rat, there are two separate, functionally distinct 5-HT projections which differ in regional distribution, axon morphology, and vulnerability to psychotropic drugs. Moreover, the results indicate the need for caution in the use of MDMA in humans. [Support: USPHS grant #NS-21011 and the Markey Fund]

251.15 **DUAL SEROTONERGIC PROJECTIONS TO FOREBRAIN IN THE RAT: TWO CLASSES OF AXON TERMINALS EXHIBIT DIFFERENTIAL VULNERABILITY TO THE PSYCHOTROPIC DRUGS p-CHLOROAMPHETAMINE (PCA) AND 3,4-METHYLENEDIOXYAMPHETAMINE (MDA).** C. Mullen*, L.A. Mamounas, E. O'Hearn and M.E. Molliver. Department of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Serotonergic (5-HT) neurons in the midbrain raphe nuclei form heterogeneous axon terminals that arborize throughout the forebrain. Two distinct types of 5-HT axons are found in cerebral cortex: (i) **fine axons** with small, pleomorphic varicosities and (ii) **beaded fibers** with large, spherical varicosities. The psychotropic amphetamine derivatives MDA and MDMA have been shown to cause degeneration of 5-HT axons in cerebral cortex and appear to ablate preferentially the fine 5-HT fibers (O'Hearn et al., NS Abs 12:1233,'86). Since PCA is also reported to damage 5-HT axons, the goal of the present study was to compare the neurotoxicity of PCA and MDA and to establish whether these drugs selectively ablate particular types of 5-HT axons. Adult rats were given multiple injections of PCA (10 mg/kg), MDA (20 mg/kg), or saline, sacrificed 1-14 days later and brain sections prepared for serotonin immunocytochemistry. Both drugs produce a marked reduction in the density of 5-HT axon terminals in forebrain, most severe in cerebral cortex, striatum and thalamus. At all survival times, fine 5-HT terminals are almost completely ablated; nearly all spared 5-HT terminals are of the beaded type with large spherical varicosities. These spared, varicose axons are concentrated in restricted forebrain locations: in the subhilar region of the dentate gyrus, the molecular layer of CA1, olfactory glomeruli, lateral entorhinal area (layer III), posterior cingulate (layer II), parietal and occipital cortex (layers I-III) and the ventricular plexus. The beaded 5-HT axons have the same regional distribution and density in control animals as in animals treated with MDA or PCA. Since both drugs cause the same neurotoxic effects, they are likely to have a common mechanism and site of action. In conclusion, this study distinguishes two classes of 5-HT axons in the rat forebrain based on selective vulnerability to neurotoxic drugs; fine axons are highly sensitive to PCA and MDA while beaded axons are resistant to these drugs. Since other studies show that the neurotoxicity of PCA and MDA depends on binding to the 5-HT uptake carrier, we suggest that there may be different subtypes of 5-HT uptake receptors that are associated with the two axon types. The results lead us to propose that there are two separate, functionally different 5-HT projections which differ in axon morphology, regional distribution, and response to psychotropic drugs. Moreover, we report elsewhere in this volume that the two 5-HT projections arise from different sets of cells located in separate raphe nuclei. [Support: USPHS grants #NS-15199, NS-21011 and MH-09538.]

251.16 **DUAL SEROTONERGIC PROJECTIONS TO FOREBRAIN HAVE SEPARATE ORIGINS IN THE DORSAL AND MEDIAN RAPHE NUCLEI: RETROGRADE TRANSPORT AFTER SELECTIVE AXONAL ABLATION BY p-CHLOROAMPHETAMINE (PCA).** L.A. Mamounas and M.E. Molliver. Dept. of Neuroscience, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

The serotonergic (5-HT) innervation of cortex arises primarily from the dorsal (DR) and median raphe (MR) nuclei of the midbrain. A recent anterograde transport study suggests that axons arising from the DR are structurally different from MR axons: DR axons are fine with small, fusiform varicosities (fine fibers); MR axons are coarse with large, spherical varicosities (beaded fibers). In addition, the substituted amphetamines MDA, MDMA and PCA have been shown to cause selective degeneration of fine 5-HT axon terminals in cortex, while sparing beaded fibers. The goal of the present study was to determine whether or not the drug-resistant (i.e., beaded) fibers in cortex have a different origin in the raphe from the drug-sensitive (i.e., fine) fibers. Assuming that only intact axons are capable of taking up and transporting an injected label, we employed retrograde axonal transport to compare the distributions of cortically-projecting raphe neurons in PCA-treated vs. control animals.

The following experimental paradigm was used: 1) adult rats were administered two i.p. injections (24 hr. apart) of either PCA (6 mg/kg; n=4) or saline (n=4); 2) after one week, the fluorescent dye Fluoro-gold (FG; 2% in 200 nL NS) was microinjected into parietal cortex; 3) one week later, the rats were sacrificed. In midbrain sections, the locations of retrogradely labeled raphe neurons were quantitatively mapped; ablation of cortical 5-HT axons was verified by immunocytochemistry.

In parietal cortex of PCA-treated rats, there was a marked loss of fine 5-HT axons with complete sparing of beaded axons, when compared to controls. FG injection sites were confined to the parietal cortex in all cases. In control animals, both DR and MR nuclei contain numerous retrogradely labeled neurons (cell ratio DR:MR, 1.2:1). After PCA treatment, the number of retrogradely labeled neurons in the DR nuclei was markedly reduced (80% decrease), when compared to controls. However, there was no decrease in the number of labeled neurons in the MR nuclei (PCA-treated rats; DR:MR, 0.26:1).

These results demonstrate that the DR projection to cortex is selectively vulnerable to the neurotoxic effects of PCA, whereas the MR projection is resistant. Also, fine and beaded 5-HT axon terminals in cortex arise from different sets of cells located in separate raphe nuclei (DR and MR, respectively). Therefore, we propose that there are two anatomically and functionally separate 5-HT projections to cortex having different 1) nuclei of origin, 2) axon morphology, 3) regional distributions and 4) pharmacological properties. [Support: USPHS #NS-15199, MH-09538]

251.17 **SEROTONERGIC (5-HT) PROJECTIONS FROM DORSAL RAPHE, RAPHE OBSCURUS, AND RAPHE PALLIDUS TO THE MOTOR NUCLEUS OF THE TRIGEMINAL NERVE: DIFFERENTIAL VULNERABILITY OF THEIR AXON TERMINALS TO p-CHLOROAMPHETAMINE (PCA).** J.-M. Fritschy*, W.E. Lyons*, M.E. Molliver and R. Grzanna (SPON: B.E. Kosofsky). Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We have demonstrated a dual innervation of the forebrain by two classes of 5-HT axons which differ in their vulnerability to neurotoxic amphetamines (O'Hearn et al., NS Abs. 12:1233) and have separate origins in the dorsal and median raphe nuclei (cf. Mamounas and Molliver, this volume). The present study was conducted to determine whether two classes of 5-HT axons in the brainstem can be differentiated by their vulnerability to PCA. The motor nucleus of the trigeminal nerve (MoV) was chosen for this analysis since it receives a dense 5-HT input. This input is of functional significance since 5-HT release induced by PCA has been shown to have a powerful influence on the excitability of motoneurons.

Retrograde transport of rhodamine (Rh) labeled latex beads was combined with 5-HT immunohistochemistry to identify 5-HT neurons that project to MoV. Control rats were compared to rats which received two i.p. injections of 10 mg/kg of PCA 24 hours apart two weeks prior to microinjections of the fluorescent tracer. After 7 days, brainstem sections were processed for 5-HT immunohistochemistry and analyzed in a fluorescence microscope. Rh labeled 5-HT cells were observed bilaterally in three raphe nuclei. In control rats, approximately 100 Rh labeled 5-HT cells were found in the dorsal raphe nucleus (DR); 400 Rh labeled 5-HT neurons were observed in the nucleus raphe obscurus and raphe pallidus. Following treatment with PCA, there was an 80% decrease in the number of 5-HT cells in the DR that were retrogradely labeled from MoV. In contrast, the number of labeled 5-HT cells in the raphe obscurus and raphe pallidus was not different from that determined in control rats.

This study demonstrates that three separate raphe nuclei give rise to the 5-HT innervation of MoV. Our study provides evidence for two pharmacologically distinct classes of 5-HT axons in brainstem as has been shown in the forebrain. Axons originating from the DR but not those originating from cells of the raphe obscurus and raphe pallidus are vulnerable to the neurotoxic action of PCA. In a separate study (cf. Berger et al., this volume) we show that the neurotoxicity of PCA is closely linked to release of serotonin from 5-HT axon terminals. The present data may explain clinical reports that, in humans, jaw clenching consistently follows ingestion of psychotropic amphetamines (e.g., MDMA). This behavioral symptom may result from massive release of 5-HT from DR axon terminals leading to enhanced excitability of motoneurons in MoV. [Support: USPHS grants NS-15199 and MH 41977].

252.1 DIFFERENTIAL EFFECTS OF N-n-PROPYLNORAPOMORPHINE (NPA) ISOMERS ON NIGRAL AND VENTRAL TEGMENTAL DOPAMINE NEURONS. R.F. Cox*, J.L. Neumeyer and B.L. Waszczak. Northeastern Univ., Boston, MA 02115

R-(-)-NPA is a potent agonist on substantia nigra (SN) and ventral tegmental area (VTA) dopamine (DA) neurons. Recent reports suggest that the S-(+)-isomer exhibits limbic-selective antagonist actions in behavioral studies (Psychopharmacol. 88: 158, 1986) and agonist activity at DA receptors in vitro (Mol. Pharmacol. 25: 18, 1984). Since the electrophysiological effects of S-(+)-NPA have not been studied, extracellular single unit recording studies were carried out to determine if NPA isomers display regional selectivity in altering firing rates of SN and VTA DA neurons.

R-(-)-apomorphine (APO), R-(-)-NPA, and S-(+)-NPA, all from single synthetic lots, were administered intravenously in logarithmically increasing doses at 1 min intervals to chloral hydrate anesthetized male Sprague-Dawley rats. Mean doses (in ug/kg) to inhibit firing rates by 50% (ID50) were:

	SN			VTA*	
	APO	R(-)NPA	S(+)NPA	R(-)NPA	S(+)NPA
ID_{50}	5.3 ±1.3	0.53 ±0.07 ^{a}	514.4 ±106.6 ^{a,b}	0.50 ±0.08 ^{a}	149.6 ±30.2 ^{a,b,c}
n	10	10	9	11	8
potency ratio	1	10	0.01	10.6	0.04

a =p<.01 vs. APO; *b*=p<.01 vs. R(-)NPA; *c*=p<.01 vs. S(+)NPA in SN

* 1 cell insensitive to R-(-)-NPA and 3 cells insensitive to S-(+)-NPA not included.

Haloperidol (HAL; 0.2 mg/kg) readily reversed inhibitions due to APO and R-(-)-NPA in both cell groups. However, HAL (0.2-0.8 mg/kg) fully reversed S-(+)-NPA-induced inhibitions for only 2 of 6 VTA cells tested; for the other 4 cells, only partial reversals were attained with doses up to 1.6 mg/kg HAL. Tests for a possible antagonist action of S-(+)-NPA revealed that doses to 0.9 mg/kg failed to reverse inhibitions due to R-(-)-NPA, although subsequent i.v. HAL fully reversed these inhibitions. A prior 40 ug/kg dose of S-(+)-NPA may, however, shift to the right the dose-response curve for R-(-)-NPA in the VTA (ID50=1.06 ug/kg ± 0.47, n=3, 0.05<p<0.1), or render more cells insensitive to R-(-)-NPA (n=3).

These results confirm that R-(-)-NPA is a potent agonist, whereas S-(+)-NPA is a weak agonist, with some antagonist potential, on midbrain DA cells. Further, S-(+)-NPA shows regional selectivity in that VTA DA cells are inhibited by lower doses than SN DA cells. While a reported 0.1% enantiomeric impurity with R-(-)-NPA would account for the inhibition of DA neurons with S-(+)-NPA, it would not explain its selective effects on VTA cells, the impaired ability of HAL to reverse these effects, nor its possible antagonist actions. Supported by NIH NS 23451.

252.2 DIFFERENTIAL EFFECTS OF DOPAMINE AGONISTS AND ANTAGONISTS ON THE SPONTANEOUS ELECTRICAL ACTIVITY OF A9 AND A10 DOPAMINE NEURONS. A.M. Kabzinski*, M.R. Szewczak*, M.L. Cornfeldt* and S. Fielding (SPON: F. Huger). Dept. of Biological Research, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

Dopamine (DA) receptors have been subdivided into D-1 and D-2 on the basis of biochemical and pharmacological studies (Kebabian and Calne, 1979). D-1 receptors are associated with the adenylate cyclase enzyme and can be labeled with (3H)-thioxanthene ligands. D-2 receptors, not linked to a cyclic AMP system, bind (3H)-butyrophenones with high affinity, whereas D-1 receptors do not. Single cell sampling techniques were used to examine the in vivo effects of DA D-1 and D-2 receptor agonists and antagonists on the activity of rat A9 (substantia nigra, zona compacta, SNC) and A10 (ventral tegmental area, VTA) spontaneously firing DA neurons.

Both D-2 antagonists, haloperidol and sulpiride, acutely increased the number of spontaneously active DA neurons in both the A9 and A10 regions as compared to controls. Chronic administration of haloperidol and sulpiride markedly reduced the number of firing DA cells below control levels in both areas. In contrast, acute treatment with the D-2 agonists, LY-171555, RU-24213 and pergolide, decreased the number of DA neurons below control levels in the SNC and VTA while repeated administration increased the number of DA neurons above controls in both areas. The DA D-1 antagonist, SCH-23390, as well as the DA D-1 agonist, SKF-38393, appeared to have no effect, after both acute and chronic administration, on the number of spontaneously firing DA neurons in the SNC and VTA. These results provide evidence that D-2 receptors, but not D-1 receptors, are involved in the control of neuronal activity of DA cells in the A9 and A10 areas.

252.3 **DEPRESSION OF DOPAMINE NEURON FIRING RATES BY THE SELECTIVE DOPAMINE AUTORECEPTOR AGONISTS, (-)3-PPP and (-)HW-165.** W.E.Hoffmann* and M.F.Piercey (G.Vogelsang, Spon.), CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Two hydroxyphenyl-piperidines, (-)3-PPP ((-)-3-(3-hydroxyphenyl)-N-n-propylpiperidine HCL, Hjorth et al., Psychopharmacology 81:89, 1983) and (-)HW-165 (trans-(4aS,-10bS)-(-)-7-hydroxy-1,2,3,4,4a5,6,10b-octahydrobenzo(f)quinoline HBr, Hjorth et al., Arch. Pharm. 333:205, 1986), each thought to be selective agonists at the dopamine (DA) autoreceptor, were electrophysiologically evaluated for their effects on firing rates of DA neurons. Male Sprague-Dawley rats were anesthetized with chloral hydrate (400 mg/kg i.p.). Extracellular glass microelectrodes were used to record from single DA neurons which were identified by their characteristic long duration (>2.5 msec.), positive-negative or positive-negative-positive action potentials, and slow firing rates (Bunney et al., JPET 185:560, 1973). Final identification required histological verification that the electrodes were located in the areas of DA cell bodies in the substantia nigra pars compacta (SNPC) or ventral tegmental area (VTA). Consistent with their proposed autoreceptor agonist roles, both agents inhibited DA neurons by a haloperidol-sensitive mechanism. However, both agents behaved as partial rather than full DA agonists. In the SNPC, both (-)HW-165 and (-)3-PPP had similar potencies (ED50's were 86 ug/kg for (-)3-PPP vs 180 ug/kg for (-)HW-165) and depressed DA neurons by similar amounts (approximately 70% for (-)3-PPP vs approximately 80 % for (-)HW-165). Although in unanesthetised animals (-)HW-165 is known to inhibit DA synthesis most effectively in mesolimbic/mesocortical regions, we found that, in our anesthetized animals, (-)HW-165 depressed firing rates of mesolimbic/mesocortical DA cells in the VTA no more effectively than it depressed basal ganglia DA cells in the SNPC. It is concluded that the selectivity of these agents for DA autoreceptors as compared to DA postsynaptic receptors is accounted for by a combination of their partial dopaminergic agonist activities and the more sensitive state of DA autoreceptors.

252.4 ELECTROPHYSIOLOGICAL ACTIONS OF CHOLECYSTOKININ AND CR 1409 ON IDENTIFIED RAT DOPAMINERGIC NEURONS. A.S. Freeman and L.A. Chiodo. Laboratory of Neurophysiology, Center for Cell Biology, Sinai Research Institute, Detroit, MI 48235.

The localization of cholecystokinin octapeptide (CCK-8) in midbrain dopaminergic (DA) neuronal cell bodies is extensive in medial A9 and in A10, sites of origin of the mesolimbic DA projection. DA cells in these areas are excited by microiontophoretically applied sulfated CCK-7 and CCK-8. These findings, combined with other data showing pronounced CCK-8 effects in the nucleus accumbens pointed to a selective action of CCK-8 on mesolimbic DA systems. On the other hand, we previously found that i.v. CCK-8 has the ability to excite as well as to modulate the apomorphine (APO) sensitivity of nigrostriatal DA cells identified with the antidromic activation mapping technique. The present study includes an examination of the effects of microiontophoretically applied CCK-8 on identified nigrostriatal DA neurons.

Microiontophoretic electrodes were guided into the lateral two-thirds of A9; the recording sites were marked and histologically identified in order to confirm electrode placements. Application of CCK-8 (9 uM, pH 8.0; 10-50 nA; 1-2 min ejection periods) produced increases of 10-50% in the firing rates of 6/12 nigrostriatal DA cells antidromically activated from the head of the caudate nucleus (8.0+0.5 msec latency; stimulation currents of 0.5-2.5 mA). These results strongly suggest that CCK-8 can directly excite a subpopulation of nigrostriatal DA neurons. Whether or not this sensitive group of DA cells also contains CCK-8 remains to be determined.

CR 1409 (lorglumide, Rotta Res. Lab.) is a potent peripheral CCK receptor antagonist. We have begun to assess the effects of CR 1409 on identified DA neurons. CR 1409 was injected in i.v. doses of 0.005-10 mg/kg while recordings were obtained from nigrostriatal or mesoaccumbens DA cells. Cumulative injections produced no marked changes in firing rate although transient decreases or increases in firing rate were occasionally observed, especially with high bolus doses. In some cases, CR 1409 pretreatment diminished the potency and efficacy of APO to inhibit nigrostriatal DA cell firing rate. Experiments are in progress to assess the ability of CR 1409 to block CCK-induced excitations. (Supported by MH-41557.)

252.5 ALPHA-2 RECEPTOR ANTAGONISTS, BUT NOT GABA, 5-HT, OR OPIATE ANTAGONISTS, AUGMENT RESPONSIVENESS OF LOCUS COERULEUS NEURONS TO STIMULATION. P. E. Simson and J. M. Weiss. Dept. of Psychiatry, Duke University Medical Center, Durham, NC 27710.

Spontaneous activity of locus coeruleus (LC) neurons is inhibited by stimulation of a variety of receptor types. Stimulation of alpha-2 adrenergic receptors (Svensson, T. H., Bunney, B. S. and Aghajanian, G. K., Brain Research, 92:291-306, 1975) and opiate receptors (Korf, J., Bunney, B. S. and Aghajanian, G. K., Eur. J. Pharmacol., 25:165-169, 1974) potently depress the spontaneous activity of LC neurons; additionally, stimulation of GABA (Cedarbaum, J. M. and Aghajanian, G. K., Brain Research, 112:413-419, 1976) and 5-HT receptors (Segal, M., J. Physiolol (London), 286:401-415, 1979) reduce LC firing rates.

The influence of alpha-2 receptors has been the most extensively studied of the inhibitory receptors. Aghajanian initially proposed that alpha-2 receptors produce the period of quiescence (PSI) that follows a burst of firing (Aghajanian, G. K., Cedarbaum, J. M. and Wang, R. Y., Brain Research, 136:570-577, 1977), although more recently Andrade and Aghajanian (J. Neurosci., 4:161-170, 1984) presented evidence that PSI is produced independently of alpha-2 receptors. Recently, we demonstrated that, in addition to whatever role these receptors play in PSI, alpha-2 receptors modulate the responsiveness of LC neurons to stimulation (Simson, P. E. and Weiss, J. M., J. Neurosci, in press). Whether or not this ability to regulate LC responsiveness is unique to alpha-2 receptors, however, has yet to be determined. The studies reported here examined whether blockade of other inhibitory receptors would produce similar alterations in the responsiveness of LC neurons as was produced by alpha-2 blockade.

In contrast with the markedly augmented responsiveness of LC neurons to stimulation following alpha-2 blockade, administration of pharmacological agents to block other receptors that inhibit LC activity did not increase responsiveness of the LC. Responsiveness of LC neurons did not change following administration of the GABA antagonists bicuculline and picrotoxin. Similarly, the 5-HT antagonists cyproheptadine and methysergide failed to augment responsiveness of LC neurons.

The opiate receptor antagonist naloxone also produced no change in responsiveness of LC neurons. In the case of opioid receptors, it could be argued that blockade of these receptors (by naloxone) would not be expected to increase responsiveness unless high levels of agonist were present. To test this, animals were implanted with pumps that released morphine continuously for over one week. Seven days after implantation of the pump, opiate receptor blockade by naloxone markedly increased spontaneous activity of LC neurons but failed to alter LC responsiveness.

These results extend our previous findings that alpha-2 receptors play a major role in regulating the responsiveness of LC neurons to excitational influences, and point to the possibility that alpha-2 receptors are unique in this regard.

252.6 ELECTRICALLY-EVOKED RELEASE OF NOREPINEPHRINE IN THE RAT CEREBELLUM: AN IN VIVO ELECTROCHEMICAL STUDY. K. Pang, P. Bickford-Wimer, G.M. Rose and G.A. Gerhardt, Depts. of Psychiatry and Pharmacology, UCHSC, Denver, CO, 80262 and Medical Research Service, VAMC, Denver, CO 80220.

The noradrenergic projection to the cerebellum originates from the pontine nucleus locus coeruleus (LC), and terminates primarily in the inner portion of the molecular layer and in the granule/Purkinje cell layers. Although this pathway has been well studied anatomically and physiologically, the release of norepinephrine following electrical stimulation of the LC has yet to be demonstrated in vivo. In the present study, we used in vivo electrochemical techniques to measure norepinephrine release following electrical stimulation of the LC.

Male Sprague-Dawley rats were anesthetized with urethane and electrochemical signals were recorded from the cerebellum using Nafion-coated graphite epoxy electrodes. The location of the LC was determined by lowering an electrode in the appropriate region and verifying that recorded neurons had broad action potentials and responded to noxious stimuli (e.g. tail pinch). Once neurons with these characteristics were found, the recording electrode was removed and replaced with a stimulator. Electrochemical electrodes were placed in the cerebellar vermis, ipsilateral to the LC stimulator. The placement of recording and stimulation electrodes was verified post-mortem. Electrochemical measurements were performed using chronoamperometric recording techniques (-0.20 to 0.45 volt pulses) with sampling rates ranging from 0.30-10.0 Hz.

Electrochemical signals from locus coeruleus stimulation (10 Hz, 10 sec, 10-30 volts) were typically less than 300 nanomolar in magnitude and lasted for 20-40 seconds following cessation of the stimulation. Signals were voltage dependent, reproducible after a five minute delay, and observed predominantly in the granule/Purkinje cell layer. Stimulation-evoked signals were not observed in the cerebellar white matter. The temporal characteristics of these signals are in excellent agreement with the electrophysiological effects of locus coeruleus stimulation.

In conclusion, these studies provide evidence for the release of norepinephrine in the cerebellum following electrical stimulation of the locus coeruleus. In addition, the data support the conclusion that in vivo electrochemical methods manifest sufficient sensitivity and temporal capabilities to measure the low levels of norepinephrine released by electrical stimulation.

This work was supported by USPHS grant AG06434 and the VA Medical Research Service.

252.7 **IN OCULO HIPPOCAMPUS/LOCUS COERULEUS CO-TRANSPLANTS: ELECTROPHYSIOLOGICAL EVIDENCE FOR FUNCTIONAL CONNECTIVITY IN VITRO** M. Mynlieff and T.V. Dunwiddie. Dept. Pharmacology, Univ. of Colo. Hlth. Sci. Ctr., Denver, CO 80262 and Denver Vet. Ad. Med. Res. Serv., Denver, CO 80222.

Previous studies have shown the feasibility of co-grafting fetal locus coeruleus (LC) and hippocampal tissue into the anterior eye chamber of adult rats, and recording extracellular activity in oculo (Taylor, et al., Exp. Br. Res., 39: 289, 1980). In the present study, we have investigated such co-transplants and single hippocampal transplants after removal from the eye chamber, and utilized both extracellular and intracellular electrophysiological analyses to assess the connections between the co-transplants.

To prepare co-transplants embryonic LC tissue from Sprague-Dawley rats was transplanted to the anterior eye chamber of sympathetically denervated 6-8 week old rats, followed four weeks later by co-transplantation of fetal hippocampal tissue. After 12-15 months in oculo the co-transplants were dissected from the eye and placed in a standard in vitro recording chamber.

Single hippocampal grafts responded in a characteristic manner to perfusion with medium containing 8.25 mM potassium and 3.3 mM penicillin; repetitive interictal spikes were observed with an average frequency of 0.54 Hz +/- 0.20 (n=5). The frequency of the interictal spikes could be modulated by noradrenergic agonists. In 5 out of 6 transplants tested, isoproterenol (1-10 uM) increased the rate, while in 3 out of 3 transplants norepinephrine (10-40 uM) in the presence of timolol (1 uM) decreased the spiking rate. Thus as we have previously shown in the hippocampal slice preparation, beta-adrenergic receptors increase spiking and alpha-adrenergic receptors reduce such activity. However, in the double transplants, interictal spikes in response to K^+/penicillin perfusion were either completely absent or occurred with a very low frequency (average = 0.08 Hz +/- 0.03, n=6), suggesting that the LC has an inhibitory influence on the hippocampal graft. Furthermore, we have been able to demonstrate that in some of the grafts, local application of GABA via micropressure ejection to LC neurons inhibited the firing of the LC neurons, but caused an increase in the activity of the hippocampus co-graft. In combination with the studies in the single hippocampal transplants, we hypothesize that the LC tonically inhibits the hippocampal co-graft via effects on alpha-adrenergic receptors. In summary, the initial electrophysiological analysis of the co- transplants in vitro indicate that there is a functional connection between the hippocampus and LC in oculo and that the integrity of this connection can be maintained in vitro.

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252.8 FUNCTIONAL COMPARISON OF THE INTRACELLULAR AND EXTRACELLULAR ELECTROPHYSIOLOGICAL CHARACTERISTICS OF DOPAMINE-CONTAINING NEURONS IN VITRO. P.D. Shepard and B.S. Bunney, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Electrophysiological studies utilizing intracellular recording techniques have provided important insights into the neurophysiological characteristics of dopamine (DA) neurons in vivo (c.f. Grace and Bunney, In: Neurotransmitters in the Vertebrate Nervous System, 1986). Although recent work has demonstrated the feasibility of conducting similar studies in vitro, as yet there are no adequate criteria which permit definitive identification of midbrain neurons as dopaminergic. In the present experiments, we have extended our earlier studies on the electrophysiological and pharmacological characteristics of presumed DA-containing neurons (Silva and Bunney, Soc. Neurosci. 12:411.16) to include a comparison of intra- and extracellular recordings from substantia nigra (SN) brain slices. Coronal slices (400 uM) were prepared from a block of tissue containing the SN. Slices were transferred to an air-interface perfusion chamber and continuously superfused (2 ml/min) with oxygenated artificial CSF. Conventional extra- and intracellular recording techniques were employed (Grace and Bunney, Neurosci.10:301, 1983). DA-containing neurons in the SN were tentatively identified by their extracellular electrophysiological and pharmacological characteristics. In agreement with previous in vitro studies (Silva and Bunney, Soc. Neurosci. 11:311.12), SN neurons which were inhibited by DA (10 uM) exhibited long duration (2.7-3.5 ms) action potentials with little variation in interspike interval. Basal firing rates of these putative DA neurons, recorded in slices maintained at 36°C, ranged from 2 to 8 impulses/s. These cells were found to be extremely sensitive to fluctuations in ambient temperature, averaging a change in rate of 1 impulse/sec/degree. In some experiments, it was possible to obtain intracellular and extracellular recordings from a single SN neuron. In agreement with similar studies conducted in vivo, (Grace and Bunney, Neurosci. 10:317, 1983), differentiation of intracellularly recorded action potentials gave rise to a waveform nearly identical to that obtained extracellularly. Spontaneously occurring intracellular action potentials (45-60 mV) were observed to arise from slow 'ramp-shaped' depolarizations and were followed by a prominent after-hyperpolarization (AHP). Brief application of hyperpolarizing current pulses (0.1-0.9 nA; 250 ms duration) resulted in electrotonic potentials characterized by a time-dependent reduction in amplitude, indicative of a slowly-developing inward (anomalous) rectification. Addition of tetraethylammonium to the bathing media significantly attenuated both the AHP and inward rectification. Supported by USPHS grants MH28849, MH25642, MH14276 and the State of CT.

252.9 DETERMINATION OF THE RECEPTOR SUBTYPE MEDIATING DOPAMINE-INDUCED ELECTROPHYSIOLOGICAL RESPONSES IN THE RAT MEDIAL PREFRONTAL CORTEX
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The mesoprefrontal dopamine (DA) system originates within the ventral tegmental area (VTA) and terminates in the deep layers of the medial prefrontal cortex (PFC). Electrophysiological studies have shown that cells within the PFC are inhibited by iontophoretic DA and by stimulation of DA cells in the VTA. Which subtype of DA receptor (D1 or D2) mediates these inhibitory responses? Simultaneous activation of D1 and D2 receptors has been reported to underly DA's effects in some postsynaptic areas (Carlson et al., Br. Res. 400:205, 1987). The purpose of this investigation was, therefore, to examine the responses of PFC neurons to selective D1 or D2 agonists and antagonists in order to determine whether the actions of DA in the PFC are mediated by one or both receptor types. Rats were prepared for extracellular recording by a low cerveau isole transection under temporary halothane anesthesia. Standard microiontophoretic techniques and intravenous administration were used to compare local and systemic drug effects.

Microiontophoretic experiments showed that the D2 selective antagonist, sulpiride, blocked DA-induced inhibition on 83% of cells tested (N=30), whereas the D1 selective blocker, SCH23390, antagonized DA on only 25% of cells (N=12). The results of agonist studies were surprising in that 87% of DA-sensitive neurons were not inhibited by the D2 selective agonist, LY171555 (N=62); 85% did not respond to the D1 selective agonist, SKF38393 (N=46); and co-iontophoresis of LY and SKF did not inhibit any of the cells tested (N=17). The non-selective agonist, pergolide, however, did induce an inhibitory response in 64% of the DA-sensitive neurons tested (N=28). In contrast, preliminary experiments using intravenous drug administration showed that LY171555, in doses as low as 5 ug/kg, produced a profound inhibitory effect on DA-sensitive PFC cells. SKF38393, in doses up to 20 mg/kg, induced only a slight decrease in the activity of DA-sensitive neurons. Furthermore, this dose of SKF did not potentiate the response of PFC cells to subthreshold doses of LY171555.

Contrary to studies in non-cortical postsynaptic areas, experiments in the PFC, involving co-administration of selective agonists, failed to provide evidence for a potentiating interaction between D1 and D2 receptors. Our studies with iontophoretic antagonists and systemic agonists suggest that in the PFC, DA is acting through a receptor with D2 characteristics. However, the predominant lack of response of DA-sensitive neurons to locally applied selective agonists suggests that DA in the PFC may be acting through a receptor which does not conform to the classical definition of either D1 or D2 subtypes.

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252.10 **STIMULATION OF THE TEGMENTAL DOPAMINE REGION MODULATES SENSORY RESPONSES OF SINGLE UNITS IN NUCLEUS ACCUMBENS AND STRIATUM.**
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Dopamine in the mesolimbic and nigrostriatal systems is thought to act as a modulator of input to neurons in dopaminergic terminal regions. Behavioral experiments have shown that alterating dopaminergic function can produce changes in sensorimotor integration. Sensory input to the striatum has been shown electrophysiologically, and we recently demonstrated that sensory stimuli evoked responses in neurons in the nucleus accumbens-olfactory tubercle region, a major terminal area of the mesolimbic dopamine system (West, C.H.K. and Michael, R.P., Soc. Neurosci. Abstr., 11: 207, 1985).

In this experiment, we tested if these sensory responses could be altered by electrical stimulation of the ventral tegmentum, within the origin of the mesolimbic and nigrostriatal dopamine systems. Single units (N = 65) were recorded in adult male rats (N = 14), anesthetized with chloral hydrate (400 mg/kg, ip) , during the application of sensory (olfactory and somatosensory) and electrical stimuli. The olfactory stimuli were ammonia, cedar oil, ethanol, acrylic solvent and rat urine, applied by cotton swab placed near the rat's nostrils. The somatosensory stimuli were light touch, tail pressure and foot pinch, tested both ipsilaterally and contralaterally to the side of recording. Electrical stimuli were applied via bipolar electrodes in the ventral tegmentum and consisted of one sec trains of square wave pulses at 100 Hz, 0.2 msec in duration at an intensity of 400-500 μA. Increases or decreases in spontaneous firing rates in response to sensory stimuli were recorded in nucleus accumbens, olfactory tubercle, striatum, preoptic area and diagonal band of Broca. More than half of all units studied were responsive to one or more types of sensory input, with 30% of units responsive to olfactory stimuli and 64% responsive to somatosensory stimuli. Responsive units that remained stable were subsequently tested with electrical stimuli, and many of these units showed a change in sensory responsiveness for a period of 1-5 min after application of a single electrical stimulus train. These changes consisted of enhancement or reduction of a sensory response and were observed in units primarily located in nucleus accumbens and striatum. Both excitatory and inhibitory sensory responses were affected by electrical stimulation, and the direction of the sensory response did not influence the type of modulatory effect obtained. The effect was most frequently observed on responses to somatosensory stimuli, especially when the response was greater on one side than on the other. Usually the response to contralateral rather than ipsilateral stimuli was altered by tegmental stimulation. Preliminary tests indicate that haloperidol (0.3 mg/kg, ip) can block the effect of electrical stimulation. Results suggested that dopamine may modulate neuronal reponses to certain sensory stimuli, which may represent a neurophysiological substrate for controling behavioral reactions directed toward sensory inputs.

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252.11 A DOPAMINE MEDIATED IPSP DUE TO AN INCREASE IN POTASSIUM CONDUCTANCE.
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Secretion of hormones from the melanotroph of the pituitary pars intermedia (PI) is under direct neural control. An inhibitory dopamine (DA) pathway from the hypothalamus has been suggested and a recent report identified an IPSP in melanotrophs possibly mediated by DA. We are now able to confirm that a hyperpolarization of the melanotroph following stalk stimulation is mediated by a DA-activated K^+ conductance. In the presence of bicuculline (100 μM), to block GABA-ergic IPSPs, stalk stimulation produced a 5-25 mV IPSP with a duration of up to 60 seconds accompanied by a decrease in cell input resistance. These effects of stalk stimulation could be mimicked by pressure-pulse application of DA (1 mM, 25-500 msec pulses, 5-15 psi) from a micropipette adjacent to the recording site. Application of GABA (in the presence of bicuculline) or saline had no effect. Both the DA and stalk stimulation induced IPSPs were abolished by dopamine D-2 antagonists (sulpiride 50 μM, domperidone 2.5 μM). When IPSPs to both stalk stimulation and DA application were obtained in the same cell, the reversal potentials for both responses were identical. We conclude that the stalk stimulation induced IPSP is mediated by DA acting at a D-2 receptor.

The IPSP reversal potential varied with $[K^+]$ from -76 ± 10 mV at 15 mM K^+ to -136 ± 10 mV in 2 mM K^+. A semi-log plot of reversal potential against $[K^+]$ was linear and showed that the reversal potential shifted by 65 mV for a 10 fold change in $[K^+]$. This is very close to the value of 62 mV predicted by the Nernst equation at the temperature of these experiments (35°C). This suggests that the conductance increase during stalk stimulation is due to increased potassium conductance.

Since DA effects on hormone release from PI are mediated by a G-protein induced inhibition of adenylate cyclase we determined the effects of pertussis toxin, a G_i inactivator, on the stalk-stimulation-induced IPSP. Rats were injected with 10 μg of toxin in 15 μL saline into a lateral ventricle. On the 3rd postoperative day recordings were made from the PI as before. In 14 cells from 5 animals GABA mediated responses were obtained to stalk stimulation, while DA mediated IPSPs were absent. Exogenous DA was also without effect. We conclude that stalk stimulation causes a DA mediated IPSP in the melanotroph due to an increase in K^+ conductance. A G-protein is integral to this effect. The melanotroph is a promising system for the investigation of dopaminergic transmission.

252.12 D-1 DOPAMINE RECEPTOR STIMULATION ENABLES FUNCTIONAL RESPONSES TO D-2 DOPAMINE RECEPTOR AGONISTS.
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Although D-1 and D-2 dopamine (DA) receptors exert opposing effects on many biochemical indices of neuronal function, recent electrophysiological and behavioral evidence indicates that these receptors also exert parallel and synergistic effects. White (EJP, 135, 1987) reported that iontophoretic (IONTO) application of the D-1 agonist SKF 38393 (SKF) enhanced the inhibition of rat nucleus accumbens (NAc) neurons produced by the D-2 agonist quinpirole (QUIN). Furthermore, pretreatment with the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine (AMPT), which produced 85% depletion of DA in the NAc (removing tonic D-1 stimulation by DA), significantly attenuated the inhibition produced by IONTO QUIN. This attenuation was reversed by IONTO SKF, suggesting that D-1 receptor stimulation enabled the response to QUIN.

In support of electrophysiological studies, D-1 receptor activation also enabled stereotyped behavioral responses to D-2 agonists in rats. The s.c. administration of SKF (4-16 mg/kg) enhanced the stereotyped behavior induced by QUIN (0.25-2 mg/kg) to the same level produced by the nonselective D-1/D-2 agonist, apomorphine (0.25-2 mg/kg). In addition, SKF restored sterotyped responses to QUIN which had been abolished by AMPT pretreatment.

In contrast to the ability of DA depletion to attenuate the inhibitory effects of QUIN on NAc neurons, AMPT failed to alter the inhibitory response of IONTO SKF on NAc cells. In addition, D-1 behavioral responses did not require D-2 stimulation since SKF-induced grooming was not abolished by either AMPT or by combined pretreatment with AMPT and reserpine (>95% depletion). In fact, SKF reinstated normal levels of grooming which were eliminated by DA depletion. Thus, it appears that D-1 receptor function is less dependent on activation of D-2 receptors.

Finally, we studied the D-2 somatodendritic impulse-regulating autoreceptor on DA neurons in the ventral tegmental area (VTA) to determine if it might also be "enabled" by D-1 receptor stimulation. In contrast to D-2 receptors in the NAc, the VTA autoreceptor did not require stimulation of D-1 receptors to express its effects. The ability of QUIN to inhibit A10 DA cells was not altered by i.v. or IONTO administration of either SKF or the D-1 antagonist SCH 23390 (i.v.), or by pretreatment with AMPT.

In conclusion, whereas D-1 receptor-mediated responses did not appear to require simultaneous stimulation of D-2 receptors, both electrophysiological and behavioral experiments indicate that functional responses of the postsynaptic D-2 receptor, but not the D-2 DA autoreceptor, require concurrent activation of D-1 receptors. (Supported by USPHS grants DA-04093 and MH-40832)

252.13 CHARACTERIZATION OF THE EFFECTS OF PUTATIVE AUTORECEPTOR-SELECTIVE DOPAMINE AGONISTS IN THE MESOACCUMBENS DOPAMINE SYSTEM. P.A. Johansen and F.J. White. Neuropsychopharmacol. Lab., Univ. of Illinois, Champaign, IL 61820.

Previous studies have shown that dopamine (DA) autoreceptors are of the D-2 subtype and are 3-10 times more responsive to DA agonists than postsynaptic D-2 receptors. Moreover, several compounds which appear to be autoreceptor-selective DA agonists (ASDAs) have been identified. Unlike classical DA agonists, ASDAs decrease locomotion in normal rats. Electrophysiological studies in the nigrostriatal DA system support an autoreceptor selective profile for several putative ASDAs. Because hyperactivity of the mesoaccumbens DA system may be involved in schizophrenia, ASDAs might have therapeutic value. Therefore, the present study determined the effects of several putative ASDAs in the mesoaccumbens DA system using extracellular single-unit recording.

The putative ASDAs (-)3-PPP, BHT 920, and (-)HW 165, and the classical DA agonist (+)3-PPP, were studied on mesoaccumbens (A10) DA neurons and their target cells within the nucleus accumbens (NAc) of chloral hydrate anesthetized rats. These drugs inhibited the firing of A10 DA cells with ID50 values of 3, 144, 640, and 790 µg/kg for BHT 920, (+)3-PPP, (-)HW 165 and (-)3-PPP, respectively. (-)HW 165 and (-)3-PPP were partial agonists since the highest dose (2.56 mg/kg) caused only partial inhibition (≈ 60%).

BHT 920 and (-)3-PPP increased NAc cell firing after i.v. injection presumably due to disinhibition resulting from autoreceptor stimulation. However, when administered by iontophoresis (IONTO), BHT 920 and (-)3-PPP caused current-dependent inhibition of NAc cells, suggesting an agonist action at postsynaptic D-2 receptors and questioning the notion of autoreceptor selectivity. Since D-1 stimulation enables postsynaptic D-2 effects in the NAc, it is possible that the lack of postsynaptic D-2 behavioral and electrophysiological effects following i.v. administration of (-)3-PPP and BHT 920 is due to the fact that ASDAs require D-1 receptor enabling to elicit postsynaptic effects. Presumably such enabling would be lost because stimulation of the more sensitive D-2 autoreceptors decreases impulse-dependent release of DA which would normally provide the necessary D-1 receptor "tone".

Preliminary IONTO and behavioral experiments appear to support this idea. Concurrent IONTO of the D-1 agonist SKF 38393 with BHT 920 or (-)3-PPP onto NAc neurons potentiated their inhibitory effects. Following i.v. BHT 920, IONTO BHT 920-induced inhibition was attenuated, but was reinstated by SKF 38393; the latter effect was reversed by the D-1 antagonist SCH 23390. Behaviorally, s.c. administration of SKF 38393 (4 mg/kg) with either (-)3-PPP (4 mg/kg) or BHT 920 (0.1 mg/kg) reversed the typical sedative response to these ASDAs, resulting in enhanced locomotion. (Supported by USPHS Research Grants MH-40832 and DA-04093)

252.14 REPEATED ADMINISTRATION OF APOMORPHINE AT LOW DOSES: EFFECTS ON THE SENSITIVITY OF SOMATODENDRITIC AUTORECEPTORS ON A10 DOPAMINE NEURONS. M.C. Jeziorski and F.J. White. Neuropsychopharmacol. Lab., Univ. Illinois, Champaign, IL 61820.

Despite clinical trials testing the ability of low doses of dopamine (DA) agonists to reverse symptoms of schizophrenia, the physiological effects of repeated administration of "autoreceptor-selective" doses are not well understood. Rebec and Lee (Brain Res., 250:188, 1982) reported that nigral (A9) DA cells in rats treated with 50 µg/kg s.c. apomorphine (APO) twice daily for 5 days exhibited excitatory or decreased inhibitory responses when challenged with i.v. APO. Because the mesoaccumbens DA system is thought to be involved in the pathophysiology of schizophrenia, the present experiments investigated the effects of repeated treatment with low doses of APO on the sensitivity of impulse-regulating somatodendritic autoreceptors on A10 DA cells in the ventral tegmental area (VTA) of chloral-hydrate anesthetized rats.

Extracellular single-unit recording techniques revealed that pretreatment with twice daily s.c. injections of 50 µg/kg APO for 7 days failed to alter the sensitivity of A10 neurons to i.v. injections of the D-2 DA agonist quinpirole (QUIN) (ID_{50} = 11.2 µg/kg) relative to control (ID_{50} = 9.8 µg/kg). However, A10 DA cells in identically treated rats exhibited reduced inhibitory responses to iontophoretically applied DA. These apparently conflicting effects could be the result of compensatory super-sensitivity of postsynaptic receptors on nucleus accumbens (NAc) neurons in response to the decrease in synaptic DA produced by repeated autoreceptor stimulation. Hypothetically, supersensitive NAc cells projecting back to GABAergic VTA interneurons would be inhibited by agonists at i.v. doses of QUIN that are normally considered autoreceptor-selective. The consequent release of inhibition of VTA interneurons would enable these cells to decrease A10 cell firing and compensate for alterations in autoreceptor sensitivity. According to this model, repeated treatment with higher doses of APO would stimulate both DA autoreceptors and postsynaptic receptors and prevent supersensitivity in feedback cells, thus isolating the subsensitive response of A10 cells to systemic administration of agonists. In fact, QUIN challenge in rats pretreated with 250 µg/kg APO on an identical 7 day schedule significantly ($p < 0.05$) decreased sensitivity in A10 cells (ED_{50} = 17.7). Additional experiments are presently ongoing to determine: 1) if ibotenic acid lesions of the NAc followed by 7 day pretreatment with the low dose APO regimen will result in subsensitivity of DA autoreceptors in response to i.v. QUIN; 2) the effects of repeated doses of other D-2 selective and non-selective DA agonists, including longer acting compounds; and 3) the comparative effects of repeated autoreceptor stimulation on A9 and A10 cells. (Supported by USPHS Grants MH-40832 and DA-04093)

252.15 ELECTROPHYSIOLOGICAL EFFECTS OF REPEATED COCAINE ADMINISTRATION ON THE MESOACCUMBENS DOPAMINE SYSTEM. D.J. Henry, M.A. Greene*, S-Y. Chen* and F.J. White. Univ. of Illinois, Champaign, IL 61820.

Behavioral evidence has demonstrated that the potent rewarding effects of cocaine are mediated, in part, by the mesoaccumbens dopamine (DA) system projecting from A10 DA cells within the ventral tegmental area (VTA) to the nucleus accumbens (NAc). Yet, little is known regarding the physiological alterations occurring within this system following repeated cocaine administration. Previous studies in our laboratory have demonstrated that cocaine exerts inhibitory effects on the activity of mesoaccumbens DA neurons. This effect appears to be due to both DA autoreceptor stimulation and to activation of NAc-VTA feedback pathways which may be engaged because of the preferential inhibition of NAc neurons (Einhorn et al., J. Neurosci, in press). The present experiments were designed to assess possible alterations of these effects following repeated cocaine administration.

Extracellular single-unit recording techniques were used to determine whether repeated cocaine administration might reduce the sensitivity of impulse-regulating A10 DA autoreceptors in chloral hydrate anesthetized rats. Following 14 days of repeated cocaine injections (10 mg/kg either 1 or 2 x/day, i.p.), the inhibitory effects of low i.v. doses of the DA agonist apomorphine (APO) were significantly reduced as compared to control rats. Thus, the ID50 values for APO were 11.05 ± 2.0 µg/kg in the control rats, 17.2 ± 4.53 µg/kg in the 1 x 10 mg/kg/day rats and > 50 µg/kg in the 2 x 10 mg/kg/day rats. Iontophoretic application of DA to A10 DA neurons in rats treated with 2 x 10 mg/kg/day also resulted in significantly less inhibition than that observed in control rats although the degree of subsensitivity was less than that observed with i.v. APO. These results indicate that A10 somatodendritic autoreceptors become subsensitive following repeated cocaine administration but also suggest that other mechanims such as feedback pathway activation may also be reduced in these rats.

Preliminary studies on NAc neurons suggest that the responsiveness of these cells to iontophoretic DA is significantly increased in rats that had received 2 x 10 mg/kg/day of cocaine. Such supersensitivity of postsynaptic DA receptors could be due to reduced occupancy of these receptors during repeated cocaine administration, perhaps as a result of diminished DA levels. However, HPLC-EC analysis revealed normal levels of DA in the NAc of rats that received cocaine at up to 2 x 30 mg/kg/day for 14 days. Therefore, it seems likely that other mechanisms are responsible for the apparent increase in the sensitivity of NAc cells to DA. Whatever the mechanism, the increased sensitivity of NAc DA receptors combined with A10 autoreceptor subsensitivity may help to explain cocaine-induced behavioral sensitization. (Supported by USPHS Research Grants MH-40832 and DA-04093)

252.16 THE EFFECT OF METHYLPHENIDATE ON THE COERULEO-CORTICAL NORADRENERGIC SYSTEM OF THE RAT: AN ELECTROPHYSIOLOGICAL STUDY. D. Lacroix and A. Ferron. Centre de recherche en sciences neurologiques (Dép. de physiologie), Université de Montréal and Dép. de psychiatrie, Hôpital Ste-Justine, Montréal, Canada, H3C 3J7.

Methylphenidate (MPH) is the drug of choice in the treatment of attention deficit disorder with hyperactivity in children. Biochemical studies indicate that MPH promotes the release and blocks the reuptake of noradrenaline (NA). Our previous studies have demonstrated that methylphenidate reduced the spontaneous firing rate of locus coeruleus (LC) neurons and decreased the number of spontaneously firing cortical units inhibited by electrical stimulation of the LC (Lacroix and Ferron, Soc. Neurosci. Abstr. 12: 479, 1986). The present study was undertaken to examine the effects of therapeutic doses of MPH on the responsiveness of cortical neurons to microiontophoretically applied NA and on sensitivity of NA autoreceptors in LC. Three groups of rats were compared: 1) controls; 2) naive rats given a single i.v. dose of MPH (650 µg/kg); 3) rats treated with MPH for 7 days (2 mg/kg, i.p., bid). In group 3, the recordings were made 18 h after the last i.p. injection of MPH. The responsiveness to NA was assessed in the sensori-motor cortex of urethane anaesthetized rats with the IT_{50} method, i.e. by measurement of the current (in nA) X time (in sec) required to obtain a 50% depression of firing rate. The sensitivity of NA autoreceptors in LC was measured by determining the effects of a single dose of i.v. clonidine (4-12 µg/kg) on spontaneous neuronal activity. After a single i.v. dose of MPH in naive rats, there was a reduction in the responsiveness of cortical neurons to NA. In rats treated with MPH for 7 days, responsiveness to NA was nearly twice lower (1286 vs 791) and the effect of i.v. clonidine on LC neuron firing was weaker than in the controls (2-fold decrease). In such MPH-treated animals, the same acute dose of MPH (650 µg/kg), who induced a 50% reduction in the spontaneous firing rate of LC neurons in controls, failed to decrease the activity of LC. These findings suggest that, following MPH treatment, cortical NA neurotransmission is markedly reduced in its efficacy. Such data indicates that the noradrenergic system may have a role in the mode of action of MPH and could explain the therapeutic effects of MPH.

(Supported by grant MA-8666 from the Medical Council of Canada)

252.17 **NOREPINEPHRINE ALTERS THE PASSIVE MEMBRANE PROPERTIES AND SYNAPTIC RESPONSES OF RAT DORSOLATERAL SEPTAL NEURONS *IN VITRO*.** K. D. Phelan, H. Hasuo*, M. J. Twery, and J. P. Gallagher. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550

The dorsolateral septum (DLS) receives projections from brainstem noradrenergic neurons and displays a high density of adrenergic binding sites. In the present study, we investigated the effects of norepinephrine (NE) on the passive membrane properties of rat DLS neurons in a submerged *in vitro* slice preparation using intracellular recording techniques. In addition, we examined the effects of NE on synaptic potentials evoked by local stimulation in DLS and in the diagonal band nucleus (DBN).

Application of NE (0.1-10 *u*M) produced concentration dependent membrane hyperpolarizations and decreased input resistance (33 of 38 cells). Superfusion of NE at the highest concentration tested resulted in membrane potential hyperpolarizations of 2-14 mV and input resistance decreases of 15-45% (n=21). The NE induced membrane hyperpolarization reversed around -90 mV indicating an increase in K conductance. No desensitization of these NE effects were observed. Tetrodotoxin had no effect on NE induced hyperpolarizations (n=4). Pretreatment with phentolamine suppressed or completely blocked the effects of NE on the passive membrane properties of these neurons (n=3). The population of NE responsive cells included DLS projection neurons identified by their antidromic activation following DBN stimulation (15 of 20 tested).

Superfusion of NE also altered the synaptic responses of DLS neurons. The late hyperpolarizing potential (LHP) evoked by local DLS (n=33) or DBN (n=2) stimulation was suppressed or completely blocked by NE. This effect was dependent on the concentration and duration of treatment. In addition, NE suppressed or completely blocked the $GABA_A$ mediated fast inhibitory postsynaptic potential (f-IPSP) arising from local stimulation of DLS (n=33) or DBN (n=14). The recovery of the f-IPSP usually occurred along with that of membrane potential and input resistance, whereas the LHP suppression could persist up to 40 minutes. Superfusion of NE also reduced the hyperpolarizing effect of baclofen.

These results demonstrate that NE alters the passive membrane properties of DLS projection neurons at a postsynaptic alpha adrenergic receptor and also depresses inhibitory synaptic potentials. The NE induced depression of the baclofen response suggests that NE is affecting the LHP (which is believed to arise from activation of a bicuculline-resistant, baclofen-sensitive $GABA_B$ receptor) through a postsynaptic site. The present evidence also indicates that NE affects the LHP and f-IPSP through separate membrane processes. These findings support the idea that noradrenergic inputs to DLS modulate intrinsic information transfer in rat septum. (Supported by MH-39163 to JPG).

252.18 **EFFECTS OF PREFRONTAL CORTEX STIMULATION ON LOCUS COERULEUS DISCHARGE.** C. Chiang, M. Ennis, V.A. Pieribone and G. Aston-Jones (SPON: R. Bartus), Dept. Biol., New York University, NY, 10003.

Previous studies report innervation of the locus coeruleus (LC) region from prefrontal cortex (FCx). Recent tracing studies in our laboratory revealed FCx innervation of central gray but no direct input to LC. However, FCx may significantly influence LC discharge through less direct pathways, providing a link between neo and paleo brain substrates of cognitive processes. Here, we assess the influence of FCx activation on LC. Bipolar electrical stimulation of medial FCx in anesthetized rats was presented through 250-um wires, and extracellular recordings were obtained from individual LC neurons with glass micropipettes.

FCx stimulation (1.0 mA, 0.5 Hz) yielded the following: Of 27 LC cells, 10 (37%) exhibited inhibition only, 9 (33.3%) were antidromically activated, and 1 was synaptically activated. Inhibition from FCx stimulation may reflect effects of collaterals from antidromically driven LC cells. To test this hypothesis, similar experiments were performed in rats 7-10 d after 6-hydroxydopamine (6-OHDA) injections to destroy LC forebrain projections. In these 3 rats, 0/16 LC cells were antidromically activated from FCx, and only 2/16 cells (11%) were even weakly inhibited (84 ms mean duration compared to 206 ms in controls). Thus, most if not all the observed inhibition from FCx in intact animals may have resulted from intracoerulear collateral interactions.

Surprisingly, a greater number of LC cells (8/16) were synaptically activated in the 6-OHDA lesioned subjects, with a mean onset of 42 ms and mean duration of 34 ms. It seems possible that the emergence of excited cells in the lesioned rats resulted from removal of antidromically-induced collateral inhibition. This possibility is supported by the finding that the onset latencies for excitation in lesioned animals was very similar to that for inhibition in intact subjects (45 ms).

While it is conceivable that such low-security activation could result from FCx inputs to distal LC dendrites outside LC proper, our results may be more consistent with a polysynaptic pathway: The excitatory responses were relatively long in latency (42 ms) and weak (<32% of the amplitude of LC responses to footpad stimulation). In contrast, FCx stimulation evoked a short latency (5 ms) and large amplitude response (more than twice that seen in LC) in the adjacent lateral dorsal tegmental nucleus. Interestingly, train stimulation of FCx (20Hz for 0.5 s) yielded more potent LC responses (3X those of single pulse tests) in 12/16 cells (mean onset = 128 ms). Additional studies are in progress to better specify the circuit mediating FCx's influence on LC.

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252.19 SPIKE FIRING OF SINGLE LOCUS COERULEUS NEURONS DURING EXPERIMENTAL EPILEPTIC SEIZURES IN THE RAT. S. Yamaguchi and M.A. Rogawski, Neuronal Excitability Section, Medical Neurology Branch, NINCDS, NIH, Bethesda, MD 20892.

A wide variety of evidence has implicated central noradrenergic systems in regulating the generation and spread of the epileptic discharge in various animal seizure models. Treatments which deplete brain norepinephrine (NE) decrease the seizure threshold and enhance the intensity and duration of seizures. Conversely, drugs that increase brain NE levels have a protective effect on seizures. Lesion studies have indicated that forebrain NE projections from the locus coeruleus (LC) may be of critical importance in this regard and, in fact, electrical stimulation of the LC in man has been demonstrated to have an antiepileptic effect. Recent biochemical studies have indicated that the activity of tyrosine hydroxylase (TOH), the rate limiting enzyme in NE biosynthesis, is increased in actively spiking areas of human epileptic neocortex [Sherwin et al., *Neurology* 34:927-933, 1984; Nadi et al., *Neurology* 37 (Suppl. 1):106, 1987]. In the present study, we sought to directly determine the firing of LC neurons during seizures. Rats were lightly anesthetized with chloral hydrate, artificially ventilated and paralyzed with gallamine. (Animals were prepared in strict accordance with *NIH Guide for the Care and Use of Laboratory Animals.*) The EEG was continuously monitored with cortical screw electrodes. Viability of the preparation was assessed with EKG and arterial blood gas measurements from a femoral catheter. Glass micropipettes filled with NaCl-pontamine sky blue solution were driven into the LC Single LC units were identified by their slow, regular firing pattern and characteristic response to noxious stimulation consisting of a brief burst of spikes followed by a prolonged pause. Recording sites were verified by postmortem histological examination. Seizures were induced by i.v. administration of pentylenetetrazol (100 mg/kg; PTZ). The firing frequency of LC neurons increased markedly during the period immediately prior to the intense electrographic seizure induced by PTZ. During the seizure, most units were quiescent. After the frank electrographic seizure, intermittent cortical spiking occurred for ~30 min. During this period, some LC neurons demonstrated bursting that coincided temporally with cortical spiking, whereas other neurons remained silent. We speculate that activation of LC neurons prior to the seizure discharge and during the afterdischarge may represent an autoregulatory mechanism, which when removed promotes epileptic activity. Since TOH is induced with increased LC activity, LC bursting as occurred during the afterdischarge may account for the enhanced enzyme activity observed in experimental seizure models and in epileptic human brain. These results are consistent with the concept that LC neurons participate in an intrinsic CNS system that dampens seizure activity.

252.20 RESPONSES OF NEURONS IN THE AVIAN LATERAL GENICULATE TO NOREPINEPHRINE. K.S. ELMSLIE* AND D. H. COHEN. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794 and Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60201.

Visually conditioned heart rate change in the pigeon has been developed as a model system for cellular studies of associative learning. In this system the avian equivalent of the dorsal lateral geniculate nucleus (LGNe) transmits effective conditioned stimulus (CS) information, and a class of LGNe neurons shows enhancement of light(CS)-evoked discharge over associative training (J. Neurosci., 6:627). This class consists of cells with increased discharge to the CS and decreased discharge to the unconditioned stimulus (US), foot-shock. The response of these neurons to the US requires an input from locus coeruleus (LC) (Neurosci. Abstr., 8:666) which must be intact for associative modification of the LGNe neurons (Neurosci. Abstr., 11:1109). Since electrical stimulation of LC can substitute for foot-shock as an effective US (Neurosci. Abstr., 9:641), the input from LC is both necessary and sufficient for transmitting US information to LGNe.

In this study neurotransmitter(s) that might mediate the responses of these LGNe neurons to the US were investigated. Norepinephrine (NE) was chosen for initial study, since it is synthesized by LC neurons and NE-containing fibers are found in LGNe. Five-barrel microelectrodes were used to record activity of single cells and to assess their responses to iontophoretic application of various agonists and antagonists.

Responses to NE of 52 LGNe neurons were characterized. Maintained activity was decreased by NE in 29 cells and increased in 11. Two responded sinusoidally, and 10 were unresponsive. Cells whose activity was decreased by NE tend to be situated posteromedially in LGNe, while those excited by NE tend to be located anterolaterally; unresponsive cells were not preferentially distributed. The alpha-agonist phenylephrine was less effective than NE in modifying maintained activity. The beta-agonist isoproterenol was relatively ineffective, and the beta-antagonist sotalol did not block the NE-induced decrease in maintained activity.

These preliminary data suggest that the decreased activity of LGNe neurons in response to application of NE is mediated by an alpha-receptor.
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253.1 BEHAVIORAL AND BIOCHEMICAL EFFECTS OF THE BETA ADRENERGIC AGONIST SOM-1122. James M. O'Donnell. Los Alamos National Laboratory, Los Alamos, NM 87545

Experiments were carried out to assess the behavioral and biochemical effects of SOM-1122. SOM-1122 inhibited the binding of ^{125}I-iodopindolol (IPIN) in vivo in cerebral cortex and cerebellum in a dose-dependent manner, indicating that the drug was centrally active following peripheral administration. This made it of interest to study its ability to bind to and activate beta adrenergic receptors in rat brain, since it has been suggested that centrally acting beta adrenergic receptor agonists might represent a new class of antidepressant drugs. SOM-1122 inhibited the binding of IPIN to membranes prepared from rat cerebral cortex (primarily beta$_1$ subtype) and cerebellum (primarily beta$_2$) in vitro in a concentration-dependent manner. As observed with other beta adrenergic agonists, guanosine triphosphate (GTP) regulated the binding of SOM-1122. This effect of GTP was greater in the cerebellum, suggesting that, like the beta agonist clenbuterol, SOM-1122 exerts somewhat greater agonistic activity at the beta$_2$ subtype of the receptor. SOM-1122 increased the level of cyclic AMP in slices of rat cerebral cortex in a concentration-dependent manner; the EC_{50} was 2.2 μM and the maximal stimulation was to 190% of control levels. Propranolol antagonized the ability of 10^{-3} M SOM-1122 to increase the levels of cyclic AMP in slices of rat cerebral cortex in a concentration-dependent manner (apparent K_I = 58 nM), indicating that the ability of SOM-1122 to increase levels of cyclic AMP was due to an interaction of the agonist with beta adrenergic receptors.

Previous work in our lab has shown that other centrally acting beta adrenergic agonists (e.g., clenbuterol and prenalterol) are behaviorally active. Furthermore, the behavioral effects of these compounds resemble those reported for established antidepressant drugs. SOM-1122 reduced the response rate and increased the reinforcement rate of rats responding under a differential-reinforcement-of-low-rate 72-sec (DRL 72-sec) in a dose-dependent manner (ED_{50} = 0.05 mg/kg). SOM-1122 also reduced response rate under both components of a multiple fixed-interval 5-min, fixed-ratio 30-response schedule (ED_{50} = 0.02 mg/kg). Pretreatment with a dose of 1.0 mg/kg propranolol antagonized the effects of 0.1 mg/kg SOM-1122 on behavior maintained under both the DRL and multiple fixed-interval, fixed-ratio schedules.

The results of the present experiments indicate that SOM-1122 is a high-affinity, partial agonist at beta adrenergic receptors. It is centrally active and appears to be somewhat beta$_2$ selective. SOM-1122 is behaviorally active and its effects on behavior are generally similar to those reported previously for other centrally acting beta adrenergic agonists as well as for proven antidepressant drugs. The behavioral effects of SOM-1122 appear to be mediated by beta adrenergic receptors since they are antagonized by propranolol.

253.2 SELECTIVE ALTERATION OF THE BEHAVIORAL EFFECTS OF THE D-1 AGONIST SKF 38393 FOLLOWING THE DESTRUCTION OF 5-HYDROXYTRYPTAMINE NEURONS. R.F. Kucharik* , P.M. McGonigle, M.S. Kreider, I. Lucki (SPON: D.J. Brunswick). Departments of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

In order to characterize the influence of 5-hydroxytryptamine (5-HT) neurons on behaviors caused by the activation of D-1 receptors, grooming behavior and oral dyskinesias induced by the selective D-1 agonist SKF 38393 were studied in rats following the destruction of 5-HT neurons by the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT, 200 ug ICV) . In control rats, SKF 38393 caused a dose-dependent increase in bouts of grooming behavior (ED50=2.8 mg/kg, IP), with a mean peak response of 20.4 ($\pm$ 2.2, 1 SEM) grooming bouts measured 10-60 min. after injection at 10mg/kg. Rats administered 0.9% NaCl showed 4.8 $\pm$ 1.4 grooming bouts during the same period. 5,7-DHT treatment enhanced the increase of grooming behavior by SKF 38393, causing a 60% increase in the peak effect at 10 mg/kg (32.0 $\pm$ 4.9) whereas the ED50 for SKF 38393 was unchanged. Although grooming bouts caused by the D-1 agonist were more frequent in the 5-HT-depleted rats, their average duration was significantly shorter. SKF 38393 also increased oral dyskinesia episodes in control rats, with a peak effect of 17.5 $\pm$ 1.3 at 10 mg/kg. In contrast to grooming behavior, however, the frequency of oral dyskinesias caused by SKF 38393 was unaltered by 5,7-DHT treatment, (peak effect = 18.0 $\pm$ 2.6). Thus, depletion of brain 5-HT content caused a selective enhancement of grooming behavior by the D-1 agonist SKF 38393.

Measurement of telencephalic monoamine content from control and lesioned rats confirmed that 5,7-DHT treatment produced an 87% reduction of 5-HT content without significant change in either norepinephrine or dopamine. The effect of 5,7-DHT treatment was also studied on the density of D-1 and D-2 receptors in various brain regions using quantitative autoradiography. D-1 receptors were measured using the specific binding of the selective D-1 antagonist (3H)-SCH 23390. D-2 receptors were meausured by the specific binding of the selective D-2 antagonist (3H)-spiperone. Dopamine receptors were studied in: 1) the ventral caudate nucleus; 2) dorsal caudate nucleus; 3) nucleus accumbens; 4) olfactory tubercle; and 5) substantia nigra. No significant change in dopamine receptors were measured in any brain region studied. The changes in behavioral effects of the D-1 agonist SKF 38393 caused by the destruction of 5-HT neurons are probably not due simply to an alteration in the number of dopamine receptors.

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253.3 YOHIMBINE ACCELERATES RECOVERY AND CLONIDINE AND PRAZOSIN REINSTATE DEFICITS AFTER RECOVERY IN RATS WITH SENSORIMOTOR CORTEX ABLATION. Richard L. Sutton and Dennis M. Feeney. University of New Mexico, Departments of Psychology and Physiology, Albuquerque, NM 87131

D-amphetamine (D-AMPH) accelerates recovery of locomotor (beam-walking) ability after unilateral sensorimotor cortex ablation (Science, 1982, 217: 855-857) and this effect is apparently mediated by norepinephrine (NE; Neurosci. Abst., 1984, 10:68). In the present pharmacological study we investigated the effects of various alpha-NE agonists and antagonists (single i.p. injections) on recovery of beam-walking in this model of rodent hemiplegia.

We first tested for effects of drug administration early (24 h) after injury. Beam-walk tests were conducted at 0.5, 1, 3, 6 and 24 h postdrug and then every other day for 15 days. Although yohimbine (YOH) at doses of 0.5 or 5 mg/kg did not significantly alter recovery, a dose of 10 mg/kg of YOH did significantly accelerate recovery of beam-walking ability as compared to saline controls. This accelerated rate of recovery was indistinquishable from that produced by D-AMPH (2 mg/kg). A common mechanism of these drugs is the release of NE and the results strengthen our hypothesis that increased NE early after injury is beneficial for recovery from motor cortex injury. No significant effects on recovery early after injury occurred after injection of clonidine (CLON; 0.1 or 0.4 mg/kg), phenylephrine (2, 4 or 8 mg/kg), or prazosin (PRAZ; 2 or 4 mg/kg). These findings suggest that studies of beta-NE agonists and antagonists might be of benefit for elucidating the mechanism of NE-mediated recovery.

To investigate the importance of NE in maintaining recovery, brain-injured and sham operated rats were administered CLON (0.1 or 0.4 mg/kg), PRAZ (2 or 4 mg/kg), or YOH (0.5, 5 or 10 mg/kg) after they recovered on the beam-walking task (day 17 after surgery). Tests on the beam were conducted at 0.5, 1, 3, 6, 24 and 48 h after drug treatment. While YOH did not affect locomotor performance, injured but recovered rats given PRAZ or CLON showed a significant, and dose-dependent, worsening of beam-walking ability relative to the effects of these drugs in sham operates. These results suggest that the integrity and functional capacities of the alpha-NE receptors are crucially involved in maintaining recovery in this model of brain injury.

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253.4 NITROUS OXIDE ACTIVATES NIGRAL NEURONS IN THE RAT N.Passino 1,V.A.Peduto 3,G.P.Carcangiu 1, V.Boi 1 and G.P.Mereu 2. Depts. of Neurosciences 1 and of Exp. Biology 2.Inst.of Anesthesiology 3, Univ.of Cagliari, Italy.

N_2O is known to produce euphoria and analgesia at low (10-30%) and anesthetic effect at high concentration (50-75%). N_2O is also illegally used for its euphoriant properties.Electrophysiological and biochemical evidences suggest that different drugs of abuse,morphine and ethanol (ETH) among others, activate dopamine (DA) transmission.On the other hand ETH, barbiturates, benzodiazepines and general anesthetics (GAs) have a marked depressant action on the activity of those Pars Reticulata (PR), putative GABAergic, cells wich in turn exert a tonic inhibitory control on DA neurons of the Pars Compacta (A9). To further investigate on this issue we have studied the effect of N_2O in comparison with halothane (HLT) on the spontaneous firing of nigral PR and A9 cells.

Rats were prepared for recording as paralyzed and locally anesthetized subjects. N_2O or HLT (mixed to oxygen) were delivered in the trachea via the respiration pump. In 10 out 12 PR cells tested N_2O (25 to 75%) produced a concentration-related activation of their firing rate up to 100% above baseline.Two cells were inhibited.On the contrary HLT (0.5-3%) inhibited all PR cells tested (N=12) up to 2% of baseline. On A9 neurons (N=14) N_2O produced a stimulation proportional to the gas concentrations,up to 60% of baseline,which was completely eliminated by apomorphine (25 ug/Kg). HLT (0.5-1%) induced a temporary stimulation while higher concentrations (1.5-3%) inhibited A9 cells (N=9).In all experiments the cell activity returned to basal value by removing the gas administration.

The results suggest that the euphoriant effect of N_2O might involve DA activation but not DA receptors blockade. They also indicate that GAs differ in their electrophysiological effect in the CNS. It has been theorized that GAs and ETH fluidize the cellular membranes thereby facilitating the Cl channel opening. In this way the unspecific action of GAs may be transduced in a specific GABA-mimetic effect. Therefore it is likely that the HLT-induced activation of A9 neurons might be secondary to the inhibition of PR cells while the elucidation of the mechanism by which N_2O stimulates both A9 and PR neurons will require further investigations.Supported by Italian MPI grant.

253.5 BRAINSTEM AND HYPOTHALAMIC NOREPINEPHRINE LEVELS FOLLOWING ACUTE AND CHRONIC STRESS IN THE BORDERLINE HYPERTENSIVE RAT (BHR). V.P. Mitchell* and J.E. Lawler. Physiology Program and Department of Psychology, University of Tennessee, Knoxville, TN 37996.

The borderline hypertensive rat (BHR), the F1 cross between SHR and WKY, develops permanent blood pressure increases when exposed chronically to tail-shock stress. The current study sought to determine whether acute or chronic exposure to such stress results in changes in brain norepinephrine (NE) levels. Forty-four male BHR were divided randomly into four experimental (E) groups, and were sacrificed following either 3 days, or 4, 10 or 16 weeks of shock exposure. An additional 44 male BHR served as age-matched controls (C). Following decapitation, brains were sliced (300 um) and sections were identified for bilateral punches of the following nuclei: A1, A2 and locus coeruleus (LC) in the brainstem, and posterior (PH), dorsomedial (DMH), ventromedial (VMH), paraventricular (PVH), anterior (AH) and supraoptic (SO) in the hypothalamus. All samples were assayed for NE using HPLC-EC. Significant ($p < 0.05$) differences between E and C groups are depicted below (Mean±SEM).

NE (pg/ug protein)

Time	Nucleus	BHR (C)	BHR (E)
3 days	A2	20.1±4.6	39.8±2.3
	PH	32.0±5.4	12.5±1.5
	LH	27.3±4.9	15.1±2.1
	SO	23.9±2.2	15.4±1.6
10 weeks	A2	34.5±6.2	63.8±7.4
	A1	10.8±1.1	17.4±2.5
	LC	23.4±5.5	56.0±8.9
16 weeks	VMH	33.0±4.8	50.8±5.3
	PVH	47.0±5.6	77.8±8.8

These data indicate that BHR respond to an acute shock stress with a decrease in hypothalamic and an increase in brainstem NE. Chronic exposure induces a temporary increase in brainstem NE and, ultimately, increases NE in the VMH and PVH, two nuclei thought to be involved in sympathetic outflow. Thus, central catecholaminergic pathways appear to be involved in the chronic response of the BHR to tail-shock stress. (Supported by HL19680 & HL01395)

253.6 LOW DOSE CAFFEINE MODULATION OF CAUDATE DOPAMINE RELEASE AS MEASURED BY IN VIVO ELECTROCHEMISTRY IN THE FREELY MOVING RAT. M.E. Morgan, D. Dunn* and R.E. Vestal*. Clin. Pharmacol. and Gerontology Res. Unit, V.A. Medical Center, Boise, ID 83702, and Dept. Med. Univ. of Washington, School Medicine, Seattle, WA

Caffeine (C) is found in numerous over-the-counter medications and foods. Theophylline (T) is used in the treatment of asthma and chronic obstructive pulmonary disease. Both of these methylxanthines (MX) are central nervous system (CNS) stimulants. Although the MX are antagonists of the action of adenosine in the brain, the precise mechanisms of CNS stimulation are unknown. Adenosine has been shown to inhibit CNS neurotransmitter release. It is not clear, however, if the consequence of adenosine antagonism by the MX is always an increase in neurotransmitter release. We have previously shown that MX-induced caudate dopamine (DA) release is dose dependent. C, 250 and 500 µmole/kg, decreased caudate DA release by 30 and 68%, respectively. In contrast, T, 250 µmole/kg, increased and 500 µmole/kg decreased caudate DA release by 25 and 50%. Since C is more lipid soluble than T, these effects may be due to differences in MX caudate concentrations. Therefore, we examined the effects of a low dose of C on caudate DA release using in vivo electrochemistry in freely moving rats. Male Sprague-Dawley rats (250-350 gm) were anthestized with choral hydrate (150 mg/kg, i.p.) and ketamine (50 mg/kg, i.p.). Stearic acid carbon paste electrodes (250 µ) were implanted into the left caudate. Ag/AgCl reference and auxiliary electrodes were implanted through separate bur holes. The entire apparatus was fixed in place with cranioplastic cement. The animals were allowed a 24 hr recovery period. Electrochemical oxidations were made with a BAS DCV-5 voltammeter with the current output processed by semidifferentiation. After achieving a steady baseline release, animals were injected with C, 75 µmole/kg, i.p. Caudate DA release was monitored for 150 min.

Per Cent Control of Mean (± SEM) Baseline Peak Heights

Time (min)	0	20	40	60	100	150
Dose (µmole/kg)						
75	99±2	123±7*	133±8*	139±11*	116±5	107±5

n= 5 experiments; * $p < 0.05$ vs 0 min

C significantly increased DA release over a 60 min period with a gradual return to baseline by 150 min. These data taken together with our previous findings indicate that there is a concentration dependent threshold effect of C on caudate DA release. Such that low doses increase and high doses decrease caudate DA release.

253.7 EEG SYNCHRONIZATION AND SEDATION INDUCED BY SIMULTANEOUS BLOCKADE OF D-1 AND D-2 RECEPTORS INSTEAD OF SELECTIVE BLOCKADE OF EACH RECEPTOR SUBTYPE. P. Bo*, E. Ongini, G. Azan* and F. Savoldi*. Inst. of Neurology C. Mondino, Univ. Pavia, School of Med., I-27100 PAVIA, Italy

Blockade of dopamine (DA) receptors by neuroleptics tends to produce sedation as shown by increased sleep time, reduction of arousing response to sensory stimuli and slowing of brain electrical (EEG) activity. We evaluated the EEG and behavioral effects of the selective compounds SCH 23390 and raclopride which block either D-1 or D-2 receptor subtype, respectively. Groups of rabbits were prepared for the measurement of EEG activity (neocortex and hippocampus). EEG was analyzed visually and by spectral power analysis. Gross behavior was also observed. The unselective neuroleptic haloperidol (0.3 mg/kg iv) induced EEG synchronization (increased slow waves and spindle activity) associated with sedation. Power density spectrum in the range of 0.1 - 20 Hz markedly increased after treatment. The D-1 antagonist SCH 23390 by itself (0.03 - 0.3 mg/kg iv) produced little or no EEG change and no evidence of sedation. Periods of slow waves occurred sporadically. Computerized EEG analysis showed moderate increases of total power density. The D-2 receptor blocker raclopride alone (1 - 3 mg/kg iv) produced weak changes of the EEG activity, mostly short periods of slow waves, and slight increases of total power. No sedation was noted. Although both selective antagonists were studied at doses above those minimally effective, they produced slight EEG and behavioral changes which were not comparable with the marked actions produced by haloperidol. Interestingly, when raclopride (1 mg/kg) was given after treatment with SCH 23390 (0.03 mg/kg) there was a marked synchronized EEG activity associated with a state of sedation and diminished responsiveness to sensory stimuli.
The data indicate that EEG synchronization and sedation, classically associated with neuroleptic treatment, do not depend upon the selective blockade of either D-1 or D-2 receptors, but, instead, require concurrent blockade of both receptor subtypes.

253.8 ONE WEEK HIGH DOSES OF HALOPERIDOL REDUCES MARKEDLY THE NUMBER OF SPONTANEOUSLY ACTIVE MIDBRAIN DOPAMINE NEURONS. Rex Y. Wang and Melissa Tsai*, Depts. of Psychiatry and Behavioral Science and Pharmacology, SUNY at Stony Brook, Stony Brook, NY 11794.

Chronic haloperidol treatment (CHAL; 0.5 mg/kg/day s.c.) has been shown toproduce a time-dependent reduction in the number of spontaneously active dopamine (DA) neurons in both the substantia nigra pars compacta (A9) and ventral tegmental area (A10). However, the reduction in the number of active DA cells/track occurred much earlier in A10; after one week, the number of active A10 DA cells/track was already significantly below control, whereas the number of active A9 DA cells/track remain unchanged until after three weeks (White and Wang, Life Sci., 32:983-993, 1983). The CHAL-induced effect was reversed by the DA agonist apomorphine (APO), suggesting that the effect was due to depolarization inactivation (DI). Acute HAL produced a rate elevation and, at higher doses, also caused DI in a subpopulation of DA cells, indicating that HAL-induced DI is dose-dependent (Hand et al., Brain Res., in press). The aim of the present study was to decide whether treatment of rats with high doses of HAL shortens the time needed for the development of DI for both A9 and A10 DA cells.

Male Sprague-Dawley rats were treated with HAL (5 mg/kg/day s.c.) for 3 or 7 days. Standard extracellular single unit recording techniques were used to monitor the DA neuronal activity and to count the number of spontaneously active DA cells per track as previously described.

Our preliminary results show that rats treated with HAL for one week resulted in an almost total absence of spontaneously active DA cells in both A9 and A10 regions. The effect produced by one week 5 mg/kg/day of HAL treatment is significantly greater than that produced by 6 to 8 weeks continuous treatment with 0.5 mg/kg/day of HAL. Even with 3 days treatment, HAL induced a marked reduction in the number of spontaneously active DA neurons; the effect was greater in A10 than in A9. The reduction of spontaneously active DA neurons caused by HAL was reversed by acute APO (100 µg/kg iv), suggesting that the HAL-induced effect was due to the process of DI. In short, high doses of HAL caused an earlier onset of DI for both A9 and A10 DA cells.

Combined, the results from the present and previous studies indicate that HAL-induced DI is not only time-dependent but also dose-dependent. If antipsychotic drug (APD)-induced DI of A10 DA cells is associated with the alleviation of schizophrenic symptoms as inferred from previous studies, our results may form the basis for further studies on "rapid neuroleptization." Attention should be paid particularly to those atypical APDs which may have lower potential for causing neurological side effects. (Supported by USPHS Grants MH-41440, 41696 and 00378 awarded to R.Y.W.).

253.9 ANTIDROMIC ACTIVATION OF MESO-ACCUMBENS DOPAMINE NEURONS FOLLOWING CHRONIC HALOPERIDOL TREATMENT. J.A. HARPER AND REX Y. WANG, Dept. of Psychology, SUNY at Old Westbury, Old Westbury, NY 11568 and Dept. Psychiatry and Behavioral Science, SUNY at Stony Brook, Stony Brook, NY 11794.

Chronic haloperidol treatment (CHAL) has been shown to cause a time-dependent reduction in the number of spontaneously active A9 and A10 dopamine (DA) neurons, and to induce an irregular firing pattern in many of the cells that remain active (White and Wang, 1983a). Both of these effects occur earlier and to a greater extent in A10 cells than in A9 cells. Since the time course for the reduction of A10 DA activity corresponds to the time course of decreased plasma HVA levels of schizophrenics and clinical improvement of schizophrenic symptoms, it was proposed that the inactivation of A10 and A9 DA neurons produced by CHAL may be related to the delayed onset of pharmacotherapy and extrapyramidal side effects (EPS), respectively. Subsequent studies employing typical and atypical antipsychotic drugs (APDs) supported this hypothesis (Chiodo and Bunney, 1983; White and Wang, 1983b). Chiodo and Bunney (1983) presented some evidence suggesting that the remaining active A10 DA cells following chronic APDs treatment are primarily, if not exclusively, mesocortical neurons. They suggest that the prefrontal mesocortical DA system may not be involved in some form of psychosis. However, this conclusion is premature because whether mesolimbic DA cells remain spontaneously active has never been systematically tested. The present study was designed to determine whether some of these remaining active DA cells following CHAL are meso-accumbens projecting cells.

Male Sprague-Dawley rats (350-450g), maintained on haloperidol (1.5 mg/kg/day) dissolved in drinking water were used. Spontaneously active A10 DA cells were identified using standard electrophysiological criteria (e.g. bi- or triphasic waveforms with a width of >2.5 ms and an inflection in the initial positive component, firing rate of 1-10 Hz etc.); these cells were then antidromically activated from the nucleus accumbens (NAc).

Many spontaneously active DA cells following CHAL can be antidromically activated from the NAc. To ensure that the results were not due to stimulating mesocortical DA fibers of passage, the experiments were repeated in CHAL rats whose prefrontal and cingulate cortices had been lesioned 1 week earlier. The cortical lesions presumably caused retrograde degeneration of mesocortical DA fiber projections because few of them have axon-collaterals. Half of spontaneously active DA neurons could still be activated antidromically. These results support the contention that a small portion of both meso-limbic and meso-cortical DA cells remain active following CHAL. (Supported by USPHS Grants MH-41440, 41696, 00378 to RYW. and RR-08180 to JAH).

253.10 SUPERSENSITIVITY TO DOPAMINE AND CHOLECYSTOKININ OF NUCLEUS ACCUMBENS CELLS FOLLOWING CHRONIC TREATMENT WITH HALOPERIDOL OR CLOZAPINE. Xiu-Ti Hu and Rex Y. Wang, Dept. of Psychiatry and Behavioral Science, SUNY at Stony Brook, Stony Brook, NY 11794.

A subpopulation of dopamine (DA) neurons in the ventral tegmental area (VTA or A10) also contain cholecystokinin (CCK); DA/CCK projections terminate primarily in the medial part of the nucleus accumbens (NAc). Lesions of ascending DA fibers in the medial forebrain bundle by 6-hydroxydopamine resulted in the development of denervation supersensitivity of NAc cells to both DA and CCK (Hu and Wang, Neurosci. Abstr. 11:743, 1985). Chronic HAL treatment (CHAL) induced an increase of binding sites for both DA and CCK. Curiously, a recent abstract by Debonnel and de Montigny (Neurosci. Abstr. 12:1319, 1986) reported that CHAL caused a supersensitivity of NAc cells to CCK but not to DA; they suggested the effect produced by DA in the NAc was mediated by an adrenoceptor other than D1 or D2 DA receptor subtypes. This view is not supported by our finding that the inhibition produced by iontophoresis of DA (ionto-DA) can be blocked by either the typical antipsychotic drug (APD) HAL or atypical APD clozapine (CLOZ). In the present study, we decided to clarify further the effects produced by chronic treatment with APDs on NAc cells.

Male Sprague-Dawley rats were treated with either HAL (0.5 mg/kg/day s.c.) or CLOZ (20 mg/kg/day, s.c.) for 4 weeks. A seven-day withdrawal period was allowed before the experiments began. Both spontaneously active cells and glutamate (GLUT)-evoked activity were studied by using the technique of extracellular recording and microiontophoresis. ID50s for DA and ED80s for CCK on NAc neurons recorded from both APD treated and control groups were systematically compared.

Our results confirm the finding that the sensitivity of NAc cells (n=18) to ionto-CCK was markedly enhanced in CHAL rats. However, unlike the report of Debonnel and de Montigny (1986), we observed that CHAL also enhanced the inhibitory responses of NAc cells (n=10) to ionto-DA. When compared to those of controls the dose-response curves for both CCK and DA shifted to the left in CHAL rats. Moreover, the responses of NAc cells to both D1 agonist SKF-38393A (n=16) and D2 agonist LY-171555 (n=13) were enhanced, suggesting that CHAL-induced DA supersensitivity may be mediated via both D1 and D2 DA receptor subtypes. Similarly, the sensitivity of NAc cells to ionto-DA (n=8) and CCK (n=14) was also strikingly increased in the CLOZ rats.

In conclusion, our results suggest that chronic treatment with APDs is effective in inducing the development of supersensitivity of NAc cells to both DA and CCK. They support the view that CCK might have an important role in mediating the therapeutic actions of APDs. (Supported by USPHS Grants MH-41440, 41696 and 00378 to RYW).

OPIATES, ENDORPHINS AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS III

254.1 DYNORPHIN A SELECTIVELY INHIBITS THE RELEASE OF OXYTOCIN FROM ELECTRICALLY STIMULATED ISOLATED RAT NEUROHYPOPHYSES. C.A. Bondy*, H. Gainer, J.T. Russell*, LNN, NICHD and LNC, NINCDS, NIH, Bethesda, MD 20892.

For some time it has been known that physiologically stimulated oxytocin (OT) release *in vivo* is under endogenous opioid inhibition and that this effect appears to occur at the level of the axon terminals of the neural lobe. Studies of the effects of various opiates on hormone secretion from isolated neural lobes *in vitro* , however, have produced confusing results, probably due to the use of non-specific opiate agonists and diverse stimulus protocols. Since it has recently been established that substantial amounts of dynorphin A are co-localized with vasopressin (VP) in the magnocellular terminals of the neural lobe and that the opiate receptors of the neural lobe are restricted to the κ subtype, we hypothesized that the dynorphin A (DYN A) co-packaged and therefore co-released with VP inhibits release from neighboring OT terminals. In order to test this hypothesis we made use of the previously defined frequency dependent pattern of VP and OT secretion in our system. Using isolated rat neurointermediate lobes(NILs) incubated *in vitro* we have shown that a constant 600 pulse stimulus delivered at a frequency of 4 Hz results in evoked release of both VP and OT that is significantly above baseline, but only ≈20% of maximal, and that secretion is further facilitated by increasing the frequency of the 600 pulses up to a maximum level of release at 12 Hz for VP and 20 Hz for OT. If the relevant opioid inhibitor is being released from VP terminals, we expected that the effect of exogenous DYN A might be evident at a submaximal release frequency (4 Hz) while obscured by high levels of endogenous DYN A release at 12 Hz. Conversely, we predicted that naloxone would have little effect at 4 Hz and maximal effect at 12 Hz where VP (and therefore DYN A) secretion is maximal. The stimulus protocol consisted of 600 pulses of 3V, 0.5ms delivered via a platinum electrode impaling the NIL. Each NIL received two periods of electrical stimulation at the same frequency (S1 and S2), with or without drugs during S2. We tested the effects of DYN A 1-8 and 1-17 and naloxone at stimulus frequencies from 4 to 30 Hz with 6-10 individual NIL's for each experiment.

Both dynorphin 1-8 and 1-17 (2μM) produced a significant reduction in OT release during the 4 Hz stimulus (<50% of control) and this effect was abolished by naloxone (10 μM). Neither form of DYN A, however, had any effect at stimulus frequencies of 12 or 30 Hz at concentrations up to 10μM. Naloxone (10μM) did not affect OT release during the 4 Hz stimulus, however it produced a highly significant amplification of OT release at a stimulus frequency of 12 Hz (≈280%). None of these agents had a significant effect on VP release at any frequency tested. A frequency dependent secretion curve (4, 8, 12, 20 &30 Hz) was established for OT and VP release in the presence of naloxone, and it was found that both hormones now attained maximal release at 12 Hz. These data support the hypothesis that DYN A released in parallel with VP during in vitro stimulations of the rat NIL inhibits simultaneously stimulated OT release. While these findings highlight the complex interactions that may occur even in relatively simple systems, their physiologic relevance remains to be explored.

254.2 OPIATE TOLERANCE ALTERS NEUROTRANSMITTER REGULATION OF THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS. D.M. Ignar and C.M. Kuhn. Duke Univ. Med. Ctr., Durham, NC 27710.

The hypothalamo-pituitary-adrenal axis of the rat is stimulated by administration of mu and kappa opioid receptor agonists. Tolerance develops to this effect following chronic administration of these agents. In order to determine the interactions of opioid neurons with other neural mediators of CRF release, a model of opiate tolerance in rats was employed.

Adult male Sprague-Dawley rats were injected with increasing doses of morphine (20 mg/kg to 40 mg/kg), the kappa agonist U50,488 (1 mg/kg to 5 mg/kg), or saline twice daily for 5 days. Challenge doses were given subcutaneously 36 hours after the end of chronic treatment. Rats were decapitated 1 hour after drug administration and blood was collected for measurement of serum corticosterone by radioimmunoassay.

Complete tolerance to morphine (10 mg/kg) or U50,488 (1 mg/kg) occurred after chronic morphine or U50,488 treatment, respectively. No cross-tolerance of morphine tolerant rats to U50,488 or U50,488-treated rats to morphine was observed.

Administration of drugs which stimulate HPA axis secretion to morphine or U50,488 tolerant rats yielded the following results: 1) The stimulatory effect of physostigmine (0.1 mg/kg) was twice the control level in morphine-treated rats while its effect was almost negligible in U50,488 tolerant rats. 2) The quipazine response of morphine tolerant rats was reduced to 50% of the control response while chronic U50,488 had no effect. 3) The effect of clonidine (0.025 mg/kg) was significantly attenuated in both morphine and U50,488 treated rats.

These results suggest that both mu and kappa receptors modulate the noradrenergic neurons involved in CRF release, whereas mu receptors may specifically regulate serotonergic neurons and kappa receptors modulate cholinergic neurons. The absence of cross-tolerance may be explained by opioid subtype specific neural adaptations which occur after chronic opiate administration.

254.3 TIME COURSE OF RETURN OF MORPHINE STIMULATION OF PROLACTIN RELEASE IN THE LACTATING FEMALE MODEL. P. Callahan, J. Janik and J. Rabii. Department of Biological Sciences, Rutgers Univ., Piscataway, NJ 08855.

We have previously reported that the lactating female model is insensitive to morphine stimulation of prolactin release. This insensitivity appears to be due to the physiological state of suckling. Post-partum females in which suckling was terminated for 4 days showed a significant morphine induced prolactin increase in 50% of the animals tested. The purpose of these studies was to further investigate the time course of this insensitivity to morphine stimulation of prolactin release. In addition, the effect of morphine administration on the activity of the TIDA neurons was determined by measuring DOPA accumulation in the median eminence of the lactating female.

Lactating females between days 4-10 post-partum were used in all studies. These dams were separated into three groups. In all groups, animals received an injection of morphine sulfate (5 mg/kg, iv) on day 4 post-partum, 2 hours after pups were removed. The pups were sacrificed and the dams were housed individually until they were again injected with morphine on day 8 (group 1), day 10 (group 2) or day 12 (group 3). Blood samples were withdrawn through previously implanted jugular cannulae, immediately prior to and 15, 30 and 45 minutes after injection. Only 50% of the dams in group 1 showed a significant morphine induced prolactin increase. 75% of the dams in group 2 and 100% of the dams in group 3 showed a significant prolactin secretory response to morphine administration. These results suggest that there is a recovery period following the lactation state which is responsible for the return of the pre-lactation sensitivity of prolactin to morphine stimulation. Additionally, it is interesting to note that DOPA accumulation in the median eminence was not altered by morphine administration in the lactating model. These results suggest that the insensitivity to morphine stimulation of prolactin release may be mediated, at least in part, by an insensitivity of the TIDA neurons to inhibition by morphine administration. (Supported by the Busch Memorial Fund.)

254.4 **CHANGING ROLE OF NE IN EOP INHIBITION OF LH SECRETION IN NEONATAL FEMALE RATS.** **E. Field, G. Kuhn and M. Doron*. Dept. of Pharmacology, Duke University Medical Center, Durham, NC 27710.**

Luteinizing hormone (LH) inhibition by CNS endogenous opioid peptides (EOP) requires functional noradrenergic (NE) neurons in prepubertal and adult female rats. Inhibitory EOP tone is particularly pronounced in neonatal females, as evidenced by the marked LH rise after naloxone (NAL). However, adrenergic stimulation does not elicit similar LH responses in female neonates, suggesting that EOP modulation of LH secretion at that age may involve neuronal pathways other than or in addition to NE. To test this hypothesis, we pretreated adult, 25 and 5 d.o. rats with NE antagonists and synthesis inhibitors before challenge with NAL and measured subsequent LH responses.

NAL (0.1 - 2.0 mg/kg sc) or saline was administered 20 min prior to decapitation in 5 d.o. and 25 d.o. females to generate a dose response curve, and a supra-maximal concentration of 2 mg/kg NAL was chosen for later experiments. In 5 d.o., 25 d.o. and adult diestrous rats, NAL induced a 12-, 4- and 2-fold rise in LH, respectively, within 20 min ($p < 0.05$), suggesting a functional but declining EOP inhibition of LH. In 25 d.o., the α-adrenergic antagonist, phentolamine (10 mg/kg ip) completely blocked the NAL-induced LH rise ($p < 0.05$). Diethyldithiocarbamate (DDC, 400 mg/kg ip), a NE synthesis inhibitor, or reserpine (0.5 mg/kg ip) also prevented the NAL-induced LH rise at this age ($p < 0.05$). DDC also blocked the post-NAL rise in LH in intact adult rats ($p < 0.05$). In 5 d.o. females, on the other hand, phentolamine, reserpine or DDC only slightly attenuated the LH rise following NAL ($p < 0.05$). However, NAL still elevated serum LH 7-9 fold after these drug treatments ($p < 0.05$). Interestingly, DDC itself induced a rise in LH in 5 d.o., but not 25 d.o. or adult, female rats ($p < 0.05$). Clonidine (CLON, 0.1 mg/kg sc), an α-adrenergic agonist, had no effect on serum LH levels in 10 d.o. female rats. In contrast, NE inhibition markedly raised serum corticosterone (CS) levels in neonatal and older rats.

These results support our hypothesis that stimulation of NE secretion mediates the rise in LH following opiate blockade in prepubertal and adult female rats, but plays at most a minor role in neonates. Furthermore, the LH rise following DDC treatment of neonatal, but not older, rats and the lack of LH response to CLON in neonates suggests that the post-synaptic α-adrenergic receptor modulating LH secretion in adults, unlike CS, is not functional in neonates. Alternatively, the balance of pre- and post-synaptic effects of NE agents on LH might change during ontogeny. In female neonates, EOP neurons may inhibit LH secretion by directly inhibiting LHRH neurons or through other pathways, e.g. serotonergic or dopaminergic, in addition to NE. **(Supported by NIDA grant #DA-02739.)**

254.5 THE MU-1 ANTAGONIST NALOXONAZINE DOES NOT BLOCK THE BETA-ENDORPHIN OR DADLE INDUCED PROLACTIN INCREASE DURING LACTATION. J. Janik, M. Baumann*, P. Callahan and J. Rabii. Department of Biological Sciences, Rutgers Univ., Piscataway, NJ 08855.

We have previously reported that lactating females are not sensitive to morphine stimulation of prolactin release. However, this model does show a prolactin secretory response to both beta-endorphin and d-ala-d-leu-enkephalin (DADLE) administration which is not reversed by naloxone. It has been reported that naloxonazine (NAZ) is a specific antagonist of the mu-1 site and blocks the morphine induced prolactin increase in male rats (Spiegel et al., Sci. 217:745, 1982). The purpose of this study was to examine the effects of this antagonist on the beta-endorphin and DADLE induced prolactin increase in the lactating female model.

Lactating females between days 6 and 10 post-partum were used in all studies. Animals received intraventricular implants on day 2 post-partum and were allowed to recover for at least 6 days following this surgery. They were implanted with jugular cannulae 24 hours prior to their use in an experiment. NAZ (20 mg/kg, iv) or saline was administered at the time of jugular cannulation. On the day of the experiment, animals were separated from their pups 2 hours prior to the administration of the opiate peptides. Blood samples were withdrawn immediately prior to and 15, 30 and 45 minutes after beta-endorphin (0.5, 2.5, 5 or 10 µg, ivt) or 5, 15 and 30 minutes after DADLE (2.5, 5 or 10 µg, ivt) administration.

All doses of beta-endorphin and DADLE produced a significant increase in circulating levels of prolactin. There was no difference between the saline and NAZ pretreated groups and NAZ alone had no effect on basal levels of prolactin. It appears that these peptides do not produce an increase in prolactin via a mu-1 opiate receptor subtype. These results confirm our previous findings that this model is insensitive to the mu agonist morphine with respect to stimulation of prolactin release. It appears likely that this particular receptor subtype does not play a physiologically significant role in the regulation of prolactin release during lactation. (Supported by the Busch Memorial Fund and the Anne B. and James H. Leathem Scholarship Fund.)

254.6 **ESTRADIOL/PROGESTERONE REGULATION OF HYPOTHALAMIC NEUROPEPTIDE mRNA LEVELS.** P. Camp and J.D. White. Dept. Medicine, Div. Endo., SUNY, Stony Brook, NY 11794.

Depending on the treatment duration, estradiol (E_2) and progesterone (P_4) can induce or block LH and prolactin (PRL) surges independently in ovariectomized (OVX) rats by acting within the hypothalamus. The goals of these studies are to determine: 1) the E_2/P_4 effect on several hypothalamic neuropeptide mRNA levels and 2) whether the mRNA levels can be correlated with E_2/P_4 effects on LH or PRL levels. One week after OVX (day 0), E_2 Silastic capsules were placed sc at 0900. Some rats also received P_4 capsules sc at 0900 on either day 0, 2 or 4. In this paradigm, serum steroid levels were within the physiological range. Rats were sacrificed at 1500 on day 0, 1, 2, 3 and 5. Control (OVX) animals were sacrificed on day 0 and day 5. Blood was collected for RIA of LH and PRL levels. Hypothalami were dissected and rapidly frozen on dry ice for mRNA determination. Total RNA was isolated using an SDS/urea extraction, repeated phenol/chloroform extraction and ethanol precipitation. RNA was separated using formaldehyde denaturing agarose gel electrophoresis and a MOPS buffer system. The RNA was transferred to nylon membrane by electrotransfer,then was covalently cross-linked to the membrane with uv light and used for northern analysis.

In the intial analysis the mRNAs coding for preproenkephalin A and for preprovasoactive intestinal peptide (VIP) were quantitated. The preliminary results suggest that estradiol initially decreases then increases preproenkephalin mRNA relative to the levels measured in OVX animals. Progesterone treatment further increases preproenkephalin mRNA levels. These steroids appear to have a similar effect on preproVIP mRNA. The effects of E_2/P_4 on tyrosine hydroxylase mRNA and preproopiomelanocortin mRNA content in the hypothalamus will also be assessed. (Supported by NIH MH-42074)

254.7 PRESENCE OF β-CASOMORPHIN IMMUNOREACTIVE MATERIAL IN PLASMA OF BEAGLE NEONATES AFTER MILK INTAKE.
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β-casomorphin-7 (BCM), a potent μ-opioid agonist, is a fragment of bovine β-casein (C). Administration of BCM elicits analgesia, enhances release of insulin and somatostatin, and induces hypoventilation. If BCM exists in plasma, it may elicit opioid activity in neonates since their blood brain barrier is not well developed. We are reporting BCM immunoreactive material (BCMIR) in the plasma of 2- and 4-wk old beagle puppies after feeding C using a sensitive, efficient new procedure for extraction of BCM. Blood (2.5 ml) was drawn at 0, 2, 4, and 6 hr after feeding 25 to 35 ml of either C or a soy protein (non-casein) based formula to puppies after a 3-hr fast. BCMIR was extracted from plasma and assayed by RIA with I^{125}-BCM and anti-bovine BCM. The extraction procedure yielded recoveries of 78±3 (mean±SD, n=12) in samples up to 0.05 p-moles per ml plasma and 90.5±2.5 with 0.5 p-moles per ml plasma. Feeding of C resulted in significant elevation of BCMIR in both age groups. In contrast, no change was noted after intake of soy protein. Baseline BCMIR was detected in all puppies. Older puppies reached peak values at 2 hr while younger puppies peaked at 4 hr and remained high at 6 hr as can be seen from table below.

HR AFTER FEED	0	2	4	6#
C (N=8) 2 WK	0.13±.01	0.14±.01	0.26±.05*	0.24±.02*
C (N=4) 4 WK	0.08±.01	0.14±.01*	0.14±.01*	-
SOY(N=5) 2 WK	0.20±.03	0.22±.01	0.23±.02	-

mean ± SD of p-moles/ml plasma, * p< .01, # n=3

The BCMIR was resistant to proteolysis though BCM added to the same plasma was rapidly degraded. Due to the negligible cross-reactivity of BCM antibody to BCM-4, 5, and 6, BCMIR is not likely to be smaller than BCM. We conclude that an increase in BCMIR was detectable in plasma of beagle neonates after C feeding, but not soy feeding; the metabolism of BCMIR appeared age dependent. We speculate that the observed levels of BCMIR may have physiological significance since BCM infusion of similar magnitude has been shown to alter pancreatic endocrine function.

254.8 OPIATE BLOCKADE IN TREATING BULIMIA - A COMPARISON OF LOW-DOSE VS HIGH-DOSE NALTREXONE.
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Bulimia, the syndrome of binge-eating and purging, is a disorder of unknown etiology. Endogenous opiates, which are known to modulate ingestive behavior in mammals, may play a role in the genesis of this disorder, and appear elevated in bulimic individuals (Fullerton DT, et al. Psychol Medicine, 16:59, 1986). Two open-studies of naltrexone, a long-acting, orally administered opiate blocker, have suggested that this medication may be of use in treating bulimia (Jonas JM, Gold MS, Lancet, i:807, 1986; Jonas JM, Gold MS, Int J Psych Med, 16(4):305-309, 1986-87), while a blind study using naloxone suggested that this short-acting opiate blocker also attenuated binging (Mitchell JE, et al., Biol Psychiatry, 21:1399, 1986). One important question which has not been answered is whether dosages of naltrexone (50-100 mg per day) which block the effects of exogenous opiates and which might therefore act at the mu receptor are effective in decreasing binging behaviors in humans. The answer to this might reveal clues as to the mechanism of action of naltrexone in bulimia, and would aid in the clinical use of this medication. We compared two dosage schedules of naltrexone in a group of 10 bulimic individuals. All patients met DSM-III criteria for bulimia, and all were binging and purging on a daily basis. Five subjects received 50-100 mg of naltrexone (low-dose) daily for 4-6 weeks, and 5 subjects received naltrexone in doses of 200-300 mg daily (high-dose) for 4-6 weeks. In the high-dose group, there were significant reductions in binging ($p < .01$), and purging ($p < .01$) at the end of the study. In contrast, there was no significant effect on binging or purging in the low-dose group with 3 subjects experiencing no change in symptoms while 2 subjects had a decrease of less than 50%. Three subjects in the low-dose group then received high-dose naltrexone. Two of the 3 experienced complete resolution of symptoms, and 1 subject reported a 75% reduction in binging and purging. It appears from these open-study design, preliminary data that dosages of naltrexone which adequately block exogenous opiates have no effect on binge-eating. One explanation of this phenomena may be that different opiate receptors are responsible for dependency and addiction (possibly mu) while other receptors underlie modulation of feeding behavior (possibly kappa). If naltrexone is to be used in treating bulimia, higher doses may be needed. Opiate blockers with greater affinity for the kappa receptor may prove superior in the treatment of eating disorders.

254.9 **COMPARISONS OF FEEDING ELICITED BY MORPHINE AND DYNORPHIN(1-13) MICROINJECTIONS INTO SELECTED BRAIN SITES.**
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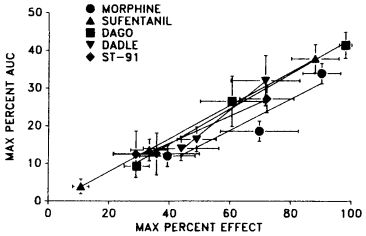
Microinjections of both morphine and dynorphin(1-13) (DYN) into the ventral tegmental area (VTA) have been shown to elicit dose-dependent feeding in satiated animals (Hamilton & Bozarth, Soc. Neurosci. Abstr. 12:412, 1986). In addition, animals will lever-press to self-inject morphine into the VTA, indicating that opioids in this region are rewarding. It was of interest to examine the effects of these two compounds in a number of other brain regions where opioids have been associated with either feeding or reward. Male, Long-Evans rats were permanently implanted with stainless steel guide cannulae aimed at the VTA, the nucleus accumbens (NAS), the paraventricular nucleus of the hypothalamus (PVN), the periaqueductal gray area (PAG) immediately dorsal to the VTA, or the substantia nigra--pars reticulata (SNR) which receives dense dynorphin innervation. Either morphine (0, 1, 3, or 10 nmoles) or DYN (0, 0.003, 0.03, 0.1, 0.3, or 3 pmoles) in a fluid volume of 0.5 μl was delivered in random dose order. Behavior was observed and recorded for 15 minutes following drug administration. Animals received all doses of both drugs; morphine and DYN administration was counterbalanced within groups.

Consistent with our earlier data, DYN in the VTA was 50,000 times more potent than morphine in eliciting feeding. Eating elicited by morphine injected into the NAS was lower than but not significantly different from VTA morphine. DYN in the NAS produced feeding duration scores similar to those for the VTA at doses up to 0.1 pmoles; however, at the two higher doses VTA DYN produced significantly more feeding than NAS injections. Morphine in the PVN failed to produce feeding; in fact, at the higher doses behavior became erratic and disorganized, and animals frequently appeared stuporous. DYN in the PVN, however, elicited a weak dose-related feeding response. Preliminary data suggest that neither drug in the PAG is effective in eliciting feeding. Morphine in the SNR did not produce feeding, and DYN in the SNR failed to elicit a robust feeding response even at 100 times higher doses than those effective in the VTA. SNR DYN injections appeared to increase grooming behavior. Drinking was not increased significantly by either compound in any of the brain regions examined.

254.10 TIME COURSE OF TOLERANCE DEVELOPMENT TO ANTINOCICEPTIVE AGENTS IN RAT SPINAL CORD.
Craig W. Stevens and Tony L. Yaksh*, Dept. of Pharmacology, Mayo Graduate School of Medicine, Rochester, MN 55905

The time course of tolerance to chronically infused antinociceptive agents in spinally catheterized rats was examined by daily hot plate testing to determine whether agents acting at the same and/or different sets of receptors display distinguishable rates of tolerance development. Male Sprague-Dawley rats (250-300 g) were tested on a 52.5°C hot plate on the morning of day 0, implanted with a lumbar intrathecal catheter connected to a continuous-delivery osmotic minipump (Alza Corp. Model 2001, 1μl/h) and tested daily thereafter at 10:00 a.m. for one week. Separate groups of rats (N = 6-8 per dose) received saline or 1 of 3 half-log spaced concentrations of μ opioid receptor agonists; morphine (2, 6 or 20 nmol/h), sufentanil (0.06, 0.2 or 0.6 nmol/h), D-ala^2,N-methyl-phe^4,gly^5-ol-enkephalin (DAGO; 0.1, 0.3 or 1 nmol/h); a δ opioid agonist, D-ala^2-D-leu^5-enkephalin (DADLE; 2, 6 or 20 nmol/h); or an α_2-adrenergic agonist, the polar analog of clonidine, ST-91 (3, 10 or 30 nmol/h). All treatment groups displayed a dose-dependent increase in hot plate latency which were maximum on day 1 and returned to saline-infused control levels by 3 to 5 days. The raw latency (in secs) was converted to maximum percent effect (MPE) by the formula: MPE = (post-pre)/(60-pre)*100. The area under the time-course curve (AUC) was calculated from these MPE-values using a trapezoidal approximation and expressed as a percent of maximum possible area under the time-course curve (max percent AUC). Thus if an infusion dose of an agent produced maximum peak response (MPE = 100%) for each day after pump implant, then the max percent AUC (integral of the time-course curve) would also equal 100%. The ratio of the peak response (max percent effect) to the integral of the time-effect curve (max percent AUC, an index of the rate constant for the development of tolerance) was independent of dose and similar for all drugs (see Fig.). This similarity of rate constants suggests the involvement of common mechanisms in the development of tolerance to these several drugs. Given the absence of a symmetrical cross tolerance between μ, δ and α_2-preferring drugs (Soc. Neurosci. Abst. 12:618, 1986) the tolerance mechanisms, though common may be independent. (NIH grant DA-02110 and Mayo Fdn.)



254.11 I.P. INJECTION OF CSF FROM OPIATE-ABSTINENT RATS PRECIPITATES ABSTINENCE SYNDROME IN OPIATE-DEPENDENT MICE. D.H. Malin, D.S. Marullo*, J.R. Lake*, V. Balasubramanyam*, J.B. Murray*, and P.A. Farley*. (SPON: S.R. Burzynski) Univ. of Houston-Clear Lake, Houston, Texas 77058.

In previous studies from this laboratory, CSF from opiate abstinent rats precipitated opiate abstinence signs when infused into the third ventricle of opiate-dependent rats. To aid the characterization of the active CSF factor, a more convenient non-stereotaxic assay procedure was desirable. We not report that i.p. injection into morphine-pelleted mice can serve as such a procedure.

Subjects were 12 30-35g male ICR mice and 24 300g Sprague-Dawley rats. Sixteen donor rats were rendered dependent by one week of subcutaneous morphine infusion (1.8 mg/kg/hr) via Alzet 2ML1 osmotic minipump. Eight donor rats were infused by the same procedure with saline alone. Under ether anesthesia, 75 µl CSF was withdrawn from the cisterna magna of each rat six hours after removal of the minipump (allowing for clearance of morphine).

Eight mice were rendered dependent by s.c. implantation with pellets containing 75 mg morphine base. Four mice served as "sham" implanted" controls. Three days after implantation, each mouse was injected i.p. with 150 µl of CSF. Four mice (Group A) received a morphine pellet followed by CSF from morphine dependent and abstinent rats. Four mice (Group B) received a morphine pellet followed by CSF from saline-infused rats. Four mice (Group C) were sham implanted followed by CSF from morphine-infused rats.

Each mouse was placed on an elevated platform and observed for abstinence signs under "blind" conditions for 45 minutes. The predominent sign observed in the first 10 minutes after injection was abdominal writhing. Shakes, tremors, seizures, ataxia and jumping from the platform were observed after 20 mins. Overall signs across all categories are presented below.

Table 1. Overall Abstinence Signs (mean + SEM)

A. Morphine Treated Donor/Morphine Treated Recipient	25.3 ± 8.1
B. Saline Treated Donor/Sham Treated Recipient	0.5 ± 0.3
C. Morphine Treated Donor/Saline Treated Recipient	1.5 ± 0.3

ANOVA revealed a significant treatment effect, $F(2,9)=9.0$, $p < .01$. Dunnett's multiple comparison procedure indicated that Group A showed significantly more signs than either of the other groups, $p < .01$. These results are consistent with the hypothesis that chronic opiate treatment induces the occurrence of an abstinence-precipitating substance with opiate-antagonist properties. (Supported by NIDA Grant R03 DA03966 and UH-CL Organized Research and Alumni Assn. Grants.)

254.12 A ROLE FOR THE NUCLEUS ACCUMBENS IN OPIATE DEPENDENCE. G.F. Koob, T.L. Wall, and F.E. Bloom, Division of Preclinical Neuroscience and Endocrinology, Scripps Clinic and Research Foundation, 10666 N. Torrey Pines Rd., BCR 1, La Jolla, CA 92037

Previous work in our laboratory has established that the region of the nucleus accumbens is an important substrate for the acute reinforcing effects of heroin in non-dependent rats with limited access to the drug. (Vaccarino, Bloom and Koob, 86:37-42, 1985). Injections of the hydrophilic opiate antagonist methylnaloxonium into the nucleus accumbens were effective in increasing heroin self-administration at significantly lower doses than those observed after injections of methylnaloxonium into the lateral ventricle or the ventral tegmental area. However, previous studies of the neural substrates of physical dependence to opiates have revealed multiple sites of action including the periacqueductal grey and the dorsal thalamus. In studies on opiate physical dependence, rats were trained on a fixed ratio-15 schedule of responding for food (45 mg Noyes pellets) and were implanted with chronic indwelling intraventricular or intracerebral cannulas aimed at the periaqueductal grey, dorsal thalamus or nucleus accumbens. The animals were made physically dependent on opiates by subcutaneous implantation of three 75 mg morphine pellets over 6 days. Very low doses of subcutaneously injected naloxone (50 µg/kg) produced a dramatic suppression of operant responding. Similar effects were observed with intracerebroventricular (ICV) administration of methylnaloxonium in doses of 60 to 250 µg. Local injections of methylnaloxonium into the periacqueductal grey or dorsal thalamus produced dose effect functions similar to that following ICV injection. Injections of methylnaloxonium into the nucleus accumbens produced a suppression of responding at significantly lower doses of methylnaloxonium (4-16 nanograms). These results suggest that the opiate receptors in the region of the nucleus accumbens responsible for the reinforcing properties of opiates may also be responsible for compensatory changes associated with opiate withdrawal such as "dysphoria". Results suggest that neuronal elements in the region of the nucleus accumbens may play an important role in the neurobiology of opiate reinforcement and dependence. (Supported by NIDA grant DA-04043 and NIAAA grant 06420).

254.13 NEURO-IMMUNOMODULATION, CENTRAL OPIOIDS AND THE IMMUNE RESPONSE TO BACTERIAL ENDOTOXIN. Q. Collard*, P.M. Dougherty*, K.J. Krajewski* and N. Dafny (SPON: J. Willmore). Dept. of Neurobiology and Anatomy, Dept. of Psychiatry and Behavioral Sciences, The Univ. of Texas Med. Sch. at Houston, P. O. Box 20708, Houston, TX 77225.

Muramyl Dipeptide (MDP), which is the smallest biologically active subunit of the lipopolysaccharide moiety of gram negative bacterial cell walls, has a variety of immunologic and neuropharmacologic activities. For example, 200.0 ug/kg MDP or its 6-O-stearoyl derivative injected systemically (i.p.) two hours before administration of 1.0 mg/kg naloxone to morphine dependent rats results in profound attenuation of the severity of the precipitated withdrawal syndrome. In addition, it has been shown that this same dosage of MDP reduces morphine-induced anti-nociception, while not affecting the development of tolerance to morphine. The goal of the present investigation is to establish that these effects of MDP are due to direct alterations of opioid activities in the CNS. To this end, we undertook an investigation of the dose-response characteristics of varying dosages of MDP upon the severity of eight signs of naloxone (1.0 mg/kg) precipitated withdrawal when injected directly into the brain via a previously implanted intracerebroventricular (i.c.v.) guide canula in 180-200 g male Sprague-Dawley rats (N = 32) made physically dependent upon morphine by subcutaneous morphine pellet (75 mg) implantation. The results of this study are compared with the effect of systemically injected MDP upon withdrawal severity. The results of these two studies indicate the following:

1) MDP attenuates the eight signs of naloxone precipitated withdrawal severity in a linear dose-related manner upon direct injection into the brain.
2) Moreover, intracranial and systemic administration of MDP result in similar effects upon opiate withdrawal severity.

These results indicate that MDP can indeed act directly upon the brain to alter CNS opioid activities. In addition, the present observation suggests that products of the immune response to bacterial infection can alter brain activity and thus provide a sensory immunologic information channel to the CNS.

254.14 IMMUNE SUPPRESSION AND CENTRAL OPIOID ACTIVITY. K. Henderson*, P.M. Dougherty*, K.J. Krajewski* and N. Dafny (SPON: R. Wiggins). Dept. of Neurobiology and Anatomy, and Dept. of Psychiatry and Behavioral Sciences, The Univ. of Texas Med. Sch. at Houston, P. O. Box 20708, Houston, TX 77225.

Cyclosporin A (CsA) is a novel undecapeptide of fungal origin with profound immunosuppressive activity. However, neuromodulatory activity for this peptide has also been suggested by the demonstration of a reduction in the severity of opiate withdrawal induced by 1.0 mg/kg naloxone following systemic (i.p.) injection of 15 mg/kg CsA. This same dosage of CsA also modifies the acute anti-nociceptive activity of morphine while not affecting the development of tolerance to morphine. These actions have been suggested to be due to actions of CsA within both the CNS and the immune system. To better define the actions of this agent upon these opioid phenomena, two studies were undertaken. First, we undertook an investigation into the dose-response characteristics of varying dosages of CsA upon eight signs of naloxone-precipitated opiate-withdrawal behavior following direct administration of the compound into the brain via a previously implanted intracerebroventricular (i.c.v.) guide canula placed within the lateral brain ventricle in 180-200 g male Sprague-Dawley rats previously made physically dependent upon morphine by subcutaneous drug pellet (75 mg) implantation. These results are compared and contrasted with those obtained for the effects of 15.0 mg/kg CsA administered systemically upon the same eight signs of withdrawal. In the second study, the interaction of CsA with morphine and naloxone upon the electrophysiologicic activity of four discrete brain areas essential for various opioid behaviors is assessed in 180-200 g male Sprague-Dawley rats previously implanted with permanent semi-microelectrodes. The results indicate the following:

1) CsA reduces opiate withdrawal severity in a "U"-shaped, dose-related manner following direct administration into the brain.
2) However, systemic administration of CsA yields a better reduction in any sign of withdrawal measured.
3) CsA alters the expected effects of morphine and naloxone upon the sensory-evoked responses recorded from the superior colliculis and the medial basal hypothalamus.

These results indicate that CsA has direct actions on the brain, in addition to its well known immune-modulatory effects. In addition, these results suggest that the effects of CsA upon withdrawal behavior may be related to an alteration of opioid activities in hypothalamic and other subcortical structures.

254.15 NEURO-IMMUNOMODULATION, CENTRAL OPIOIDS AND THE IMMUNE RESPONSE TO VIRUS. J.R. Lee*, P.M. Dougherty*, K.J. Krajewski* and N. Dafny. Dept. of Neurobiology and Anatomy, and Dept. of Psychiatry and Behavioral Sciences, The Univ. of Texas Med. Sch. at Houston, P. O. Box 20708, Houston, TX 77225.

It was reported that Interferon-alpha (IFN), a family of glycoproteins associated with the immune response to viral infection, can alter CNS activity and thus may provide a means for the immune system to transmit information into the brain. For example, 150.0 IU IFN/g.b.w. injected systemically (i.p.) two hours prior to administration of 1.0 mg/kg naloxone in morphine-dependent rats results in a dramatic attenuation in the severity of the signs of opiate withdrawal. This same dosage of IFN (injected i.p.) alters the development of tolerance to morphine while not affecting its acute pharmacologic properties of anti-nociception, catalepsy and catatonia. The goal of the present report is to establish that these effects of IFN are due to direct alterations of opioid activities in the CNS. To this end, we undertook an investigation of the dose-response characteristics of varying dosages of IFN upon the severity of eight signs of naloxone-precipitated withdrawal, when injected directly into the brain via a previously implanted guide canula within the left lateral ventricle (i.c.v.), in 180-200 g male Sprague-Dawley rats made physically dependent upon narcotics by subcutaneous implantation of a 75 mg morphine base drug pellet. Since these methods are identical to our previous studies with systemically injected IFN, except for route of peptide administration, a comparison of the effect of i.p. versus i.c.v. IFN upon withdrawal severity precipitation by naloxone is also presented. The results of these two studies indicate the following:

1) IFN reduces opiate withdrawal severity in a "U"-shaped, dose-related manner upon direct administration into the brain.
2) Systemic administration of IFN is more efficacious than intracranial administration of the agent in reducing the severity of all eight signs measured.

These results indicate that IFN can indeed act directly upon the brain to alter CNS opioid activities. However, IFN has peripheral sites of action in addition to the brain which yield an enhanced alteration of opioid phenomena. This suggests that products of the immune response to viral infection can alter brain activity by both direct and indirect means and thus provide sensory immunologic information to the CNS.

254.16 **EFFECT OF PRENATAL ETHANOL ON ADULT OPIOID MEDIATED ANALGESIA.** **R.A. Baker, and W.J. Shoemaker. Neuroscience Program, Univ. of Connecticut Health Center, Farmington, CT 06032.**

Prenatal ethanol exposure produces a variety of behavioral, neuroanatomical and gross morphological changes. One such change is significantly higher levels of β endorphin in mid and hind brain regions of newborn rats (Shoemaker et al., 1983, Monogr. Neural Sci. 9:130). Previous studies using a tail-flick test have reported enhanced analgesia after foot-shock stress in rats prenatally exposed to alcohol (Nelson et al., 1985). In the present study two different forms of stress-induced analgesia (SIA) were employed: 24 hr. food deprivation and 5 minute room temperature swim (RTS). Analgesia was measured as latency to lick the hind paw on a hot plate at 55± .5°C measured with a hand-held stop watch. Pregnant rats were fed one of four diets from day 7 of gestation until delivery. One group (BSP) was pair fed the same liquid diet with a second (BSA) group whose diet was 5% ethanol. The other two controls received a more nutritious liquid diet and standard laboratory rat chow. All offspring were fostered to control mothers at birth and weaned at 28 days of age. Testing was performed at 3 to 4 months of age on a 17 by 19 cm hot plate in a 34 cm high clear plexiglass chamber at 55°C. No animal was tested more than once per day at any test condition. In all test situations, BSA male offspring showed the highest level of analgesia. That is, at baseline conditions, after 24 hour food deprivation stress and after a 5 minute forced swim in 21°C water, male animals prenatally exposed to ethanol had the longest latencies compared to pair-fed controls or to other well nourished control groups. Although females demonstrated similar baseline levels of analgesia and also similar quantitative responses to stress as the males, there were no differences among pre-natal ethanol exposed and several control groups in response latencies to any of the conditions in females. The 24 hour food deprivation stress, believed to generate an opioid mediated analgesic response (McGiven, et al., 1979), produced only a small increment in analgesic latency above baseline. Five minute forced swim at 21°C, another opioid mediated analgesic stress, produced more robust increases in paw lick latency (nearly double in most groups; more than double in BSA males). We found the latencies after RTS to be highest immediately following the swim (within 2 minutes) compared to assessments made at 15 minutes post stress. One interesting observation regards the sex differences seen in these antinociception tests. Similar to what we and others have observed in a variety of behaviors, the male-female differences between the prenatal alcohol offspring is much attenuated compared to controls

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254.17 PERINATAL EXPOSURE TO MORPHINE INDUCES HYPERINNERVATION BY MET-ENKEPHALIN AND SUBSTANCE P NEURONS IN THE CNS. A.M. Di Giulio, B. Tenconi*, A. Mannavola*, P. Mantegazza*, °P. Restani*, °C.L. Galli* and °A. Gorio. Dept. of Medical Pharmacology and °Inst. of Pharmacological Sciences, Univ. of Milano, 20129 Milano, Italy.

The endogenous opioid systems seem to be implicated in the natural trophic regulation of the growth and development of the CNS. Evidence in support of this hypothesis has been gathered by in vivo and in vitro studies. The perinatal exposure to morphine or to its congeners causes a retardation in somatic and neurobiological development in human infants and in laboratory animals. In addition, cultured cells treated with narcotics or with opioid peptides show a clear reduction in growth rate. This effect is stereospecific and it is blocked by coadministration of narcotic antagonists. Recent data have shown an increase in brain size and an enhancement of proliferation of both neurons and neuroglia following the administration of naltrexone to newborn rats. All these data indicate that opiate receptors mediate a trophic response, an effect observed both in neurons and glia.

In order to gain more informations on the physiological role of the endogenous opiate system in the control of bra on, and in the attempt to understand the mechanism by which narcotics impair cell function and growth in the developing neural system, we have followed the development of enkephalin containing neurons in various brain areas of rats exposed to morphine during the fetal life and lactation. In the same experimental conditions, we have also monitored the development of substance P containing neurons. The results obtained indicate that the perinatal exposure to morphine markedly influences the rate of CNS development by causing a significant hyperinnervation by both met-enkephalin and substance P neurons, an effect particularly evident in the nucleus caudatus, cortex and pons-medulla oblongata. At birth the levels of both peptides are higher than normal in morphine-treated animals, and such a difference is magnified with growth.

254.18 ACUTE HYPOTENSIVE, BRADYCARDIC EFFECTS BUT CHRONIC PRESSOR ACTIONS AFTER INTRAVENOUS INFUSION(S) OF MORPHINE TO CONSCIOUS, UNRESTRAINED RATS. J.A. Thornhill, C. Townsend & L. Gregor. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask. Canada, S7N 0W0.

Experiments were designed to (1) investigate and compare the hemodynamic (blood pressure, BP and heart rate, HR) changes in conscious unrestrained, male Sprague-Dawley rats following acute versus repeated intravenous (iv) infusion of morphine sulphate (MS) and (2) the possible receptor mechanisms mediating those acute and chronic responses. Conscious rats (~300 g) received an acute infusion (136 ul/min), via the femoral vein catheter (surgery done 48 hr prior under sodium pentobarbital anesthesia) of the following drugs: (A) sterile physiological saline for 15 min, followed by (B) morphine sulphate, BDH (1.47 mg/ml) for 15 min totalling 7.5 mg/kg then (C) naloxone HCl, DuPont Chemicals (0.735 mg/ml) for 10 min totalling 2.5 mg/kg. BP was monitored via femoral arterial catheter (into abdominal aorta) in conjunction with PD23ID BP transducer and Grass 7B polygraph. Chronic morphine responses were measured after the same iv infusion doses of morphine in rats previously given twice daily subcutaneous injections of MS (5 mg/kg) for 3 or 6 days before retesting. Other pretreatment studies were done whereby equimolar doses (0.35 umoles) of atropine, free base or phentolamine HCl were given iv 5 min before an acute or repeated infusion of MS. Acute MS infusion caused a transient but profound hypotension related to marked decrease in HR. After 1.0 min of MS infusion the bradycardia and hypotension lessened. Subsequent naloxone infusion caused an initial increase in MHR and mean arterial pressure (MAP) but after 5 min, BP and HR returned to control levels. Pretreatment with atropine blocked the acute bradycardic and hypotensive effects of MS. Infusion with the same dose of MS in the 3 day, chronically-treated MS group caused MAP to transiently increase over the first few minutes. Subsequent naloxone infusion 10 min later caused both MHR and MAP to rise again in this group. Animals previously injected for 6 days with MS showed a greater initial pressor response to the 7.5 mg/kg infusion dose of MS with MHR increasing during the latter stages of the 15 min period. Interestingly, chronic MS animals pretreated with phentolamine before iv infusion of MS had the subsequent pressor effects of MS blocked. These results suggest that the hemodynamic responses of conscious rats following acute versus repeated iv infusion of MS are different. The acute, classical bradycardic and hypotensive effects of MS are antagonized by muscarinic receptor blockers while the pressor actions of repeated MS infusion are antagonized by α receptor antagonists like phentolamine suggesting a possible vasoconstrictor action.

This work was sponsored by the Canadian Heart Foundation.

254.19 LOCOMOTOR ACTIVITY SHOWS PROGRESSIVE INCREASES FOLLOWING REPEATED EXPOSURE TO A STRESSOR SIMILAR TO THOSE OBSERVED FOLLOWING REPEATED EXPOSURE TO MORPHINE. M. Leyton* and J. Stewart (SPON: N. White). Center for Studies in Behavioral Neurobiology, Psych. Dept., Concordia Univ., Montreal, Quebec, Canada, H3G 1M8.

The present experiments were done to determine whether a parallel could be found between the progressive increases in locomotor activity observed following repeated exposure to morphine and the possible effects of repeated exposure to a stressor, and whether previous repeated exposure to a stressor would alter the locomotor responses to morphine.

Rats were exposed to 20-min. sessions of mild, inescapable foot-shock and were observed in this environment for a further 60 min. Immediately after shock animals displayed a freezing response (5 min.) followed by increasing activity that in the later part of the session exceeded that of a no-shock control group. Over five such sessions the initial freezing remained, but the period of hyperactivity progressively lengthened in duration.

Two weeks following this stress regimen these animals were tested in the absence of shock. At this time, previously shocked animals again displayed a freezing response that was followed by 20 min. of increased activity relative to the no-shock group. On subsequent tests animals were administered either 0.5 or 5.0 mg/kg i.p. of morphine sulphate and monitored for activity. Under the lower dose of morphine the difference between the previously shocked animals and the no-shock group was similar to that seen under saline. With the higher morphine dose, however, previously stressed rats were more active than the no-shock group throughout the entire period of observation.

To determine whether the changes in responsiveness to morphine might be observed in animals tested in an environment other than that in which they were shocked, other animals were exposed to 30 min. sessions of shock on consecutive days and monitored in a different environment 20 h. later. Shocked animals were found to be more active in the neutral environment than were animals in a no-shock group throughout the four post-shock test days. In subsequent tests given under saline and after repeated morphine injections this difference between groups was maintained. Thus, the stress-induced changes in locomotor activity were relatively permanent and not dependent on explicit re-exposure to the shock environment. In a final test given under saline, the non-shocked animals were found to display an activity profile strikingly similar to that which had been displayed by the previously shocked animals prior to their exposure to morphine. This comparison suggests that repeated exposure to either shock or morphine has similar effects on locomotor activity.

254.20 MORPHINE SELF-ADMINISTRATION, EEG AND BEHAVIOR IN MORPHINE POST-ADDICT RATS CHRONICALLY TREATED WITH METHADONE OR PENTAZOCINE. Gerald A. Young, Avraham Naharin* and Naim Khazan. Dept. of Pharmcol. and Toxicol., Univ. of Maryland Sch. of Pharm., Baltimore, MD 21201.

Previously, we found that morphine and methadone post-addict rats exhibited severe immediate abstinence, and a long-lived protracted abstinence reflected by abnormal EEG and behavioral arousal, rather than EEG slow-wave bursts and stuporous behavior, in response to morphine challenges (Psychopharmacologia 29: 271-276, 1973). Protracted abstinence has been ascribed as a major factor in relapse to opioid abuse. We also found that, in contrast to methadone substitution, ethylketocyclazocine (EKC) substitution for morphine in self-administering rats resulted in miminal signs of abstinence upon EKC withdrawal (Pharmacol. Biochem. Behav. 19: 711-713, 1983). Furthermore, EKC challenges in EKC post-tolerant rats demonstrated a lack of protracted abstinence (Eur. J. Pharmacol. 125: 265-271, 1986). Therefore, we hypothesized that kappa opioid maintenance in morphine post-tolerant rats, in contrast to methadone (mu) maintenance, might lead to a protracted abstinence syndrome of lower severity and to a lower tendency for morphine self-administration. Adult female Sprague-Dawley rats were made tolerant to and physically dependent on morphine by a series of automatic i.v. injections of increasing doses over seven days. After two weeks of morphine withdrawal, rats were given either a series of automatic i.v. methadone injections or automatic i.v. pentazocine (kappa) injections. Two weeks after withdrawal, all rats were returned to their experimental cages and given the opportunity to lever press for 10 mg/kg injections of i.v. morphine on an FR-1 schedule of reinforcement. Morphine self-injections in methadone post-tolerant rats produced primarially EEG and behavioral arousal. In contrast, morphine self-injections in pentazocine post-tolerant rats produced EEG slow-wave bursts and behavioral stupor. Thus, differences in protracted abstinence were found, while establishment of morphine self-administration was similar in the two groups. It is suggested that the use of a more complex schedule of reinforcement may reveal differences in morphine self-administration in the methadone vs. pentazocine post-tolerant rats. (Supported by NIDA Grant DA-01050.)

NEURONAL DEATH II

255.1 PRELABELED RED NUCLEUS AND SENSORY-MOTOR CORTEX NEURONS 10 WEEKS AFTER SPINAL CORD TRANSECTION. R.L. McBride, E.R. Feringa and J.N. Pruitt, II.* Veterans Administration Medical Center and Medical College of Georgia, Augusta, GA 30910.

Strategies to induce axonal regeneration of axotomized CNS neurons in adults must consider the time course of changes in those neurons. While our previous studies supported the hypothesis that red nucleus and sensory-motor cortex neurons die after spinal cord transection, the possibility remains that they survive in an inactive, atrophic state. A major problem in these studies is the positive identification of axotomized neurons mixed in larger neuron pools.

In this study we have prelabeled sensory-motor cortex and red nucleus neurons projecting to the lumbar enlargement with the retrogradely-transported fluorescent dye Fluoro-Gold (Fluorochrome, Inc.) prior to spinal cord transection. Ten 7 week-old female rats were anesthetized with ketamine and xylazine. Five ul of Fluoro-Gold was injected into the L-5 cord segment of each rat. Four days later, five of the rats were reanesthetized and the spinal cord completely transected at T-9. Ten weeks after transection all rats were anesthetized and perfused with saline followed by 10% formalin. Labeled neurons were counted on 30 um frozen sections using a fluorescence microscope. In the cortex, every fifteenth section was counted (7 sections per rat) and in red nucleus every other section was counted (22 sections per rat). In cortex, 1250.5±105.1 (mean ±SEM) neurons were counted in control rats and 1681.5±164.4 in transected rats. In red nucleus, 1631.2±147.6 labeled neurons were counted in control rats and 1401.8±118.6 in transected rats. Differences between control and transected rats are not statistically significant.

Three weeks after a T-9 spinal cord transection, we found no difference from controls in the number of sensory-motor cortex or red nucleus neurons prelabeled by an L-5 injection of Fluoro-Gold. Previous studies interpreted as showing cell death were based on greatly reduced anterograde (tritiated proline) transport, counts of cells labeled by retrograde HRP transport in areas containing axotomized neurons, and total neuron counts on H&E stained sections. The disparities in results of this experiment and previous studies could be due to an altered physiological state of the neurons. However, we are also investigating the possibilities that: (1) there are qualitative and/or quantitative differences in labeling by HRP and Fluoro-Gold at different spinal cord levels, or that (2) the injection of L-5 with Fluoro-Gold results in death of some neurons.
Supported by VA Medical Research Service.

255.2 VENTRAL HORN MOTOR NEURONS 10 WEEKS AFTER SPINAL CORD TRANSECTION. E.R. Feringa, R.L. McBride, and J.N. Pruitt, II.* Veterans Administration Medical Center and Medical College of Georgia, Augusta, GA 30910.

In preliminary studies, based on counts of neurons on H&E stained sections, we reported loss of smaller ventral horn neurons of L-5 following a T-9 spinal cord transection in adult rats (J Neuropath Exptl Neurol 44:355, 1985). Subsequently, Eidelberg (Soc Neurosci Abst 12:1422, 1986) reported loss of large ventral horn neurons of L-5 after cord transection. This cell loss suggested that some neurons, deprived of descending input, died. This could be of clinical significance in amyotrophic lateral sclerosis and in efforts to provide functional recovery after spinal cord injury. Because we have been unable to confirm our earlier results, we again studied the fate of ventral horn neurons, this time labeling the motor neurons supplying the sciatic nerve prior to sacrifice.

We transected the spinal cord at T-9 in six seven-week old female rats anesthetized with ketamine and xylazine. Ten weeks later, under anesthesia, the right sciatic nerve of these rats and five age and sex matched isogeneic controls was severed above the popliteal fossa and the proximal cut end soaked for one hour in a 1% aqueous solution of the retrogradely transported fluorescent dye true blue (Sigma). Four days later the rats were deeply anesthetized and perfused with saline followed by 10% formalin.

Frozen sections, 30 um thick, of the L-5 cord segment were cut, mounted on slides and studied under a fluorescence microscope. Labeled neurons were counted on every fifth section (36 sections per rat). The mean number of labeled ventral horn neurons in control rats was 393.0±76.0 and in transected rats was 436.5±41.9. These studies fail to demonstrate a loss of ventral horn neurons supplying the sciatic nerve 10 weeks after spinal cord transection. Marked shrinkage of neurons could have resulted in miscounting of neurons in the earlier studies. We are currently using morphometric analysis of the labeled neurons to assess this possibility.
Supported by VA Medical Research Service.

255.3 FATE OF MOTONEURONS AFTER LIMB AMPUTATION IN POSTNATAL MICE. L. Lee Crews* and Donald J. Wigston (SPON: J. Manning). Department of Physiology, Emory Univ. School of Medicine, Atlanta, GA 30322.

The response of motoneurons to injury is variable. Whether they degenerate or recover to restore function depends on the extent of the injury, the species of the animal, and its age. We have characterized the response of motoneurons to limb amputation in mice between postnatal day 14 (P-14) and adulthood because we are interested in the possibility that neurons continue to depend on peripheral targets for survival during postnatal development. Our protocol entailed labeling the motoneurons that project to the biceps brachii muscles in both forelimbs with a long-lasting marker, followed by unilateral amputation at the midhumeral level and subsequent examination of the labeled motoneurons.

Motoneuronal cell bodies were labeled by pressure-injecting the fluorescent retrograde tracer Fluoro-Gold (1 µL; 2%) into the biceps muscles of anesthetized mice at P-12, P-19, and in adulthood. Two days later, animals were reanesthetized and one forelimb was amputated. One to six weeks post-amputation, the animals were killed by intracardial perfusion of fixative. The spinal cord (segments C1-T3) was immediately dissected, sectioned frozen, and viewed with epi-illumination (Leitz A cube). Fluoro-Gold-labeled motoneurons on the amputated side were then compared with those on the uninjured side.

The result of this comparison, for all ages studied, was that the fluorescent motoneurons on the amputated side were both fewer in number and smaller than those on the unamputated side. In general, the younger the animal at the time of the injury, and the longer the post-amputational survival time, the greater the extent of motoneuronal atrophy and death. We believe the consistent response to injury of motoneurons within each age group will allow us to use this system as an in vivo assay for testing potential sources of growth factors that might affect motoneuron survival.

255.4 DO CORTICOSPINAL PROJECTION NEURONS DIE AFTER SPINAL TRANSECTION IN THE NEONATAL RAT? C.A. Bates and D.J. Stelzner. Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, NY 13210.

It has been demonstrated that axonal lesion in the immature animal can lead to extensive retrograde degeneration and cell death. A previous study analyzing cortical neuronal density suggests that the corticospinal projection system does not respond to axonal lesion in the immature animal with the loss of corticospinal projection neurons (Ramirez & Kalil, JCN:1985). We have looked more directly at the corticospinal projection neurons after axotomy.

0.5ul of Fluorogold (2%) or Fast Blue (2%) were injected bilaterally into the upper thoracic spinal cord of rats on postnatal day (PND) 3 or 4. On PND 6, the spinal cord was transected above the injection site between C4 and C6 and the animals were allowed to survive until the weanling stage. Control animals were sacrificed on PND 6/7 or allowed to reach weanling stage without transection. The animals were sacrificed by transcardiac perfusion with 10% formalin under ether anesthesia. The brain and spinal cord were sectioned at 30um and the sections viewed with epifluorescent light microscopy using UV light.

In all cases, there was extensive fluorescent labeling of cells in the parietal cortex of the rat. In animals sacrificed as weanlings, the cells are located in layer Vb and their areal distribution is similar to the normal distribution of corticospinal projection neurons in weanling controls. In animals sacrificed on PND 6/7, the areal distribution appears to extend more laterally than in either the normal or transected animals. These cells may be "destined" to die as suggested by Humphrey et al (Neurosci Abs:1986) or they may die as a result of damage to their axons incurred during the injection. Nonetheless, the "normal" areal distribution of corticospinal projection neurons in parietal cortex is unaffected by spinal transection. Although all corticospinal axons projecting to the injection site are severed, a large proportion of the neurons certainly survive. This differs from the rubrospinal system in which spinal hemisection in the neonate leads to a massive loss of cells in the red nucleus (Prendergast & Stelzner, JCN:1976). The survival of corticospinal neurons may be related to their ability to send collaterals to multiple targets during development. Alternatively, their axons may remain above the lesion site forming aberrant connections. Supported by NS079740 (CAB) and NS14096 (DJS).

255.5 A TEST OF THE COMPETITION HYPOTHESIS. EFFECTS OF SUPERIOR CERVICAL GANGLION LESIONS ON POSTNATAL TRIGEMINAL CELL DEATH. Tim O'Connor and Derek van der Kooy, Neurobiology Research Group, Dept. of Anatomy, University of Toronto, Toronto, Ontario, Canada. M5S 1A8.

The trigeminal ganglion provides the major sensory innervation for the cerebral vasculature and the superior cervical ganglion (SCG) provides the major sympathetic innervation of the same target. In the adult it has been suggested that transmitter levels in these two projections may be modulated by the competition for available nerve growth factor at the cerebral vessels. Many more trigeminal ganglion cells have axons projecting to the cerebral vasculature in neonatal rats than adults. Approximately 50% of the cells with neonatal prjoections die between posntatal day (PND) 5 and PND 90. We asked whether SCG lesions at birth could decrease postnatal trigeminal cell death as would be predicted by the competition theory. At the day of birth (PND 0) rats had their left SCG removed. At PND 3 True Blue (a fluorescent tracer that remains in labeled cell bodies for months without significant loss) was applied to the left middle cerebral artery (MCA). Rats were sacrificed at 5, 25, 55 and 90 days of age. Non-lesioned controls received the same PND 3 True Blue applications and the same survival times. A second group of SCG lesioned animals did not have True Blue applied to their left MCA until 2 to 4 days prior to sacrifice. This allowed determination of the number of trigeminal cells that had maintained axons innervating the MCA at the various times of sacrifice. A second group of non-lesioned siblings received the same True Blue applications 2-4 days prior to sacrifice.

Preliminary results suggest that SCG lesions do not prevent cell death, but instead may promote it. That is, more of the trigeminal cells that project to the MCA at PND 5 have died by PND 90 in the SCG lesioned animals compared to their non-lesioned sibling controls. However, more of the cells that survive in the SCG lesioned group maintain their axon projection to the vasculature compared to the non-lesioned group. In the non-lesioned group, fewer trigeminal cells die, but more of those that survive eventually retract their axons from the vasculature. Thus, a loss of the competition from the SCG does not prevent trigeminal cell loss, but prevents axon retraction from a population of surviving trigeminal cells that normally retract their axons. Thus, the loss of a competitive influence postnatally can result in two seemingly opposite effects. The loss of competition augments the amount of neuronal cell death and yet also permits maintenance of axons that the surviving perikarya normally retract. The augmentation of cell death contradicts the competition hypothesis, although the decrease in axon retraction supports its intent.

255.6 EARLY LOSS OF UBIQUITIN-PROTEIN CONJUGATE IMMUNOREACTIVITY PRECEDS DELAYED NEURONAL DEATH IN THE RAT HIPPOCAMPUS FOLLOWING TRANSIENT CEREBRAL ISCHEMIA. K.G. Magnusson and T.W. Wieloch, (SPON: S.R. Whittemore) Lab. for Exp. Brain Research, Lund Hospital, University of Lund, S-221 85 Lund, Sweden.

Ubiquitin is a small protein widely distributed in all eucaryotic cells, which has been demonstrated to participate in the ATP-dependent breakdown of proteins, and implicated as a factor in DNA transcription, thus playing a key role in cell survival.

Transient cerebral ischemia causes selective neuronal damage in certain areas of the brain, while other areas are resistant to the insult. In some areas such as the CA1 region of the hippocampus neuronal necrosis is not observed until several days postischemia. Although transmitter mediated processes have been shown to influence the developement of ischemic neuronal damage, the intracellular processes underlying these events are unknown.

We have studied the changes in ubiquitin-protein conjugate immunoreactivity (UIR) in rat brains subjected to reversible cerebral ischemia. Ischemia of a duration of 15 minutes was induced in male Wistar rats under isoflurane/nitrous oxide anesthesia, using the bilateral common cartotid occlusion model combined with hypotension. Following 0, 30 min, 2h, 6h, 24h, 48h, and 72h of recovery, the animals were perfusion fixed and sectioned on a vibratome. The sections were processed for immunochemsitry using a polyclonal antibody directed against ubiquitin-protein conjugates (gift from Dr A. Haas, University of Wisconsin, Milwaukee) and stained using the avidin-biotin-peroxidase method.

In normal control brains UIR was found in all neurons of the hippocampus. Cell body structures as well as dendrites were stained. During ischemia no marked changes were observed. Thirty min-2h following recirculation UIR was lost in the dendrites of the pyramidal and granule cells in the hippocampus. By 6h postischemia UIR in the cell soma vanished. In the dendrites of the dentate gyrus (DG) UIR recovered in the outer two thirds but was absent in the inner third of the molecular layer. Over the period of 1-3 days UIR recovered, first in the DG, then in the CA3 pyramidal neurons, and finally in CA2. CA1 neurons never recovered UIR at any time postischemia. In the early recovery phase the interneurons showed extensive UIR which persisted up to two days postischemia. Our data demonstrate:

1. A rapid loss of UIR postischemia in all hippocampal pyramidal and granule cells, while interneurons show a relative increase in UIR.
2. UIR recovers postischemia (DG>CA3>CA1) and is inversely related to the susceptibility of the neurons to ischemic damage (CA1>CA3>DG). The recovery within the dendritic field is laminated and correlated with the distribution of NMDA and adenosine A1 receptors.
3. UIR never recovers in irreversibly damaged neurons, and thus precedes delayed neuronal death.

We propose that the loss of UIR postischemia reflect a loss of ubiquitination of proteins. The postischemic loss of ubiquitination seems not to be due to lack of ATP but rather to a receptor mediated phenomenon. An irreversible loss of ubiquitination in the CA1 region could lead to an excessive accumulation of proteins (heat shock proteins, regulatory proteins), receptor dysfunction, and disturbances in DNA transcription leading to cell death.

255.7 DELAYED INTRAVENTRICULAR NGF INFUSION REVERSES AXOTOMY-INDUCED LOSS OF MEDIAL SEPTUM CHAT-POSITIVE NEURONS. T. Hagg*, H.L. Vahlsing*, M. Manthorpe, and S. Varon. Dept. of Biology, Univ. California San Diego, La Jolla, CA 92093.

Unilateral aspirative fimbria-fornix transection in the adult rat brain causes large reductions in the number of recognizable ipsilateral medial septum cholinergic neurons, i.e. neurons stainable for choline acetyltransferase (ChAT) or acetylcholinesterase (AChE). The cholinergic cell loss is accompanied by a substantial reduction in the number of cresyl violet stainable neurons. Several investigators have recently reported that Nerve Growth Factor (NGF) prevents most of the cholinergic cell number reduction and also some of the cresyl violet cell loss. The question addressed in this study was whether the disappearance of ChAT-positive neurons represents i) the actual death of the axotomized cholinergic neurons, which NGF infusion is able to prevent, or ii) a reduction in their ChAT (or AChE) content, which NGF might not only prevent but possibly also reverse. NGF or vehicle was continuously infused into the ipsilateral ventricle for 14 days beginning immediately, 7 or 14 days after fimbria-fornix transection. The rats were then examined at 7, 14, 21 and 28 days post-lesion for ChAT-positive medial septum neurons. In the absence of NGF treatment, the number of ChAT-positive neurons dropped to about 35% of contralateral, unlesioned numbers after 7 days and then to a baseline of about 15% after 14 days or longer. When NGF treatment was started immediately after fimbria-fornix transection, 75% of the ChAT-positive neurons survived. When treatment was begun on day 7 or 14, between 50% and 70% survived. Thus delayed administration of NGF causes an increase in the number of ChAT-positive neurons, indicating a reappearance of these neurons. These data suggest that the apparent loss of cholinergic neurons induced by the fimbria-fornix transection may be largely a loss of ChAT-stainability rather than actual neuronal cell death, at least within the time frames examined.

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255.8 INHIBITORS OF PROTEIN SYNTHESIS AND RNA SYNTHESIS PREVENT NEURONAL DEATH CAUSED BY NERVE GROWTH FACTOR DEPRIVATION.

D.P. Martin, R.E. Schmidt, P.S. DiStefano, O.H. Lowry*, and E.M. Johnson, Jr. Dept. of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

A paradigm was developed to study the mechanism by which nerve growth factor (NGF) permits the survival of dependent neurons. Dissociated sympathetic neurons from E21 rats were grown in vitro for 7 days in the presence of 50 ng/ml 2.5S NGF. Cultures were then deprived of NGF by adding 1% polyclonal antiserum against NGF. The first changes appeared 18-24 hours after deprivation when the neurites became thin and discontinuous. By 30-36 hours about half the neuronal population had died and those that remained had small cell bodies which appeared dark under phase contrast microscopy. By 48 hours the culture dish was covered with degenerated neuronal debris and very few intact neurons. Replacement of the NGF at this point had no ameliorative effect and the debris gradually lifted off the dish over the next several days.

The earliest observable ultrastructural change was disruption of the neurites 12-18 hours after deprivation. The ultrastructure of the dying cell body was characterized by an accumulation of "lipid droplets", changes in the nuclear membrane, and dilation of the rough endoplasmic reticulum. Mitochondrial and lysosomal alterations did not appear to be critical factors.

The death of NGF-deprived neurons was characterized biochemically by assessing [^{35}S]methionine incorporation into TCA precipitable counts and by measuring the release of the cytosolic enzyme adenylate kinase into the culture media. Methionine incorporation began to decrease about 12 hours post-deprivation and was maximally depressed by 36 hours. Adenylate kinase began to appear in the culture media about 30 hours after deprivation, reaching a maximum by 54 hours.

Neuronal death caused by NGF deprivation was entirely prevented by inhibiting protein or RNA synthesis. Cycloheximide, puromycin, actinomycin-D, and ultraviolet irradiation all prevented neuronal death subsequent to NGF deprivation as assessed morphologically with phase contrast microscopy and biochemically by assessing methionine incorporation and adenylate kinase release into the media. Inhibitors of lysosomal function (leupeptin and chloroquine) did not prevent death. Protein or RNA synthesis must be inhibited within 12-15 hours of NGF deprivation to prevent cell death. Since at this time no morphological changes were observed, these results suggest that cells are "committed" to die prior to significant morphological change. We propose that NGF, and presumably other neurotrophic factors, maintain neuronal survival by suppressing an endogenous, active "death program".

255.9 ROLE OF CATALASE IN PROTECTION BY NERVE GROWTH FACTOR FROM HYDROGEN PEROXIDE. K. Werrbach-Perez*, L. Apffel*, G. Jackson* and J.R. Perez-Polo. Dept. of Human Biological Chemistry and Genetics, Univ. of Texas Medical Br., Galveston, Texas 77550

The nerve growth factor protein, NGF, has been shown to play a central role in the regulation of cell death phenomena for some neurons of the peripheral nervous system and lesioned neurons of the basal forebrain. NGF may also function in an immunoregulatory capacity and has been shown to act as a neurite promoting and tropic factor for some peripheral neurons.

It has been suggested that under in vitro conditions CNS neurons behave as if in a lesion induced state in terms of trophic dependence on the free radical scavenging enzyme catalase and also that there is a shift in energy metabolism for these cells resulting in a further dependence on catalase. Here we report that NGF protects the rat pheochromocytoma cell line PC12 and the human neuroblastoma cell line SK-N-SH-SY5Y from free radical induced death using 6-hydroxydopamine as a generator of quinones and hydrogen peroxide, a hydroxyl generator as well as hydrogen peroxide directly applied to the cells. Catalase mimics this effect in a specific fashion whereas superoxide dismutase has no effect and DMSO confers some partial protection. After screening several cell lines only those lines with neuronal properties were found to have relatively low levels of endogenous catalase and glutathione transferase activity. It was thus of interest to find that NGF specifically induces catalase and glutathione transferase activity but has no effect on superoxide dismutase levels in these cells and that this NGF protection is abolished by a small molecular weight inhibitor of the catalase enzyme. These results are in agreement with the hypothesis that under the metabolic conditions of tissue culture, NGF protects from peroxidative damage and subsequent cell death by inducing catalase. Whether similar events take place during early neuronal development and provide a mechanism for the regulation of neuronal cell death is not known but we would maintain that such a hypothesis is in agreement with our in vitro data.

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255.10 NATURALLY OCCURRING SOMATIC MOTONEURON DEATH IN THE SPINAL CORD OF THE TURTLE EMBRYO (Chelydra serpentina). S. E. McKay*[1], R. R. Provine[2], and R. W. Oppenheim[1] (SPON: B. T. Troost). Dept. Anatomy, Wake Forrest Univ. Sch. of Med., Winston-Salem, NC 27103[1], and Dept. of Psychol., Univ. Maryland Baltimore County, Catonsville, MD 21228.[2]

The death of motoneurons in the spinal cord of the snapping turtle was described to learn more about neurogenesis in this rarely studied organism and to evaluate the generality of findings about motoneuron death from the widely studied chick embryo. Eggs were collected from clutches laid in the laboratory by eight wild caught females, and incubated at 30° C. Two to four embryos per age were sampled at 8-, 10-, 12-, 14-, 15-, 18-, 20-, 24-, and 60-days of embryonic age. (Hatching occurs at 60 to 70 days at 30° C.) Spinal cords were fixed in Carnoy's solution, sectioned at 8-12 µm and stained with thionine. Pycnotic (dying) cells in the ventral horn region were counted in every tenth section.

Pycnotic motoneurons were rare in pre-motile 8- and 10-day embryos. Pycnotic neurons were more numerous by 12 days, the age of onset of the first axial body movements, after which their numbers increased gradually until 15 days, the age of onset of limb movement. The number of dying motoneurons increased sharply between 15 and 18 days. After reaching a peak at 18 days, the number of pycnotic neurons declined gradually until 24 days. Pycnotic neurons were rare at 60 days, a few days before hatching. The above developmental trends in pycnotic motoneuron numbers were characteristic of brachial, thoracic, and lumbar cord regions.

It is significant that both the turtle and chick embryos experience substantial motoneuron death during the first half of incubation and that the peak rate of neuronal death coincides with the onset of limb movement. (For chick data, see Hamburger, V., and Oppenheim, R. W., Neurosci. Commentaries, 1 (1982) 39-55.) This concurrence of events in two organisms that differ so greatly in evolutionary history, morphology, physiology, locomotion, and length of incubation, suggests that the correlation between the onset of neuromuscular function, especially limb movement, and motoneuron death may be a general feature of vertebrate spinal cord development.

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255.11 THE PREVENTION OF NATURAL MOTONEURON CELL DEATH BY SOMATOSTATIN. C.L. Weill. Depts. of Neurology and Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112.

Somatostatin (SRIF) appears in the spinal cord before the onset of synaptic transmission, and about 24 hr after the peak of natural motoneuron cell death. Thus, could SRIF regulate the extent of motoneuron cell death?

Chick embryos (Harco X Partmer red) were treated with SRIF on embryonic days 5 through 9 by daily injections onto the egg shell membrane, sacrificed on day 10, their body weight and embryonic stage determined, the lumbar spinal cord dissected, fixed in Carnoy's, stained en bloc, embedded in paraffin, and serially sectioned transversely at 10 um. All dark staining cells associated with the lateral motor column (LMC) containing at least one nucleolous were counted at 400 x. Uncorrected data are presented as the mean +/- SEM with n noted. Significance was assessed using the Mann-Whitney U-test and correlations assessed by evaluating Spearman's Rho.

The number of available motoneurons per LMC on embryonic day 6 is 22,838 +/- 731 (n=6). This number declines by 35% to 14,815 +/- 472 (n=16) by day 10. Treatment with 0.5 nmole of SRIF increased survival by 13.2% relative to control to 16,767 +/- 367 (n=9), while 2.5 nmole increased survival by 22.3% to 18,113 +/- 367 (n=7). Thus, SRIF prevents the death of 24.3% and 41.1% respectively, of those cells that would normally die by day 10. The increase in survival is statistically significant at the 95% confidence level; P=0.012 at 0.5 nmole and P=0.001 at 2.5 nmole. Body weight for controls was 2.417 +/- 0.039 gm (n=45) and for 0.5 nmole SRIF 2.717 +/- 0.064 gm (n=9, P=0.004) and 2.5 nmole, 2.580 +/- 0.034 gm (n=9, P=0.059). Toe length for controls was 5.13 +/- 0.03 mm (n=30) and for 0.5 nmole SRIF 5.22 +/- 0.05 mm (n=9, P=0.092) and for 2.5 nmole SRIF 5.24 +/- 0.05 mm (n=9, P=0.031). Thus, a significant increase in body weight was observed at 0.5 nmole of SRIF, while a significant increase in toe length was observed at 2.5 nmole of SRIF. The number of surviving motoneurons did not correlate with either body weight or toe length in controls, however body weight did correlate with toe length (rho = 0.6795, n=12, P=0.015). Cell number also did not correlate with either body weight or toe length with SRIF treatment, but body weight did correlate with toe length; 0.5 nmole SRIF, rho = 0.8809, n=9, P=0.002 and 2.5 nmole, rho = 0.9357, n=9, P<0.001.

These data demonstrate that SRIF at doses in the nanomolar range effect a statistically significant increase in the number of motoneurons that survive natural motoneuron cell death, thus suggesting a possible role for SRIF in motoneuron development. Supported by NIH grant NS18642.

255.12 REDUCTION OF NATURALLY OCCURRING MOTONEURON DEATH BY SERA FROM PATIENTS WITH DENERVATING DISEASE. L.J. Haverkamp, R.W. Oppenheim, S.H. Appel, D. Prevette, and J.L. McManaman. Dept. of Neurology, Baylor College of Med., Houston, TX 77030 and Dept. of Anatomy, Wake Forest Univ. School of Med., Winston-Salem, NC 27103.

Immune factors have been implicated in the pathogenesis of ALS, with the occasional reports of detrimental effects of ALS sera on cultured neurons supporting such an involvement. We have applied an in vivo system - naturally occurring cell death of motoneurons (MN) in the developing chick embryo - to the hypothesis of blood-borne factors acting upon motoneurons in ALS.

Sera from ALS and control patients, heat-inactivated and extensively dialyzed, were applied daily to the chorio-allantoic membrane from E6 through E9. On E10, the embryos' spinal cords were processed for histology and cell counts performed. Contrary to prediction, 5 of 11 ALS sera resulted in a significant rescue of motoneurons in the lumbar lateral motor column (LMC) during the cell death period. The active sera resulted in a range of 1500-4500 more motoneurons (N=4-5, $p<.05$ for each serum) compared to saline controls (MN number = $12\text{-}13 \times 10^3$). Counts of neurons in the DRG and symapthetic ganglia, and measurements of motoneuron nuclear diameters reveled no effect of active ALS sera on these parameters, while numbers of thoracic motoneurons were significantly increased and numbers of pyknotic neurons in the LMC significantly decreased.

This rescuing effect is not confined to ALS, however, but was also produced by 3 of 10 control sera so far tested. The active sera were obtained from patients with potentially denervating disorders, namely denervating polyneuropathy, and Guillain-Barre' and post-polio syndromes.

These results indicate that sera from a sizable proportion of patients with ALS and other denervating diseases may induce an alteration of normal motoneuron-target communication, possibly through effects on the levels of target-derived neurotrophic factors. In support of this possibility, we have also been able to ameliorate motoneuron death by applications of extracts of E9 chick embryo muscle.

255.13 **CELL DEATH IN THE DEVELOPING MOUSE CEREBELLUM: FURTHER STUDIES OF NUMERICAL MATCHING BETWEEN PURKINJE AND GRANULE CELLS IN +/LC CHIMERAS.** M.W. Vogel, K.Sunter*, and K. Herrup. Dept. of Human Genetics. Yale Med. School. New Haven, CT 06510.

Recent studies of neuron-target populations have emphasized the importance of quantitative matching as a rationale for naturally occurring cell death. Herrup and Sunter (J.Neurosci. ,7:829, 1987) found that the relationships between granule and Purkinje cells in sg/sg chimeras and inbred mice are colinear. In +/Lc chimeras, however, Wetts and Herrup (Dev. Brain Res., 10:41,1983) found up to a four-fold increase in the ratio of surviving granule to Purkinje cells over wild type values and proposed a curvilinear function relating granule to Purkinje cell numbers. Purkinje cells are a primary site of gene action in both mutants; however, while sg/sg Purkinje cells fail to develop granule cell synapses, +/Lc Purkinje cells degenerate beginning in the first postnatal week of development after synaptogenesis has begun. To clarify the role of these transient Purkinje:granule cell relationships, we have counted both cell types in sagittal sections of 14 additional half-cerebella of +/Lc chimeras and +/+ and +/sg control chimeras.

The granule to Purkinje cell relationships in inbred mice and in sg/sg and control chimeras were found to be colinear. Linear regression of the combined control and sg/sg chimera data shows that the y-intercept is not significantly different from zero ($P>0.05$). The analysis of additional +/Lc chimeras, however, revealed that the relationship between Purkinje and granule cells in the +/Lc chimeras is also best described as linear ($R^2=0.94$). The slopes for the regression equations of +/Lc chimeras and the sg/sg chimeras are not significantly different ($P>0.05$). This result suggests that the numerical matching between granule and Purkinje cells follows the same function in both experimental situations. However, the Y-intercept of the +/Lc chimera line falls at 7.2 million granule cells. The implication of this is that this number of granule cells are present in every +/Lc chimera in addition to those present due to numerical matching. These data are consistent with two hypotheses. First, the increased survival of granule cells may reflect a pleiotropic effect of +/Lc gene action that allows increased granule cell survival. Second, Caddy *et al* (Neurosci. Abst. 12:1583, 1986) have shown that the dendrites of wild type Purkinje cells in +/Lc chimeras appear de-afferented. The increased granule cell survival may reflect a Purkinje cell deafferentation response that allows increased granule cell survival.

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255.14 EVIDENCE OF WEAVER GENE ACTION IN THE PREMIGRATORY GRANULE CELL. R. Smeyne and D. Goldowitz. Dept. of Anatomy, Thomas Jefferson University, Philadelphia, PA 19107

Neurological mutants provide a useful tool to study development. By analyzing abnormal phenotypic expression of mutant genes, one can dissect out important developmental events. One such mutant is the homozygous weaver mouse (wv/wv) which is characterized by a loss of granule cells. The granule cell loss is attenuated in the heterozygous weaver mouse (wv/+). It has previously been shown that the granule cell is a primary site of gene action. The cause of the weaver granule cell death is unknown, although it has been speculated that the granule cell death is due to the cells' inability to migrate from the proliferative external granule layer (EGL) to the internal granule layer. The purpose of this study is to isolate a time-point when the weaver granule cell phenotype is first expressed, thereby suggesting the mechanism by which granule cells die.

From the day of birth (PO) through post-natal day 8 (P8), wv/wv, wv/+, and +/+ mice were deeply anesthesized and perfused with modified Bouin's solution. Brains were removed, dehydrated, blocked in the midsagittal plane and embedded in paraffin. Serial sections were cut at 6 u and every 20th section was mounted and stained. The sections were analyzed for total cerebellar area, total area of the EGL, total number of cells in the EGL, number of mitotic figures in the EGL, and number of dead/dying cells in the EGL.

Examination of the sections revealed that as early as P4, significant differences were found between the three genotypes in all parameters studied except for the number of mitotic figures. At P2, the only significant difference found between genotypes was the number of dead/dying cells found in the EGL. This difference was also seen at PO.

The defect seen in the weaver mutant mouse has been described as a migration defect. In this study, the three different genotypes (wv/wv, wv/+, and +/+) can be differentiated as early as PO based upon the percentage of dead/dying cells present in the EGL. Since granule cell migration begins around P4, the ability to delineate differences between the 3 genotypes prior to the start of granule cell migration suggests that the impaired granule cell migration is not the first step which is affected by the weaver gene. Prior to migration, the granule cell must undergo its terminal differentiation, which includes exit from the cell cycle as well as process extension. This study suggests that the weaver gene may cause one of these earlier developmental processes to go awry.

256.1 DEVELOPMENTAL EXPRESSION OF CHOLINE ACETYLTRANSFERASE IN DROSOPHILA. L.A. Carbini*, V.J. Maines* and P.M. Salvaterra. Division of Neurosciences, Beckman Research Institute/City of Hope, Duarte, CA 91010.

Genes and the action of their products can potentially be regulated at a number of different control points. In this study we have measured the steady state levels of choline acetyltransferase (ChAT) mRNA throughout the developmental stages of Drosophila. We have also determined the levels of ChAT protein by Western blots and the amount of enzyme activity at the same developmental stages and compared these parameters with the mRNA levels.

Total RNA samples were used for Northern or dot blot analysis and probed with a ChAT specific cRNA. ChAT mRNA could be detected approximately 6-7 h after oviposition and the maximum amount of ChAT mRNA was found in 24-25 h old organisms. ChAT mRNA decreased by the 3rd larval instar, increased during late pupation and decreased again in adult flies. The relative proportions of ChAT mRNA in 1st larval instar, 3rd instar, pupae, and adult were 1: 0.6: 0.9: 0.3 when expressed per organism. Northern analysis showed a single major RNA band with a Mr of approximately 5.0 Kbase pairs and two minor bands of 9.0 Kb and 11.0 Kb (probably pre-spliced mRNA) in all developmental stages.

A pattern of increasing activity was observed throughout development. A sharp increase was seen from the 1st larval instar up to the 3rd larval instar with a less marked increase in the pupal and adult stages. In contrast to ChAT mRNA levels, no decrease was observed for ChAT activity during pupation. This may reflect the relative turnover rates of mRNA and protein. Western analysis of ChAT polypeptides showed a single major protein band with a Mr of 75 Kdaltons throughout development.

The single size observed for ChAT mRNA and protein throughout development make it unlikely that regulation occurs by splicing or postranslational modifications. If ChAT protein turnover is slower than mRNA turnover, the enzyme activity levels seem to reflect the availability of mRNA which may indicate that the major control point for active ChAT expression is at the level of transcription. Supported by NIH-NINCDS.

256.2 **REGULATION OF CHOLINE ACETYLTRANSFERASE ACTIVITY IN CULTURED EMBRYONIC RAT SPINAL CORD.** D. Lombard-Golly*, A. Chalazonitis and J.A. Kessler. Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Neuronal development and neurotransmitter expression are influenced by the environment of the neuron. The present study was undertaken to define factors regulating development of cholinergic spinal cord neurons, and to determine whether the two principal populations of cholinergic spinal cord neurons, motor neurons and intermediolateral autonomic neurons, are regulated by the same factors. Levels of the biosynthetic enzyme, choline acetyltransferase (CAT), were examined in dissociated cell cultures of embryonic rat spinal cord to define cholinergic development. Initial experiments determined that optimal neuronal survival was achieved using embryos at day 14.5 of gestation, and all subsequent studies used spinal cords at this stage. During the first week of culture, CAT activity was strikingly dependent upon cell density. Cultures established with $0.75x10^6$ cells per 35 mm dish contained no detectable CAT activity after 7 days in culture, whereas cultures established using 10^6 cells contained 1.14 nmol product per mg protein per hr (nmol/mg/hr), and cultures using $1.5x10^6$ cells contained 2.09 nmol/mg/hr. However, by 14 days CAT activity was almost identical (2 nmol/mg/hr), at all three densities. Thus the differences in CAT activity in younger cultures apparently reflected density dependent differences in regulation of CAT rather than differences in cholinergic neuron survival. Development of CAT activity was examined in cultures of ventral (motor neuron enriched) and mediodorsal (intermediolateral neuron enriched) spinal cord. Levels of CAT were high in ventral cord cultures (3.3 nmol/mg/hr after 14 days in culture) compared to whole cord cultures, whereas levels of CAT in mediodorsal cultures were extremely low (0.47 nmol/mg/hr). Current studies are directed towards defining differences in regulation of CAT activity and cholinergic neuron survival in cultures of ventral versus mediodorsal spinal cord.

256.3 **CHARACTERIZATION OF A MEMBRANE ASSOCIATED MOLECULE WHICH STIMULATES CHOLINERGIC AND PEPTIDERGIC TRANSMITTER EXPRESSION IN SYMPATHETIC NEURONS.** V. Wong and J.A. Kessler. Depts. Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Neuronal contact with other cell membranes and with extracellular matrix (ECM) plays an important role in regulating neuronal growth and development. The present study was undertaken to characterize cell surface molecules that influence cholinergic and peptidergic transmitter expression. Treatment of cultured sympathetic neurons with membranes prepared from adult rat spinal cord stimulated levels of choline acetyltransferase (CAT) activity and induced substance P (SP). The active molecule was extracted from spinal cord membranes by 4M NaCl, suggesting that it is a membrane associated molecule. Treatment of sympathetic neurons with the 4M NaCl extract of this membrane-associated neurotransmitter stimulating molecule (MANS) for 1 week increased CAT activity 5-fold and induced SP to a level of 23 fg/neuron. Moreover, simultaneous treatment with nonneuronal cell-derived soluble factors strikingly facilitated the effects of MANS on both CAT activity and SP expression (see Kremer, Wong, and Kessler, this volume). Tyrosine hydroxylase (TH) activity in the same cultures was not affected. MANS increased CAT activity in a dose dependent manner, effective at a dose as low as 800ng/ml and achieving saturation at 3.25ug/ml. MANS was also solublized from spinal cord membranes by 100mM octylglucoside (OG), but not by 50mM OG or 0.1% Triton. CAT-SP-stimulating property of MANS was destroyed by treatment with trypsin or heat-inactivation, but was stable at 37 C. Testicular hyaluronidase, a glycosidic enzyme, also destroyed MANS activity. Our observations suggest that MANS is a membrane associated molecule with an active site that appears to include 2 domains: (1) a protein which is sensitive to trypsin, and (2) a glycosaminoglycan (GAG) which is sensitive to hyaluronidase.

256.4 **FACILITATORY INTERACTIONS BETWEEN SOLUBLE AND MEMBRANE-ASSOCIATED TRANSMITTER STIMULATING FACTORS OCCUR AT THE mRNA LEVEL.** N.E. Kremer, V. Wong, J.A. Kessler. Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Neurotransmitter phenotypic expression is influenced by the environment of the neuron. The present study examines facilitatory interactions between soluble and membrane-associated factors in the regulation of sympathetic neuron transmitter expression. Previous studies have shown that coculture of sympathetic neurons of the rat superior cervical ganglion (SCG) with ganglion non-neuronal cells induces expression of substance P (SP, Kessler, 1984) and elevates choline acetylytransferase (CAT) activity (Patterson and Chun, 1974). Treatment of cultured sympathetic neurons with plasma membranes derived either from ganglion non-neuronal cells or from spinal cord also induced SP expression and elevated CAT activity, indicating that neuronal contact with other membranes regulated transmitter expression. Treatment of sympathetic neurons with medium conditioned by ganglion non-neuronal cells or with rat fibroblast conditioned medium (RFCM) increased CAT activity but failed to induce expression of SP. Simultaneous treatment with membranes and RFCM resulted in a striking facilitatory increase in both SP and CAT.

A membrane-associated neurotransmitter stimulating molecule (MANS) which mimics the presence of non-neuronal cells (cf Wong and Kessler, this volume) has been solubilized from rat spinal cord membranes. Treatment of sympathetic neurons with MANS induced SP expression and elevated CAT activity. Treatment with both MANS and RFCM synergistically elevated levels of both SP and CAT. This facilitatory interaction was clearly seen at the mRNA level. Preprotachykinin (PPTI, encoding substance P) mRNA was measured using Northern blot analyses. Pure neuronal cultures contained no detectable PPTI mRNA, whereas neurons cocultured with non-neuronal cells had high levels of the mRNA. Pure neuronal cultures treated with RFCM also did not contain PPTI mRNA, but RFCM potently increased mRNA levels in neurons cocultured with non-neuronal cells. Treatment of pure neuronal cultures with MANS induced PPTI mRNA reproducing the effects of non-neuronal cells, and simultaneous treatment with RFCM and MANS resulted in further increases in mRNA levels.

Thus neuronal contact with other membranes or with MANS induced expression of SP and elevated CAT activity. Soluble factors in RFCM also increased CAT activity but did not induce SP expression. However, RFCM strikingly facilitated the effects of membrane contact or MANS treatment on both SP expression and CAT activity. These observations indicate that the effects of soluble factors and cell-cell contact interact in determining neurotransmitter expression.

256.5 LOSS OF THE CAPACITY OF INTRINSIC ENTERIC NEURAL PRECURSORS TO EXPRESS A CATECHOLAMINERGIC PHENOTYPE IS CORRELATED WITH INVASION OF THE DEVELOPING CHICK GUT BY SYMPATHETIC NERVES T. P Rothman, H. M. Mackey*, and M. D. Gershon. Department of Anatomy and Cell Biology, Columbia Univ., Coll. of P & S, New York, NY 10032.

The adult enteric nervous system (ENS) contains noradrenergic sympathetic axons derived from neurons located outside the bowel; however, there are no intrinsic catecholaminergic neurons. On the other hand, transient catecholaminergic (TC) cells can be found in the enteric mesenchyme during development of the mammalian, but not the avian gut. These TC cells disappear from the mammalian bowel on the day the sympathetic innervation reaches the developing bowel. The current experiments were done to assess the catecholaminergic potential of avian enteric neural precursors and to determine whether this capacity is lost coincidentally with the arrival of the sympathetic nerves in the gut. We tested the hypothesis that crest cells that colonize the avian bowel are able to express a catecholaminergic phenotype, but fail to do so when they develop within the enteric mesenchyme. Accordingly, the chick small intestine (days E7-E9) was dissociated with trypsin (0.25%) in Ca^{2+}/Mg^{2+}-free solution and the separated cells were plated on collagen and grown *in vitro*. Cultures were grown in an enriched medium containing chick embryo extract (10%) and horse serum (20%). As noted previously (Mackey et al., 1986), many cells from day E7 bowel expressed tyrosine hydroxylase immunoreactivity (TH-IR). TH-IR was not expressed by cells in similar cultures from gut dissociated at days E8 or E9. The timing of the arrival of the sympathetic nerves in the bowel *in situ* was studied radioautographically, following incubation of tissues with ^{3}H-norepinephrine (^{3}H-NE; 0.02-0.2 μM) in the presence of the MAO inhibitor, pargyline (0.1 mM). At day E 7 no nerves were labeled in the foregut or in its surrounding connective tissue. At day E8, ^{3}H-NE labeled nerves in the connective tissue dorsal to the esophagus, gizzard, small, and large intestines. Moreover, labeled nerves were also found within the dorsal mesenchyme of the developing gizzard, intestines, and pancreas. Labeling did not extend fully around the circumference of the gut at any level. Neural uptake of ^{3}H-NE was antagonized by desmethylimipramine (1-100 μM). Intensity of neural labeling was concentration-dependent, but extraneuronal labeling was seen in tissues incubated at the higher concentration of ^{3}H-NE (0.2 μM). Extraneuronal, but not neuronal, labeling was inhibited by normetanephrine (10 μM). By day E9, ^{3}H-NE-labeled nerves were found in both myenteric and submucosal plexuses around the entire circumference of the bowel, although labeling was much more intense in the caudal than proximal intestine. It is concluded that crest cells that migrate to the avian gut are capable of expressing a catecholaminergic phenotype, although they do not do so *in vivo*. This capacity can be revealed by dissociating the bowel and growing the enteric neural precursors out of the enteric microenvironment, *in vitro*. As in mammals, the ability of avian crest cells to express a catecholaminergic phenotype is lost coincidentally with the arrival of sympathetic nerves in the gut. These observations are consistent with the idea that sympathetic nerves influence phenotypic expression by enteric neural precursors. Supported by NIH grants, HD 21032, HD 20470, NS 15547, and by the MOD (1-747) and the Robert Wood Johnson Fdn.

256.6 IN SITU HYBRIDIZATION OF mRNA FOR PREPROTACHYKININ (PPT) AND SOMATOSTATIN (SOM) IN ADULT RAT DORSAL ROOT GANGLIA (DRG). Deborah B. Henken[1], Alan Tessler[1,2], Marie-Francoise Chesselet[1], Alan Hudson[2], Frank Baldino, Jr.[3], and Marion Murray[1]. [1]The Medical College of Pennsylvania, Philadelphia, PA, [2]Philadelphia VA Hospital, Philadelphia, PA, and [3]E.I. Dupont Co., Wilmington, Delaware.

Peptide synthesis is regulated by transcriptional and translational mechanisms. As a first step in distinguishing these mechanisms we have compared the distribution of mRNA's for specific peptides with the existing immunocytochemical evidence. Immunocytochemical techniques permit the localization of peptides within discrete populations of DRG neurons and have served as useful methods for examining the regulation of neuronal metabolism during regeneration. DRG neurons are commonly subdivided into small and large; peptidergic neurons are among the small neurons. We have used hybridization probes to identify the neurons which contain mRNA for the precursors of the tachykinins substance P and substance K (preprotachykinins, PPT) and for somatostatin (SOM). For PPT, antisense (test) and sense (control) RNA probes were labelled with ^{35}S-UTP during transcription from cDNA's cloned from a human brain library in an SP6-containing vector (Affolter et al., 1986). The cDNA fragment was a 345 base region containing exons 2 through 6 of the PPT gene. For SOM, a 39-base synthetic oligonucleotide probe complementary to the 3' coding region of SOM_{28} mRNA, was 3'-end labelled with a-^{35}S dATP and terminal transferase. Following in situ hybridization, specific mRNA-containing neurons in lower lumber DRG of young adult Sprague-Dawley rats (200-250 gm) were visualized with light microscopic autoradiography. Neurons which contained the mRNAs in each DRG were mapped and the areas of both labelled and unlabelled neurons were measured. The antisense probe for PPT mRNA labelled an average of 17% of the total cell population. No somata were labelled with the sense probe. The average cell area for the labelled population was 420 um^2. The synthetic oligonucleotide for SOM mRNA labelled 10% of the total cell population with an average cell size of 560 um^2. Both these populations are in the size range of small DRG neurons. However, cells that contain PPT mRNA are significantly smaller then those that contain SOM mRNA (Student's T-test; $p<.05$), in agreement with previous studies using immunocytochemistry (Price, 1985). These results suggest that immunocytochemical methods for localizing PPT and SOM can be correlated with in situ hybridization for localizing PPT and SOM mRNA's and that these techniques can be used to study the mechanisms that regulate metabolic changes.

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256.7 THE DEVELOPMENT OF AXONS CONTAINING TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE SPINAL CORD OF THE NORTH AMERICAN OPOSSUM, DIDELPHIS VIRGINIANA. R.R. Pindzola, R.H. Ho, and G.F. Martin. Department of Anatomy and Neuroscience Program, The Ohio State University, Columbus, Ohio 43210.

We have previously used the peroxidase antiperoxidase technique (PAP) to study the distribution of tyrosine hydroxylase immunoreactive axons (presumably catecholaminergic) in the spinal cord of the adult opossum (Pindzola et al., Neurosci. Abstr., 12: 1547, 1986). In addition, we have combined retrograde labelling methods with immunohistochemistry to determine the origin of these axons. The results of those studies have been used as endpoints for developmental analysis.

Opossums are born 12 days after conception, in a very immature state. At birth, axons showing tyrosine hydroxylase immunoreactivity (TH), using the PAP method, are present in the spinal cord where they are limited primarily to the marginal zone. Such axons appear most numerous in the dorsolateral marginal zone, the region containing most of them in the adult animal. By postnatal day (PND) 6 (24 mm snout-rump length, SRL), TH axons are obvious in the intermediate zone and some are found within the presumptive intermediolateral cell column. A few TH axons are found in the developing dorsal and ventral horns by estimated PND 15 (35mm SRL). By estimated PND 44 (76 mm SRL), the pattern of TH innervation in the spinal cord resembles that in the adult. The density of TH axons at some stages of development appears greater than in the adult.

At birth TH cell bodies are present in most, if not all, of the brainstem areas providing TH projections to the spinal cord in the adult animal (Pindzola et al., Neurosci. Abstr., 12: 1547, 1986). Since there are few TH perikarya in the spinal cord at birth, we assume that most of the TH axons in the spinal cord originate within the brainstem. TH perikarya are found more frequently in the spinal cord at estimated PND 10 (28 mm SRL) and they become even more numerous and widespread at later ages before diminishing to adult levels.

In summary, our results suggest that: 1) TH axons of brainstem origin are present in the spinal cord of the newborn opossum, and 2) TH axons innervate the developing grey matter over an extended period of time. These data, considered in light of those published previously on the development of serotoninergic projections to the spinal cord (DiTirro et al., J. Comp. Neurol., 213: 241-261, 1983), indicate that monoaminergic axons are among the first from the brainstem to reach spinal levels. Even so, anatomical evidence for direct control over postsynaptic targets occurs considerably later. (Supported by NS-10165)

256.8 MOLECULAR SPECIES OF SOMATOSTATIN IN DEVELOPING RAT RETINA. D.M. Ferriero and S.M. Sagar. Neurology Service, V. A. Medical Center and Department of Neurology, University of California, San Francisco, CA 94143.

Somatostatin (SLI) has been found by radioimmunoassay (RIA) and immunocytochemistry (ICC) in the retina of many vertebrate species. In the rat and rabbit the major species of SLI present is somatostatin-14 (SS-14). In the guinea pig and cow, the major species is somatostatin-28 (SS-28) and in the fish and chick, there are equal amounts of both forms. The functional implication of the species differences in chromatographic patterns is unknown; in particular, it is unknown whether the SS-28 species or any other fragment of the prosomatostatin molecule has a function different than that of SS-14. In the guinea pig SS-28 is the only species present from embryonic through adult life. In the chick the molecular forms differ in the embryo from the adult. It is unknown currently which species predominates in the fetal rat retina, or whether the species changes as development proceeds.

We have previously shown that SLI appears as early as E16 in high quantities (4.5 ng/mg protein) and decreases to barely detectable levels at an early postnatal age (P4 = 0.09 ng/mg protein); gradually returning to adult levels (0.46 ng/mg protein) after eye opening. Gel permeation chromatography was performed on retinal extracts corresponding to the above developmental times to address the issues of molecular species preservation through ontogeny. We have found that there are three forms present at E16: a large MW species, SS-28 and SS-14. This is true at E19 as well, but at P4, the SS-14 disappears completely. In the adult retina, there is a large MW species present, with SS-14, but no SS-28, as previously reported.

Therefore, there is a switch during retinal development from SS-28 and SS-14 to SS-14 only in adult retina, with a transient phase of SS-28 expression only at a time when SLI is barely detectable by ICC and RIA. Possibly, differential gene processing or other posttranslational events generate different products during development to cue the developing retina. The relationship of the switch (SS-28 → SS-14) and the decline of SLI prior to synaptogenesis is intriguing and may illustrate a developmental role for SLI.

256.9 CHANGES IN THE IMMUNOSTAINING PATTERN OF CHICK SPINAL CORD 5-HT NEURONS GROWN IN CULTURE OVER DIFFERENT LENGTHS OF TIME. R.R. Maez*, P.C. Allgood* and J.A. Wallace (SPON: G. Wild). Dept. of Anatomy, University of New Mexico School of Medicine, Albuquerque, NM 87131.

Serotonergic (5-HT) neurons are found in the spinal cord of chick embryos from 7 days of incubation (E7) through hatching (E21). In a detailed study of the number of 5-HT neurons in the chick spinal cord at different embryonic ages, we have reported changes in the number of anti-5-HT immunostained cells during embryogenesis (Brain Res. Bull. 17:297, 1986). Changes in 5-HT cell numbers include a drop of over 40% of total cord 5-HT cell counts determined at E17 from the plateau of approximately 3,500 5-HT neurons that were estimated to occur from E9 to E12. Subsequent to E17, 5-HT cell counts increased at the time of hatching back to the values obtained for the earlier ages, suggesting that the reduced number of spinal cord 5-HT cells detected at E17 was not due to cell death. Here we report initial qualitative observations on changes in the immunostaining patterns of spinal cord 5-HT neurons grown in vitro over two time periods that would approximate the in ovo ages where the 5-HT cell counts in the chick spinal cord would be at their early plateau or at their later reduced levels. For comparison with other central 5-HT neurons, cultures of chick embryonic brainstems were grown for similar time periods under identical culture conditions. Spinal cords from embryos at E8, or brainstems from animals at E6, were removed and dissociated with trypsin. Cells were plated onto four-chambered Lab-Tek Tissue Culture glass slides coated with collagen, at a density of 5 x 10^5 cells per chamber. The slides were incubated with 5% CO_2 and air at 37°C, and cultured for either 5 or 10 days. The cultures were then fixed and stained by anti-5-HT immunocytochemistry using the ABC peroxidase technique. Spinal cord and brainstem cultures incubated for 5 days demonstrated deeply-stained 5-HT neurons of various morphologies. In contrast, spinal cord cultures incubated for 10 days possessed numerous examples of dark, immunostained axonal endings that were connected either to unstained or to only very lightly-stained cell bodies. This specific anti-5-HT staining pattern was rarely encountered within spinal cord cultures incubated 5 days or within brainstem cultures incubated for 10-12 days. The frequent occurrence of cultured spinal cord cells which do not fully express the 5-HT phenotype in their perikarya, yet have 5-HT immunostaining in their axonal processes, may mimic the in ovo situation at E17, wherein fewer spinal cord 5-HT cell bodies could be detected immunocytochemically. These findings may provide an in vitro model to study factors regulating neurotransmitter phenotype expression in neurons of the CNS. Supported by NSF grant BNS-8511079 and NIH grant RR-08139.

256.10 CHARACTERIZATION OF A NEURECTODERMAL SEROTONINERGIC CELL LINE. H. Tamir, M. D. Gershon, S. H. Hsiung, and K. P. Liu. NYS Psychiat ric Inst. and Dep'ts. of Psychiatry and Anatomy and Cell Biology, Columbia University, P&S, New York, NY 10032

The parafollicular (PF) cell of the thyroid gland is embryologically derived from the neural crest. In adults, PF cells contain calcitonin and also, in some species, serotonin (5-HT), calcitonin gene related peptide (CGRP), and somatostatin. PF cells have been isolated from sheep thyroid. The secretory vesicles of these cells co-store calcitonin and 5-HT and contain, in addition, a 45 kDa protein that specifically binds 5-HT (SBP). The same protein is found in serotoninergic neurons in the brain and in the gut. Since enteric serotoninergic neurons and thyroid PF cells are derived from the same region of the neural crest (vagal), it is possible that the two types of cell arise from a common precursor that expresses an endocrine phenotype when it develops in the thyroid and a neural phenotype when it develops in the bowel. The recent observations that PF cells are induced to extend neurites and to exress CGRP in preference to calcitonin when treated with nerve growth factor (NGF) supports this hypothesis. Moreover, following treatment with NGF, PF cells do not survive unless grown on enteric smooth muscle. Since further analysis of the plasticity and serotoninergic neural potential of PF cells would be greatly facilitated if experiments could be done on an appropriate cell line, we characterized the neural and serotoninergic properties of human medullary (PF-derived) thyroid carcinoma cells (TT cells, first isolated by Leong et al.). TT cells were grown on plastic in an enriched medium containing 10 % fetal bovine serum. The cells were found to display calcitonin, CGRP, somatostatin, and 5-HT immunoreactivities, but they did not contain substance P. The cellular concentration of 5-HT was measured in washed cells by high pressure liquid chromatography and was found to be 1-5 fg/cell. When incubated with ^{3}H-5-HT (1.0 μM), TT cells concentrated the amine (~5- to 25-fold). This uptake was inhibited by fluoxetine and zimelidine (1.0 μM), specific inhibitors of the neuronal uptake of 5-HT. When incubated with ^{3}H-L-tryptophan (50 μM), TT cells produced ^{3}H-5-HT and ^{3}H-5-hydroxyindoleacetic acid; therefore, TT cells must contain both tryptophan hydroxylase and monoamine oxidase. TT cells also were found to contain a ~45 kDa SBP (binds ^{3}H-5-HT; K_{D1} = 8 nM; K_{D2} = 23 nM). ^{3}H-5-HT binding to TT cell SBP was inhibited by 1.0 μM reserpine, 140 mM Na^+, 1.0 mM Ca^{2+}, and 1.0 μM thiol reagents. These properties are similar to those of neural SBP. The SBP of TT cells also cross-reacted with antisera generated against 45 kDa SBP purified from rat brain and could be shown in TT cells by immunocytochemistry. It is concluded that cultured TT cells express serotoninergic neural properties, not all of which (uptake of ^{3}H-5-HT) are expressed by PF cells *in situ*. Supported in part by grants NIMH 37575; NS12969.

BIOCHEMICAL AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT I

257.1 VITAMIN A-INDUCED SUPPRESSION/ENHANCEMENT OF PROTEIN GLYCOSYLATION AND NEURULATION. Y. Ersahin*, L.I. Nelson*, R.G. Higbee, D.G. McLone, and P.A. Knepper. Division of Neurosurgery, Children's Memorial Hospital and Northwestern University Medical School, Chicago, IL 60614.

Glycoconjugates play major roles in critical cellular functions, e.g., cell migration and cell-to-cell adherence, which are involved in neural tube closure. Previous studies from our laboratory reported biochemical methods for isolating neuroepithelium before, during, and after completion of neurulation (Johnson et al., Neuroscience Abstr., 12:1360, 1986). In this study, the effects of maternal administration of Vitamin A on protein glycosylation were correlated to the process of normal and abnormal neurulation. Pregnant mice were given IP injections of 1,000 or 5,000 IU of Vitamin A (Aquasol parenteral; USV Pharmaceutical Corp.) diluted with deionized water on the evening of day 8 (day 8.5; at the time of initial closure of the neural tube) and on the morning of day 9. Control mice received IP injections of deionized water. On day 12, the embryos were photographed with dark-field illumination, and placed in 100% ethanol for 24 hours at 4°C to stabilize the tissues for microdissection of the neuroepithelium. The lower IU dose of Vitamin A resulted in a low incidence of neurulation defects and the higher IU dose resulted in defects of all live embryos.

The neuroepithelium was solubilized in lysis buffer for one- and two-dimensional polyacrylamide gel electrophoresis, and Western blots using peroxidase-coupled lectins. Proteins were detected by silver staining. Two-dimensional maps of Vitamin A-treated neuroepithelium indicated that 15 spots (approximately 13% of observed proteins) varied, i.e., appeared, disappeared, or changed in their coordinates from the control catalog. In Vitamin A-treated defective embryos with cranial cysts, a marked suppression in glycosylation was observed in two Con A-binding proteins, 20 and 30 Kd, whereas in Vitamin A-treated normal embryos, a marked enhancement of the glycosylation was present in these proteins. Similarly, in Vitamin A-induced caudal neural tube defects occurring with dysgenesis of the caudal cell mass, a reduction of the glycosylation was also observed in the 20- and 30-Kd proteins. Additional studies are in progress to further characterize the glycosylation patterns by additional peroxidase-coupled lectins.

These results indicate that (1) changes in protein glycosylation parallel the events of neural tube closure; and (2) abnormal neuroregulation in the Vitamin A-induced defects may involve abnormalities in the glycosylation of certain proteins in the neuroepithelium.

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257.2 COMPARISON OF c-SRC EXPRESSION AND HYPERTHYROIDISM IN CEREBELLAR MUTANTS. A. Messer, J.S. Brugge*, B. Eisenberg*, A. Lustig*, P. Maskin* and D.I. Martin. Wadsworth Ctr. for Laboratories and Research, NYS Dept. of Health, Albany, NY, 12201; School of Public Health Sciences, SUNY, Albany, NY; Dept. of Microbiology, SUNY, Stony Brook, NY.

In order to understand the mechanisms involved in cerebellar development, a knowledge of the hierarchy of commands used to create the mature cerebellum would be useful. The present studies examine three different aspects of cerebellar development, combining mutants which specifically and reproducibly perturb the normal sequence with thyroid hormone (T4) and/or the proto-oncogene c-src. Mutants were staggerer (sg/sg), missing or abnormal Purkinje cells from birth, granule cells degenerate shortly after migration; Lurcher (Lc/+), degeneration of all Purkinje cells during weeks 2-5, subsequent loss of most granule cells; weaver (wv/wv), degeneration of granule cells due to intrinsic failure to migrate.

A neuron-specific form of pp60c-src has been reported (Brugge et al, Nature 316, 524). The loss of this altered form (designated pp60c-src(+) due to its slightly larger size) generally correlated with the loss of granule cells and Purkinje cells from the cerebella of mice carrying the sg and Lc mutations, with the most pronounced changes observed in cerebella from the more severely affected sg mice. The expression of c-src(+) in wv mice is qualitatively and quantitatively quite different. From the earliest time points, there was a significant reduction in the levels of c-src(+), with no further loss of this form during the period of maximal neuronal differentiation. This suggests an early, predegenerative absence of c-src in wv, which is defective in granule cell migration.

Hyperthyroidism is known to cause premature proliferation and differentiation in the cerebellum. However, T4 does not seem to accelerate the peak of thymidine kinase activity in sg mice, consistent with the hypothesis that intact Purkinje cells mediate this process. In Lc mice, on the other hand, a very mild degree of induced hyperthyroidism leads to significant changes in levels of the amino acids taurine, glutamate, aspartate and GABA at P14, as well as changes in the developmental patterns of these amino acids from P14 to P28. These results suggest that in Lc cerebellum, T4 actually accelerates the program of Purkinje cell degeneration.

Thus, a mutation intrinsic to granule cells affects neuronal c-src presymptomatically, while two mutations intrinsic to Purkinje cells show c-src levels which apparently reflect total neuronal degeneration, yet show differential responses to hyperthyroidism.

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257.3 TWO FORMS OF $pp60^{src}$ ARE DIFFERENTIALLY EXPRESSED IN DEVELOPING CNS. Otmar D. Wiestler* and Gernot Walter*. (SPON: A.E. Traynor). Department of Pathology, University of California, San Diego, La Jolla, CA 92093.

$pp60^{c\text{-}src}$, the protein-tyrosine kinase encoded by the proto-oncogene c-src, is highly active in the brain. It was shown recently that two forms of src protein, detectable in CNS tissue, are generated by differential splicing of c-src mRNA. These two proteins, designated pp60+ and pp60 were studied during CNS development. We found differential regulation of pp60+ and pp60 in distinct areas of the developing mouse brain and at different stages of neurogenesis. One form, pp60, was seen at a low level at embryonic day E9. Its activity increased 50-fold in forebrain and midbrain reaching a plateau in the perinatal period. A trace amount of the other form, pp60+ was first detected in E10 embryonic brains. Between E10 and E18, this protein increased approximately 200-fold in forebrain and midbrain. In the cerebellum, the time course and the level of expression of pp60+ and pp60 were markedly different with constant amounts of both proteins throughout development. These regional and temporal differences in pp60+ and pp60 protein-tyrosine kinase activity corresponded to similar changes in the amounts of pp60+ and pp60 protein. Therefore, c-src appears to be regulated at the level of protein synthesis rather than by changes in its specific kinase activity. We also assayed the c-src kinase in two mutant mouse strains, staggerer and weaver, with postnatal degeneration of cerebellar granule cells. The results indicate that granular neurons provide the main source of pp60+ in the cerebellum. In situ hybridization and immunocytochemical studies are in progress to determine the cellular and subcellular localization of the two proteins in the developing and mature CNS.

257.4 TIME OF APPEARANCE OF mRNA FOR GLUTAMIC ACID DECARBOXYLASE (GAD) DURING RAT BRAIN DEVELOPMENT. R.W. Bond* and D.I. Gottlieb (SPON: G. Cole). Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Glutamic acid decarboxylase (GAD) catalyzes the formation of GABA and is localized primarily in neurons utilizing GABA as a transmitter. It is thus a good marker for studying how neurons become different from one another. Recently, a cDNA which encodes feline GAD has been obtained by A. Tobin's laboratory (Kaufman et al., Science 232: 1138, 1986); this cDNA has been generously given to us. We have used this as a probe to study the appearance of GAD mRNA in the developing rat brain.

The feline GAD cDNA was subcloned as a 2.1 kb EcoR I-Hind III fragment into the Bluescribe plasmid (pBS-Stratagene, Inc.). ^{32}P-labeled antisense RNA was transcribed using T3 RNA polymerase. mRNA was prepared from rat brains by standard methods using guanidine hydrochloride extraction followed by isolation of $PolyA^+$ RNA. The $PolyA^+$ RNA was run on formaldehyde agarose gels and transferred to nitrocellulose (Northern blot). As reported by Tobin's lab (Soc. Neurosci. Abstr. 12: 1458) the cDNA hybridizes to a 3.7 kb mRNA from brain. There is no hybridization to rat muscle or liver RNA.

To study the expression of the GAD gene during development we prepared $PolyA^+$ RNA from whole brains of rats of the following ages: embryonic day 15 (E15), E18, postnatal day 1 (d1), d6, d20, and d60. Northern blots of these preparations showed that a substantial level of mRNA for GAD was present by E15. Two major features of the developmental pattern of GAD mRNA were noted. First, in the adult brain the only detectable band was at 3.7 kb, while at E15 an additional band of higher molecular weight was present at a level of about 30% of the main band. The relative amount of this additional band decreased steadily with age. Second, the amount of GAD mRNA increased markedly between E15 and d1. In the d1 brain, the amount per total $PolyA^+$ RNA was similar to the adult. This is in marked contrast to the levels of GAD enzyme activity in whole brain. At birth the specific activity of the enzyme is about 15% that of the adult. Adult levels are reached about three weeks after birth. The regulatory mechanisms which account for this disparity are not known.

The feline GAD clone has been used to isolate a cDNA from a rat brain λGT-11 library. The clone contains a 2.1 kb insert. Hind III cuts this into a 1.3 and a 0.8 kb piece. The 0.8 kb piece is from the 3' end of the cDNA and does not hybridize with the feline clone. This is consistent with the presence of a 3' untranslated portion of the GAD mRNA (A. Tobin, personal communication; Kobayashi et al., J. Neurosci., in press). The 5' 1.3 kb piece recognizes the same rat mRNA as the feline clone does. (Supported by NIH grant NS12867.)

257.5 **DEVELOPMENT OF SUBSTANCE P mRNA IN SEROTONERGIC CELLS OF THE MEDULLARY RAPHE** Bruce Daugherty*, Ronald P. Hart, and G. Miller Jonakait, Dept. Biological Sciences, Rutgers University, Newark, N.J. 07102

Substance P (SP) is co-localized with serotonin (5-HT) in cells of the medullary raphe nuclei. Few studies have addressed the issue of coordinate regulation of these co-localized neurotransmitters during development. In order to begin to examine the factors that affect the development of multiple neurotransmitters within individual brain nuclei, we have undertaken a study to document the initial expression and subsequent development of SP in the raphe. Since the earliest event in gene expression is transcription, we have measured mRNA specific for the SP preprohormone at different times during development.

A discrete midline segment of the medulla was dissected from rats of gestational days 14 (E14) through adulthood. Total RNA was isolated and subjected to Northern blot analysis using a radioactive cDNA probe for rat preprotachykinin (pGem 2-31-1, kindly provided by J. Krause, Washington University, St. Louis, MO). Northern blots revealed a single hybridizing band about 1100 base pairs in length. Quantitation of SP RNA was performed by densitometric scanning of the Northern blots.

SP message was barely detected at E14 (0.6 densitometric units [DU] per raphe), the earliest age examined. Maximum levels of SP message were detected at postnatal day 13 (P13; 101 DU per raphe), falling thereafter to adult levels of about 18 DU per raphe. The sharpest increase occurred between E21 and P2.

In order to determine whether SP message measured in postnatal raphe nuclei was co-localized with 5-HT, 3-day-old rat pups were injected intracisternally with saline or with 5,7-dihydroxytryptamine (5,7-DHT; 50 ug) to selectively destroy 5-HT neurons. All pups were pretreated with desipramine (20 mg/kg) to prevent neurotoxic effects to catecholamine neurons. After four weeks, the dorsal raphe was removed and assayed for tryptophan hydroxylase (TPH) to monitor 5-HT neuronal destruction. The medullary raphe was divided at the midline; one half was used for total RNA isolation for Northern analysis, the other half for SP determination by radioimmunoassay. Consistent with the studies of Towle *et al.* (1985), 5,7-DHT injection resulted in a 80-98% reduction in TPH activity with no concomitant change in SP peptide. By contrast, however, SP message declined by about 30%. Reasons for the maintenance of SP message are under investigation. (Supported by NS 23687. GMJ and RPH are Johnson & Johnson Discovery Research Fellows.)

257.6 **SEROTONIN, SUBSTANCE P AND TRH DEVELOPMENT IN ORGANOTYPIC TISSUE CULTURE OF EMBRYONIC MOUSE BRAIN.** G. Miller Jonakait and Sandra Schotland*, Dept. of Biological Sciences, Rutgers University, Newark, N.J. 07102

Substance P (SP) and thyrotropin-releasing hormone (TRH) are co-localized with serotonin (5-HT) in cells of the medullary raphe nuclei (Johansson *et al.* 1981). While several studies have documented the initial appearance and subsequent development of 5-HT, SP and TRH in the central nervous system of mammals, few studies have addressed the issue of coordinate regulation of co-localized neurotransmitter substances during embryonic development. In order to examine the factors that control development of multiple neurotransmitters within individual brain nuclei, we have grown presumptive raphe nuclei in organotypic tissue culture, an environment in which mammalian embryonic brain is easily accessible and manipulable.

Tissue was obtained from E13 mice. A discrete midline segment extending from mesencephalon through rhombencephalon was dissected intact or was separated into "midbrain" (MB) and "medullary" (MO) fragments. Tissue was explanted onto collagen coverslips and grown for up to 2 weeks in Maximow depression chambers. Tryptophan hydroxylase (TPH), the rate-limiting enzyme in 5-HT biosynthesis, was barely detectable at explantation. After one week in culture, however, TPH activity registered 24±2.5 ng 5-hydroxytryptophan formed/hr/culture in the undivided cultures. After 2 weeks, TPH activity had increased almost 2.5-fold above the 1-week level. Immunocytochemical analysis of the cultures confirmed a widespread distribution of 5-HT-positive cells and fibers throughout the explant. Substance P, monitored by radioimmunoassay (Kessler *et al.*, 1981), was detected after two days in culture, and attained a level of 111.7±9.8 pg/culture after two weeks. TRH activity (Manaker *et al.*, 1985) was 56.0±2.2 pg/culture after two weeks *in vitro*. Therefore, developmental increases in TPH, SP, and TRH occur in culture, mimicking the condition *in vivo*. Whether SP and TRH are co-localized with 5-HT in culture awaits immunohistochemical analysis.

In four separate experiments, MB and MO fragments, when grown apart on separate coverslips, developed 1.57-2.26 times the TPH activity that developed in the undivided piece. By contrast, SP activity was additive, the amount of SP present in the undivided piece being equal to the sum of the MB and MO fragments. MB and MO fragments grown in the presence of 1 uM pargyline did not develop increased TPH activity, suggesting that 5-HT itself may be inhibiting TPH development. These data indicate that factors (including, possibly, 5-HT itself) affecting the development of TPH in the cultures are not acting simultaneously to affect SP. (Supported by NS 23687. GMJ is a Johnson & Johnson Discovery Research Fellow.)

257.7 CHEMICAL DIFFERENTIATION OF SOMATOSTATIN NEURONS IN RAT NEOCORTEX: IMMUNOHISTOCHEMISTRY, IN SITU HYBRIDIZATION AND NORTHERN BLOT ANALYSIS. C.C.Naus, F.D.Miller*, J.H.Morrison and F.E.Bloom. Division of Preclinical Neuroscience and Endocrinology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The chemical differentiation of somatostatin (SS) neurons in rat neocortex was characterized by molecular biochemical and morphological methods. A cDNA specific for pre-prosomatostatin mRNA was used to probe a Northen blot of adult rat brain regional poly(A)+ RNA, and the results agree, in general, with known distribution patterns of SS neurons. Northern blot analysis of whole brain poly(A)+ RNA shows an increase in pre-prosomatostatin mRNA from barely detectable at E14, to unquestionally detectable at E16, peaking at P1, to decline through P23 to the adult level. Similar analysis of poly(A)+ RNA isolated form cerebral cortex at various times postnatally shows an increase between P9 and P15, with a slight decrease in the adult. Immunohistochemical analysis of immunoreactivity to SS14, or its N-terminally extended form, SS28, reveals a significant development of this system by late gestation (E20). At this point SS28(1-12), the predominant SS form detected, is mainly in neurons of the subplate, with less detectable immunoreactivity in the intermediate zone and cortical plate. By P2, neurons in the subplate exhibit detectable SS28 and SS28(1-12). Although cellular immunoreactivity is no longer detectable in the cortical plate or marginal zone at P2, a very dense plexus of SS28(1-12) fibers is seen in the subplate, marginal zone and intermediate zone, with relatively few fibers in the cortical plate. At P8 to P10, the neuronal immunoreactivity remains largely restricted to the subplate, and immunoreactive fibers appear lessened. By P12, a dramatic shift in immunoreactivity occurs with supragranular SS28 neurons now prominent, and SS28(1-12) neurons and fibers greatly diminished. There is a gradual decrease in the number of SS28 neurons from P12 to adult, when these neurons exhibit a bilaminar distribution; neurons containing SS28(1-12) are now sparsely distributed throughout the cortex, while SS28(1-12) fibers densely innervate layer I and V-VI. A similar pattern of development can be seen using in situ hybridization with a probe specific to pre-prosomatostatin mRNA. Furthermore, individual cortical neurons appear to increase their transcription of the SS gene from E20 to P12, as revealed with in situ hybridization. These results demonstrate the dynamic nature of the chemical differentiation of neocortical SS neurons, at the level of gene transcription, mRNA translation and subsequent post-translational modification of these peptides. Supported by MRC and Huntington Society of Canada, NIH grants NS22347, AA06420 and McNeil Laboratories.

257.8 DEVELOPMENT OF PEPTIDERGIC SYSTEMS IN THE SPINAL CORD OF THE BRAZILIAN GRAY SHORT-TAILED OPOSSUM: FUNCTIONAL AND MORPHOLOGICAL STUDIES. C.A. Fox*, C.D. Jacobson and S. Jeftinija. Dept. of Veterinary Anatomy, Iowa State University, Ames, Iowa 50011.

The distribution of neuroactive peptides in the spinal cord as well as their effects on spinal neurons have been studied in adult animals. However, very little is known about the functional and anatomical development of these systems. To study this we have utilized the Brazilian gray short-tailed opossum, Monodelphis domestica, a small, pouchless, marsupial easily bred in the lab. Initial results on methionine-enkephalin (m-ENK) localization in the spinal cord of adult animals indicate that the spinal cord contains a large amount of m-ENK like immunoreactivity. The areas which had the most abundant concentrations of m-ENK were in laminae I, V and X. Also, there are scattered collections of m-ENK like immunoreactivity in the ventrolateral cell groups in the ventral horn. In general, these results are in agreement with those found for the North American opossum (DiTirroet al., JCN 213, 1983). To test the responsiveness of the developing spinal cord neurons to peptides an in vitro spinal cord preparation of 11-13 day old pups was used (day 1=day of finding pups). This period equals that of the perinatal rat. After laminectomy under ether anaesthesia, the lumbosacral segment of the spinal cord was removed. This preparation was pinned in a Sylgard-lined chamber and continuously perfused with 95% O_2/5% CO_2, at 31±1°C. Intracellular recordings of neurons were obtained using glass micropipettes filled with 3M K-acetate (100-120MΩ). The passive and active electrical properties were investigated by injecting depolarizing and hyperpolarizing current pulses. Neuronal input resistance (105-223MΩ) and time constant (5.6-15.7ms), were measured at a resting membrane potential of 55-62mV. The threshold for an action potential produced by depolarizing pulses was between -40 and -50mV. The amplitude of the action potential measured from threshold to peak was 42-55mV; the duration measured at the threshold was 2.3 to 4.8ms. Bath application of substance P ($5x10^{-6}$M) produced a reversible depolarization associated with an increase in synaptic activity. Similarily, an excitatory depolarization was observed in response to vasoactive intestinal polypeptide (10^{-5}M) and thyrotropin releasing hormone (10^{-6}M). In addition, glutamate and N-methyl-D-aspartate had an excitatory action. Acetylcholine, nicotine and muscarine produced a depolarization associated with an increase in synaptic activity. (D-Ala, D-Leu)enkephalinamide (10^{-5}M), however, produced a reversible hyperpolarization of 2-5mV lasting between 4-8 minutes. These results indicate that the Brazilian opossum is a model in which the sensitivity of spinal cord neurons to putative neurotransmitters during development can be studied. Supported by NIH grants HD-16148 and 2S07 RR07034.

257.9 DEVELOPMENTAL EXPRESSION OF DARPP-32 IN MOUSE CORPUS STRIATUM. N.L. Rosen, I. Shalaby, T. Kurihara, M.E. Ehrlich, H.C. Hemmings, Jr. and P. Greengard. The Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

DARPP-32 is a neuron-specific phosphoprotein, which is a substrate for cAMP-dependent protein kinase. It is found in dopaminoceptive neurons that possess D_1 receptors, particularly the medium spiny neurons of the basal ganglia. It is hypothesized that DARPP-32 is involved in the processes of neuromodulation that follow D_1 receptor-stimulated generation of cAMP. We have developed a quantitative Western blot assay for DARPP-32 that is linear in the range of 2-100 ng of DARPP-32, as well as a DARPP-32 cDNA clone. With these tools we have looked at the appearance of DARPP-32 and its mRNA in the basal ganglia of embryonic and postnatal mice.

The corpus striatum was dissected from CD-1 mice (Charles River) sacrificed at ages between embryonic day 14 and adult. Pooled samples were sonicated for protein determination in 1% SDS at 70°, followed by heating at 95° for 5 min. Samples of 0.25 mg protein were subjected to SDS-PAGE and proteins blotted onto nitrocellulose paper. The blots were developed by successive incubations with monoclonal antibodies against DARPP-32, rabbit anti-mouse IgG, and I-125 labelled protein A. Radiolabelled bands located by exposure to X-ray film were cut and counted in a γ-counter. Appropriate DARPP-32 standards were included for quantitation. Total cellular RNA was prepared from tissue that had been dissected onto dry ice. Ten microgram RNA samples were separated by electrophoresis on a 1.2% formaldehyde gel, transferred to nitrocellulose and hybridized with an antisense ^{32}P-RNA probe transcribed from a full length DARPP-32 cDNA (T. Kurihara et al., J. Neuroscience, in press). Relative DARPP-32 mRNA levels were determined by densitometry of the autoradiogram.

The amount of DARPP-32 mRNA increased dramatically between birth and the end of the first week of postnatal life, and then more slowly approached adult levels by the end of the third week. The amount of the phosphoprotein itself increased markedly throughout the first three postnatal weeks, reaching near-adult levels by the end of the third week. Thus, the major increase in expression of DARPP-32 occurs after the arrival of dopaminergic input during fetal life and immediately following the cessation of neuron production (R.R. Sturrock, J. Anat. 130:243, 1980). It correlates closely with the appearance of adenylate cyclase activity, and the beginning of formation of dendritic spines. (Supported by USPHS NS 00988 and MH 40899 and by a grant from the American Parkinson Disease Association.)

257.10 DEVELOPMENT OF THE BASAL FOREBRAIN CHOLINERGIC NEUROTRANSMITTER SYSTEM. L.J. Thal, D.M. Armstrong, E. Gilbertson, S.R. Deputy, F.H. Gage. Dept. Neurology, San Diego VA Med. Ctr., Dept. Neurosciences, Univ. of California, San Diego, La Jolla, CA 92093

As a correlative to our anatomical investigations into the development of forebrain cholinergic neurons we employed biochemical methods to assay choline acetyltransferase (ChAT) activity within discrete anatomical loci of the developing forebrain. Fetal tissue was obtained from timed pregnant Sprague-Dawley rats. At least four brains, obtained from one or more litters, were examined from each of the following times: embryonic day 14 (E14), E18, birth, postnatal day 1 (PD1), PD5, PD10, PD16, PD20, PD30, PD60 and PD180. From prenatal brains five anatomical regions were assayed for ChAT: medial septum/diagonal band (MS/DBB); magnocellular preoptic/substantia innominata (MgPO/SI); striatum; anterior cingulate/frontoparietal cortex (ACg/FrPa); and hippocampus. Similarly, seven regions were examined from postnatal brains: MS; DBB; MgPO/SI: medial striatum; lateral striatum; ACg/FrPa; and hippocampus.

ChAT activity was first detected on E18 in the MS/DBB and MgPO/SI (10 nmoles ACh/hr/mg protein) (Cholinergic perikarya were first detected by immunohistochemical methods on E14, four days earlier. In the striatum ChAT levels were barely detectable until after birth, thereafter enzyme activity increased first in lateral then in medial striatum. Following birth and continuing through the first month, all forebrain regions exhibited a dramatic rise in ChAT activity reaching levels beyond that observed in the adult. This rise in enzyme activity correlates with our anatomical observations of intense amounts of peroxidase reaction product during the first three weeks of development. In the hippocampus and neocortex ChAT activity was first detected several days later than in the MS/DBB or MgPO/SI. These target areas, however, also exhibited a dramatic increase in enzyme levels following birth. In summary, our findings support dynamic postnatal changes in ChAT expression.

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257.11 IMMUNOCYTOCHEMICAL LOCALIZATION OF TRANSFERRIN AND MALATE DEHYDROGENASE IN THE DEVELOPING RAT NERVOUS SYSTEM. G.J. Markelonis, T.H. Oh, T.L. Dion*, B.S. Bregman and M.A. Pugh*. Dept. Anatomy, Univ. Maryland Sch. Medicine, Baltimore, Maryland 21201.

Transferrin accumulates within neurons of the developing nervous system of humans, sheep, pigs and chickens. To assess the relationship of this accumulation with the ontogeny of oxidative metabolism, we studied the immunocytochemical localization of transferrin (Tf) and the mitochondrial form of malate dehydrogenase (mMDH) in developing neural tissues by the peroxidase-antiperoxidase method. Rabbit antirat Tf was obtained commercially and gave a single band of reaction product (MW = 80 kd) on Western blots. Antibodies to porcine heart mMDH were elicited in a rabbit. Western blot analysis showed that this antiporcine mMDH antibody reacted with the mMDH from porcine, rat or avian tissue but not with the cytosolic MDH from several species. Tf was first detected in rat brain neurons at about the 18th embryonic day and reached a peak at about the 6th postnatal day. All neurons were immunoreactive with large neurons throughout the brain showing a strong reaction for Tf. From this time onward, the level in brain neurons gradually decreased until adulthood. However, Tf immunoreactivity still remained strongly evident in capillary endothelial cells. The localization of Tf within rat spinal cord neurons peaked as early as the first postnatal day and remained elevated to the 6th postnatal day. By contrast, reactivity for Tf within dorsal root ganglia (DRG) neurons was intense as early as the 18th embryonic day and diminished only gradually. Mitochondrial MDH, a marker for oxidative metabolism, appeared to reach a peak after the crest of intraneuronal Tf had been observed. For example, brain and spinal cord MDH immunoreactivity increased with intense staining in the cell bodies and fibers of neurons from the 6th to the 13th postnatal day; immunoreactivity gradually diminished into adulthood. The gradient of reactivity was low in some areas of the brain but more intense in areas containing large neuronal cell bodies such as the red nucleus. This occurred after the peak of intraneuronal Tf at day 6 and suggested a precursor-product relationship. By contrast, immunoreactivity for neuron-specific enolase, a glycolytic enzyme, showed a developmental pattern that differed from either Tf or MDH in that reactivity appeared later in development and was less intense. These data suggest that as cerebral metabolic rates begin to increase as early as 5-6 days after birth in the rat, an increase in mMDH occurs coincident with the onset of oxidative metabolism. Furthermore, this rise in intraneuronal mMDH follows the peak of intraneuronal Tf and suggests that Tf supplies the iron required for the synthesis of other mitochondrial ferroproteins. Supported by the NIH (NS 20490-GJM and NS 15013-THO).

257.12 **EFFECTS OF PRENATAL 8-OH-DPAT & TFMPP ADMINISTRATION ON THE DEVELOPMENT OF SEROTONERGIC NEURONS & RECEPTORS** A.Shemer, E.Van Bockstaele, P.M.Whitaker-Azmitia, E.C.Azmitia Dept.of Biology. Washington Square Center for Neural Science, New York University,New York, N.Y.10003. and Dept. of Psychiatry, SUNY at Stony Brook, Stony Brook, N.Y.11793

Serotonin has been shown to have an important role to play in the development of the brain. We have shown previously in an in vivo and in vitro model that the serotonergic agonist 5 Methoxytryptamine (5-MT) has a biphasic effect on the outgrowth of serotonergic neurons. A low dose of 5-MT (0.1 mg/kg) administered to pregnant rats produces an inhibition of neurite outgrowth in neonates while a high dose (3 mg/kg) produces enhanced outgrowth in target areas of serotonergic innervation.This biphasic response may be acting through two different $5HT_1$ receptors : the $5\text{-}HT_{1a}$ has been observed on midbrain raphe cell bodies, a possible autoreceptor; the $5\text{-}HT_{1b}$ has a tenfold lower affinity and appears to be located in target areas.

In order to further establish the role of these two high-affinity receptors during development we decided to investigate the effects of prenatal 8-Hydroxy-2 (DI-n-Propylamino) Tetralin (PAT) a specific $5\text{-}HT_{1a}$ agonist and 1-(3-(trifluoromethyl) phenyl)-piperazine (TFMPP) a specific $5\text{-}HT_{1b}$ agonist. Both drugs were administered daily at 1mg/kg i.p.to pregnant Sprague-Dawley rats from day 12 of gestation to birth. The neonates were killed on day 1 and 15 postnatally. The high affinity uptake of 3H-5HT (50nM) was performed in a synaptosomal preparation . Receptor binding was carried out in a membrane preparation using 3H-5HT (0.5nM - 20.0nM).

TFMPP, working through the $5\text{-}HT_{1b}$ receptor replicated the enhanced outgrowth to target areas observed after a high dose of the agonist 5MT. High affinity uptake into the forebrain was significantly increased on D1 & D15. PAT treated rats showed no significant changes in uptake measures. Receptor binding reflected differential effects of the prenatal PAT and TFMPP administration : the PAT caused reduced binding in the high affinity component of the $5\text{-}HT_{1a}$ receptor, whereas the TFMPP did not produce a similar inhibition.

These results suggest that the $5\text{-}HT_{1a}$ and the $5HT_{1b}$ receptors are functional prenatally in the rat and produce differential effects on the development of serotnergic neurons. Supported by NSF grant 5-259-272.

257.13 DEVELOPMENTAL CHANGES OF NEURAL CELL ADHESION MOLECULE (N-CAM) IN RETINAL AFFERENTS TO OPTIC TECTUM. P. Streit and I. Kulka*, Brain Research Institute, University of Zurich, CH-8029 Zurich, Switzerland

At which stage of ontogeny does the 'adult' (A) form of N-CAM appear in a well defined 'in vivo' system, which allows analysis at the level of a particular neuronal cell type? Is this appearance correlated in time with certain events in the development of the system investigated? Which mechanism brings about the developmentally regulated change from the 'embryonic' (E) to the A form, the so- called 'E to A conversion'? - Intraocular injection of [^{35}S]-methionine in chicken embryos at different stages of development led to biosynthetic labeling of N-CAM which was axonally transported in the retinotectal pathway. After different survival times, N-CAM was immunoprecipitated from detergent extracts of tectal tissue by a monoclonal antibody selected to react with both forms of the molecule. Labeled N-CAM was autoradiographically analysed following SDS-polyacrylamide gel-electrophoresis. - Labeling was found almost exclusively in the tectum contralateral to the injected eye in this mostly crossed projection. Newly synthesized and transported A form of N-CAM could first be observed at incubation day 16, i.e. after the time of synapse formation, after the periods for generation and degeneration of retinal ganglion cells and with the onset of myelination in this pathway. If tracer was injected on day 15, labeled N-CAM was immunoprecipitated in its E form even on day 19. No processing of the E form to the A form of N-CAM seemed to occur following axonal transport and incorporation into the cell surface membrane in this 'in vivo' system. 'E to A conversion', thus, seems to be the result of a change of programs for the neuronal synthetic machinery. If a relatively slow turnover of N-CAM is assumed, the coexistence of E and A forms can be expected at the cellular level for a period of several days and may result in graded adhesion forces. Besides such a modulatory function of the E to A conversion, the newly appearing A form could have an inductive action in the process of myelination, or it could be involved in the stabilization of synapses.

257.14 NEURONAL REGULATION OF MEMBRANE ACETYLCHOLINE (ACh) RECEPTORS AND SODIUM (Na) CHANNELS IN DEVELOPING RAT MUSCLE. L. Bambrick and T. Gordon. SPON: P. Braun), Dept. of Biochemistry, McGill Univ., 3655 Drummond Drive, Montreal, Quebec, H3G 1Y6 and Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alberta, T6G 2H7.

During the development of mammalian skeletal muscle in vivo, the muscle membrane becomes specialized with incorporation of voltage-sensitive Na channels and the restriction of high densities of ACh receptors to the neuromuscular junction. This differentiation is nerve-dependent (Sherman & Catterall, J. Gen. Physiol., 80: 753, 1982). We have studied the effects of denervation and presynaptic blockade of ACh release with botulinum toxin (BoTX) to investigate the mechanism of the neuronal regulation of these membrane proteins.

Triceps surae muscles were denervated by sciatic nerve section or their neuromuscular transmission blocked by subcutaneous injection of BoTX at 1, 7, 14, 21 and 28 days after birth. Three to 7 days later, muscles were homogenized for assay of Na channels and ACh receptors using ^{3}H-saxitoxin (^{3}H-STX) and ^{125}I-alpha-bungarotoxin (^{125}I-BTX) as specific radioligands in equilibrium binding and saturation binding assays, respectively. Both denervation and BoTX-blockade inhibited or reversed the normal developmental increase in Na channel and decrease in extra-junctional ACh receptor numbers measured relative to muscle protein, weight or fiber diameter. The effects of denervation were always greater than those of BoTX-blockade, in contrast to our earlier work with adult rats in which BoTX-blockade was as effective as denervation in altering the numbers of Na and ACh receptors in the muscle membrane (Bambrick & Gordon, J. Physiol., 382: 69, 1987).

Both results are consistent with the idea that neuronal control of Na channels and ACh receptor incorporation into the muscle membrane is mediated via functional neuromuscular transmission. The finding that BoTX-blockade, which is complete for evoked but not for spontaneous ACh release, is less effective in neonatal animals suggests that membrane depolarization may be sufficient to control the incorporation of Na channels and to decrease the incorporation of ACh receptors into the developing membrane. Depolarizations in response to the small, spontaneous release of ACh in BoTX-treated junctions would be insignificant in the adult. However, in the neonatal muscle in which depolarization would be enhanced by high input resistance, longer ACh receptor open times and lower levels of acetylcholinesterase, membrane depolarization may be sufficiently large to exert a significant trophic effect in controlling membrane protein synthesis.

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258.1 ELECTROENCEPHALOGRAPHIC NEUROTOXICITY STUDY OF THE FIRE ANT POISON AMDRO. G.M. Strain and M.C. Graham*. Veterinary Physiology, Pharmacology, and Toxicology, Louisiana State University, School of Veterinary Medicine, Baton Rouge, LA 70803.

Amdro (American Cyanamid, AC 217,300) is a novel insecticide of the amidinohydrazone class marketed for treatment of red imported fire ants and roaches. Amdro has low toxicity (male rat oral LD50 = 1131 mg/kg, techical grade) but because the fire ant formulation is in soybean oil on corn meal it is highly attractive to domestic and wild animals. Seizures have been observed in dogs, cats and birds after accidental poisoning, and neurotoxicity has been reported after long-term dosing in calves (Evans et al, Am J Vet Res 45:1023; 1984). No neurotoxic effects were detected with somatosensory, auditory, or visual evoked potentials in F-344 and hooded male rats during 10 days of recording after a single oral dose of Amdro concentrate (Strain and Graham, Soc Neurosci Abstr 12:93; 1986). We now report the effects of Amdro on the electroencephalogram of male F-344 rats. A single dose was used to simulate an actual poisoning, since technical Amdro has a 1 hour half-life in sunlight and repeated exposures are unusual.

Subjects were divided into two treatment groups: vehicle control (oleic acid in soybean oil) and Amdro concentrate in vehicle. The Amdro concentrate dose was equal to 0.75 X LD50 of technical Amdro. EEG were recorded from chronic screw electrodes before dosing, and at hours 1, 4, and 8 and at days 1, 2, 3, 4, and 7 after dosing. Recording span was chosen based on the elimination rate of Amdro in the rat (90% in 9 days). EEG were recorded on FM tape and later analyzed on an Apple-based IQS EEG 4000 spectral analysis system. Six 10-second artifact-free epochs recorded between left and right cortex were averaged for each rat; a grand average was then computed for each treatment group at each measurement time. EEG power was calculated in 1 Hz bands from 0-30 Hz, but most of the power and all of the variation occurred in the 0-10 Hz range.

The EEG power spectrum in control rats showed two peaks, at 4 and 7-8 Hz. Dosing with Amdro produced an increase in power in the low frequencies and an approximately 1 Hz shift to the left of both peaks. The effect was maximum at 24 hours, but did not resolve until day 7. The rats receiving vehicle control displayed similar frequency and power changes, but the change in EEG power was less marked and the frequency shift had resolved by day 2. Thus, both treatments produced sedative effects. Initial effects could be attributed to "postprandial" lethargy, but would not have persisted for subsequent days; circadian activity rhythms should also have affected the pre-dosing and day 7 EEG recordings. Therefore, the results likely reflect a previously unrecognized CNS depressant effect of oleic acid and an additional sedative effect of Amdro.

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258.2 CYTOTOXIC MECHANISM OF *Pyrularia* THIONIN. K.-P. Shaw, F.C. Kauffman, V.P. Vernon*[1], and E.X. Albuquerque. Dept. Pharmacol. & Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201 & [1]Dept. Chem. Microbiol. Brigham Young Univ. Provo, Utah 84602.

Pyrularia thionin (PT) isolated from *Pyrularia pubera* Michx. (Santalaceae) is a 47-amino-acid, basic peptide which has high homology with wheat purothionin and mistletoe viscotoxin. It has been used for many years for the treatment of neoplastic disease and has been suggested as a possible chemotherapeutic agent for cancer patients (Vernon *et al.*, *Arch. Biochem. Biophys.* 238:18, 1985). The purpose of these experiments was to characterize the cytotoxicity of PT on spleen lymphocytes and hepatocytes from rats by a simple dye extraction assay and to study the effect of PT on the electrophysiological properties of frog sartorius muscles. The IC_{50} of PT for cell viability based on dye uptake by isolated hepatocytes and lymphocytes, were 0.15 μM and 40 μM, respectively. PT (0.5 μM) potentiated the contraction of indirect twitch of the sartorius muscle by 20% within the first 5 minutes and caused complete inhibition within 30 minutes. The membrane potential of sartorius muscle was depolarized by 80% with PT 80 nM and by 60% with PT 60 nM. Alteration of membrane potential in the presence of PT was a calcium-dependent phenomenon. Preincubation with verapamil (10 μM), a calcium channel blocker, for one hour and subsequent addition of PT (60 nM) for another hour protected the membrane from depolarization. After washout of verapamil, treatment with PT (60 nM) for one hour depolarized the membrane potential from 95% to 40% of control values. No protection of membrane depolarization occurred in the presence of either indomethacin (100 μM), a cyclo-oxygenase inhibitor, or tetrodotoxin (330 nM), a sodium channel blocker. However, preincubation of sartorius muscles with the phospholipase A_2 inhibitor, dexamethasone (1 to 100 μM for 3 hours), and continuous presence during one more hour exposure to PT (60 nM), preserved the membrane potential at a level of 80 to 90% of control values. A 37% increase in the frequency of miniature endplate potential was recorded during the first 10 minutes of exposure to PT (0.05 μM). In summary, the data suggest that the cytotoxic effect of PT may be directly related to calcium influx due to the activation of phospholipase A_2. Different membrane-phospholipid compositions may underlie different sensitivities to the toxin. (Supported by Directed Research Initiative Funds from the Univ. Maryland and NIDA Grant DA 02804).

258.3 GANGLIOSIDE TREATMENT REDUCES PERIPHERAL NERVE ELECTROPHYSIOLOGIC ALTERATIONS INDUCED BY VINCRISTINE ADMINISTRATION. F. Di Gregorio*, G. Favaro*, C. Panozzo*, A. Schiavinato*, E. Lini* and M.G. Fiori. Fidia Research Laboratories, 35031 Abano Terme (PD), Italy

Vincristine (VCR) is an antiblastic drug used against several types of tumors. Doses and duration of VCR treatment are limited by the drug side effects, which often affect the peripheral nervous system. To study the possibility of peripheral nerve protection against VCR neurotoxicity, the effect of simultaneous treatment with a mixture of bovine brain gangliosides (Cronassial®, Fidia) was tested. The rabbit was chosen as experimental animal since it resulted much more sensitive to VCR than smallest laboratory animals. A first group of rabbits was treated once a week for five consecutive weeks with 0.2 mg/kg VCR (Lilly) i.v. and with either phosphate buffer solution (PBS) or ganglioside mixture dissolved in PBS (50 mg/ml, at daily doses of 1 ml/kg given i.v. six times a week for the entire period of VCR administration). Controls were injected i.v. with PBS. A second group of rabbits was treated according to the same protocol, but the VCR dose was raised to 0.25 mg/kg. At the end of the treatment animals were sacrificed, and sciatic nerve and its branches dissected out. Compound action potential (CAP) amplitude and area, and nerve conduction velocity (NCV) were measured by in vitro standard technics. Other nerve segments were processed for light and electron microscopy. Treatment with 0.2 and 0.25 mg/kg VCR + PBS reduced NCV by 17.6% and 23.7% respectively. Monophasic CAP area and peak amplitude were affected in a dose-dependent manner, decreasing respectively by 22.3% and 34.6%, after 0.2 mg/kg VCR + PBS administration, and by 35.3% and 45.6% after 0.25 mg/kg VCR + PBS. Histologic and ultrastructural observation demonstrated the presence of nerve fiber degeneration which was more frequent at the high VCR dose. Morphometric analysis on sural nerves myelinated axons of control and 0.2 mg/kg VCR-treated rabbits, evidenced the increased frequency of tiny fibers, coupled with decreased frequency of myelinated axons of larger size. This could suggest that axon regeneration and remyelination take place in rabbit VCR neuropathy, as a consequence of degenerative events. Ganglioside treatment was effective in reducing alterations of all the electrophysiologic parameters, in 0.2 mg/kg VCR-treated rabbits, being the NCV reduction limited to 11.8%, CAP area to only 3.9%, and CAP peak amplitude to 18.7%. Ganglioside protective action on CAP area and amplitude was maintained at the high VCR dose as well, the decrease being limited to 21.6% and 25.2%, respectively. These observations suggest that ganglioside treatment could reduce the loss of functional nerve fibers which affects rabbit sciatic nerve after VCR administration.

258.4 CYANIDE-INDUCED NEUROTOXICITY: ATTENUTATION BY CHLORPROMAZINE. E.U. Maduh*, B.K. Ardelt*, J.D. Johnson*, J.L. Borowitz* and G.E.Isom* (SPON: G. Yim). Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.

Chlorpromazine (CPZ) has been demonstrated to be an effective cyanide antidote with its greatest efficacy displayed when combined with the classic antidotes, sodium nitrite and sodium thiosulfate (Kong et. al., Toxicol. Appl. Pharmacol. 71: 407 3). Since the presumed site of cyanide lethality is the central nervous system, the objective of the present study was to determine the interaction of chlorpromazine with cyanide in neural systems. Tremors produced by KCN administration to mice are correlated with increases in brain calcium and accompanied by peroxidation of brain lipids. These toxic manifestations are prevented by pretreatment with diltiazem, a calcium channel blocker. This study examines the ability of CPZ to alter the KCN-induced increase in cellular calcium and neuronal lipid peroxidation. KCN (1-10 mM) increased [Ca]i in rat pheochromocytoma (PC-12) cells as indicated by the fluorescent dye quin II. This effect was blocked by diltiazem or CPZ (0.1 mM) added 15 min to the cells prior to addition of KCN. Peroxidation of brain lipids in mice administered KCN 15 mg/kg (sc), as determined by the conjugated diene method, was blocked by pretreatment with CPZ or diltiazem. Furthermore, production of lipid peroxidation in fresh mouse brain slices (0.3mm) induced by 0.1 mM KCN was blocked by 0.1 mM diltiazem or 0.1 mM CPZ. These observations suggested CPZ protects neuronal cells against damage by cyanide by preventing calcium influx into the cells and by decreasing lipid peroxidation of membranes. (Supported in part by PHS grant ES04140).

258.5 EFFECT OF 1,1,2,TRICHLOROETHYLENE ON THE HIPPOCAMPAL FORMATION OF THE DEVELOPING RAT. L.G. Isaacson*, T. Kravetz*, D.H. Taylor*, and R.J. Pfohl*. (SPON: R.G. Sherman). Dept.Zoology, Miami Univ., Oxford, OH 45056.

1,1,2 Trichloroethylene (TCE), an industrial solvent and degreaser, has been detected in the drinking water of several municipalities and poses a potential health hazard to the general population. Previous behavioral and biochemical studies in our laboratory indicate that the hippocampus of the developing rat may be affected by exposure to TCE. The present study utilizes immunocytochemical and histochemical techniques in order to assess damage to the hippocampal formation which might result from exposure to TCE in rat pups while in utero and during lactation.

Dams were given drinking water containing 312 or 625 ppm TCE while pregnant and during lactation. Thus, rat pups were exposed to TCE while in utero and postnatally through dam's milk. At post-natal day 21, they were sacrificed by aortic perfusion. Frozen sections were processed for choline acetyltransferase (ChAT) immunocytochemistry. Alternate sections were stained for the presence of myelin using a modification of the Heidenhain procedure (J. Neurosci. Meth.7:289-294). Also, selected sections were stained for the presence of degenerating axons and terminals using a modification of Fink-Heimer silver degeneration stain.

Examination of sections from TCE treatment groups stained for the presence of myelin revealed a dramatic decrease in staining activity in the stratum lacunosum-moleculare of the dorsal hippocampus, an area which has been shown to receive input from the entorhinal cortex. The axons of the mossy fiber pathway and the fornix appeared unaffected. Following Fink-Heimer staining, degenerating axonal fibers were observed in the stratum lacunosum-moleculare and in the stratum radiatum of treated animals. No difference in ChAT immunoreactivity, either in neuronal or axonal immunolabeling, was ascertained at the light microscopic level.

These results indicate that the area of the developing hippocampal formation which receives input from the entorhinal cortex appears to be affected by exposure to TCE. In contrast, cholinergic input, which originates primarily from the septum, appears to remain intact. Thus, the changes observed in the hippocampus may explain the behavioral dysfunctions observed in rats exposed similarly to TCE.

258.6 INTRACORTICAL AF64A INFUSION IN THE RAT INDUCES AN ATROPHY AND LOSS OF CHOLINERGIC CELL BODIES WITHIN THE NUCLEUS BASALIS MAGNOCELLULARIS (nBM). P.R. MOUTON* and G.W. Arendash (SPON: R. Shannon). Section of Physiology and Development, Department of Biology, University of South Florida, Tampa, Florida 33620.

Ethylcholine mustard aziridinium ion (AF64A), a selective irreversible inhibitor of high-affinity choline uptake located on cholinergic terminals, has been proposed as a useful agent in the development of animal models for the cholinergic hypofunction and memory loss characteristic of senile dementia of the Alzheimer's type (SDAT). Previously we have reported that following low dose intracortical AF64A infusions, specific decreases in cholinergic markers occur within the cerebral cortex through at least 10 weeks post-infusion, resulting in a variety of learning/memory deficits with a similar time course. It is not known, however, whether such treatment is toxic to the cell bodies of cholinergic neurons located in the nBM, which provides the primary cholinergic innervation to the fronto-pairetal (FP) cortex. We report in the present histochemical analysis that a significant atrophy and loss of cholinergic neurons in the nBM occurs by 3 weeks following AF64A infusion into the rat FP cortex.

Adult male Sprague-Dawley rats received two 1.0 ul infusions of AF64A (1 nmole/1 ul) or vehicle into the FP cortex bilaterally. We have previously reported that, at the dose and volume of AF64A used in the present study, only minimal non-specific tissue damage occurs in the neuropil adjacent to infusion sites and no decreases are seen in monoaminergic or peptidergic (somatostatin or neuropeptide Y) markers within the cortex. Three weeks after such AF64A or vehicle infusion, all animals were pre-treated with di-isopropylfluorophosphate (DFP: 2.0 mg/kg, i.m.) and sacrificed five hours later. Following brain perfusion and removal, 30 um serial sections were cut through the level of the nBM utilizing a freezing microtome. Sections were histochemically stained for acetylcholinesterase (AChE) followed by Nissl counter-staining for cell body visualization. Widths and lengths of dark AChE-positive cell bodies within the anterior and middle nBM were made in addition to AChE-positive cell counts in the same nBM regions. A highly significant 37% decrease in the cross-sectional area of nBM cholinergic neurons was observed in AF64A-treated rats compared to vehicle-treated animals (412 ± 23 vs. 649 ± 32 um^2). Moreover, the density of cholinergic neurons within the nBM of AF64A-treated rats was significantly lower (by 35%) compared to that of controls.

These data indicate that AF64A can be a specific cholinotoxin for a sizable number of neurons comprising the nBM-to-cortex cholinergic pathway. Furthermore, those nBM cholinergic neurons that survive cortical AF64A treatment apparently do so in an atrophic state. The data support a use of AF64A in the development of animal models for SDAT based on cholinergic dysfunction.

258.7 THE EFFECTS OF PYRETHROIDS ON PAIRED PULSE INHIBITION IN THE HIPPOCAMPUS OF THE RAT. M.E. Gilbert, C. Mack* and K.M. Crofton*. Northrop Services Inc, RTP, NC 27709 and U.S.EPA, Neurotoxicology Division, RTP, NC 27711.

The pyrethroid insecticides have been divided into two classes on the basis of their biochemical actions and behavioral indices of toxicity. Both types of pyrethroids have effects on Na conductance, and Type II pyrethroids have been reported to have GABA-antagonistic effects through interaction with the picrotoxin binding site. Cismethrin (CSM) and deltamethrin (DLT) are Type I and Type II pyrethroids, respectively. We attempted to demonstrate a dissociation between the pyrethroid types by testing their actions on GABA-mediated inhibition in the perforant path-dentate circuit of the hippocampus in the conscious, unrestrained rat. We anticipated a decrease in GABA-mediated recurrent inhibition with DLT that would be manifested in a larger response to the second pulse of the pair at brief IPIs (20-50ms) following treatment with DLT but not CSM. A bipolar stimulating electrode was placed in perforant path and a recording electrode in the hilus of the dentate gyrus. One week after surgery, animals were delivered pairs of stimulus pulses (biphasic square waves, each 0.1ms in duration, once every 10s) at interpulse intervals (IPI) of 20-1000ms, before and 3-5h after oral administration of 10-15mg/kg of DLT or 20-30mg/kg of CSM in a corn oil vehicle. These dose levels produced strong behavioral reactions typically reported for the pyrethroids (e.g. profuse salivation, tremor, writhing movements). Intensity of the stimulation was initially chosen to produce a response 75% of the maximal population spike (PS). After dosing, the amplitude of the PS was altered, so intensity was adjusted to equate the size of the spike in the pre- and post-dosing tests. The PS evoked by the two pulses was measured and the amplitude of the response to pulse 2 expressed as a percentage of the amplitude of the response to pulse 1. This percentage was then compared between baseline and post-dosing tests. DLT (N=5) actually enhanced paired pulse inhibition up to and including the 500ms IPI, an effect opposite to a picrotoxin-like action. CSM (N=6) had no effect upon paired pulse inhibition. These findings suggest that DLT does not act through a GABA antagonistic mechanism in this system. It is unlikely that DLT is acting as an agonist of GABA-mediated inhibition since all but the longest IPIs were affected. Pyrethroids also act on neuronal membrane ion channels, prolonging Na permeability through formation of a modified open state of the Na channel. Type II pyrethroids produce a depolarizing block of nerve activity which has been attributed to summation of long lasting depolarizing afterpotentials. This action of pyrethroids on Na channel kinetics may account for effects on paired pulse inhibition in the hippocampus.

258.8 THE SUPPRESSIVE EFFECTS OF ANESTHETIC, HALOTHANE, ON REACTIVE SYNAPTOGENESIS IN THE RAT DENTATE GYRUS. E. Uemura, E.D. Levin, R. DeLuna, and R.E. Bowman. Dept. of Veterinary Anatomy, Iowa State Univ. Ames, IA 50011, Dept. of Psychology, UCLA, CA 90024, Dept. of Psychology, Univ. of Wisc. Madison, WI 53715.

Halothane is one of the most widely used inhalation anesthetic agents. It is typically used at 1.5% concentration (15,000 ppm) in conjunction with nitrous oxide. We report that in rat received entorhinal electrolytic lesions, halothane exposure considerably slows down reactive synaptogenesis of the rat dentate gyrus, but also that the initial delay in the reactive synaptogenesis did not result in permanent deficits in synaptic pupulation. Male rats (n=72, 60 days old Sprague-Dawley) were used in the study. Rats were separated into three groups: unlesioned control (n=24), lesioned control (n=24), and experimental (n=24). Starting on the day after unilateral entorhinal lesioning, the experimental group were exposed to 100 ppm halothane (8 hours/day) for 15 days. At days 2, 15, 25, and 30 post-lesion, six rats from each group were killed for electron microscopic quantitation of synapses. Tissue from the outer molecular layer of the dentate gyrus were block-stained with 1% ethanolic phosphotungustic acid, and number of synapses per unit volume of tissue was calculated.

The synaptic density was similar in the unlesioned control ($F=1.795$, $df=3,20$, $P>0.18$). However, in both the lesioned control and experimental groups, a significant loss of synapses was observed in the dentate gyrus ($F=38.0$, $df=6,60$, $P<0.0001$). The maximum loss occured at two days after the entorhinal lesions. Although the synaptic replacement followed with age in these two groups, the replacement was notably faster in the lesioned control. The lesioned control rats recovered about 73% of the lost synapses by day 15 post-lesion. By day 30 post-lesion, synaptic density in those rats reached 88% of the unlesioned control value. The experimental group demonstrated the inhibitory effect of halothane on reactive synaptogenesis in the dentate gyrus ($F=71.64$, $df=3,20$, $p<0.0001$). The rate of synaptic recovery was very low during the time rats were exposed to halothane. Only 17% of the lost synapses were restored by the day 15 post-lesion. However, suppression of reactive synaptogenesis for 15 days did not induce a permanent synaptic deficit. Rapid recovery of the synaptic population followed when halothane exposure was terminated. Thus, by day 30 post-lesion, the synaptic density in halothane-exposed rats attained the lesioned control values ($t=1.6479$ $df=20$, $p>0.12$). This suppressive action of halothane suggests its utility as a research tool for delaying synaptogenesis during selected developmental epochs or recovery from brain lesions, to study the relationship between synaptic and behavioral recovery. Supported by March of Dimes grant 15-56.

258.9 REMAK AND SCHWANN CELL SUSCEPTIBILITY TO LOCAL ANESTHETIC-INDUCED INJURY IN RAT SCIATIC NERVE. M.W. Kalichman, H.C. Powell*, R.R. Myers*. Anesthesia Research, V-151, VA Med Ctr, San Diego, CA 92161.

Local anesthetics can be neurolytic (e.g. Myers et al., Anesthesiology 64: 29, 1986); however, little is known about the etiology or mechanisms of the resulting nerve injury. Using a model developed in this laboratory, numerous studies have been conducted examining the neurotoxicity of a representative group of local anesthetic agents including chloroprocaine, etidocaine, lidocaine, and procaine. Surveys of the tissue exposed to toxic concentrations of these agents suggested the possibility that the severity of Schwann cell injury was dependent on the type of Schwann cell. Two types of Schwann cell are found in peripheral nerve: Remak cells which do not myelinate associated nerve fibers and Schwann cells (SC-M) which myelinate nerve fibers. To test the hypothesis of differential sensitivity to nerve injury, tissue was examined from a study in which 3 concentrations of each of 4 different local anesthetics were tested. Test solutions were injected near the sciatic nerve of female Sprague-Dawley rats. After 48 hours, the nerves were removed and prepared for light and electron microscopy. One-micron-thick sections from a representative block from each nerve were evaluated by light microscopy and ultrathin sections for electron microscopy were cut from blocks that were sufficiently well-fixed. Five of these blocks were randomly selected for this study. Electron microscopy was done on a Zeiss 10 electron microscope at 2000x.

Remak cells, SC-M, and the number of injured cells were counted in each negative representing a 41 X 44 um non-overlapping cross-section of the largest fascicle from the selected nerve block. Cytoplasmic vacuolization (VAC) and the disruption of the basal lamina (BLD) occurred with approximately twice the frequency in Remak cells (58% and 62%) compared to SC-M (22% and 20%). Conversely, lipid droplets were significantly more frequent in SC-M (9% vs. 2%). Chi-squared calculations for comparisons of each of these three pairs of proportions indicated a highly significant difference in susceptibility between SC-M and Remak cells ($P \ll 0.001$).

VAC and BLD are significant pathologic features of Schwann cell necrosis. The relative absence of lipid droplets in the cytoplasm of Remak cells supports the hypothesis that these inclusions are indicative of a protective mechanism in the SC-M. Since other histopathological and functional evidence is consistent with the hypothesis that the relative potencies of local anesthetics as neurolytics and as anesthetics are comparable (Co Tui et al., J Pharmacol Exp Ther 81: 209, 1944), it is worth noting that the present results demonstrate greater injury to those Schwann cells associated with small, pain-carrying fibers -- a mechanism consistent with local anesthesia.

258.10 NEUROTOXICOLOGICAL ASSESSMENT OF 3-ACETYL PYRIDINE IN RATS. V.C. Moser,[1] K.F. Jensen,[2] J.P. O'Callaghan,[2] and R.C. MacPhail.[2] [1]Northrop Services, Inc, RTP, NC 27709 and [2]U.S. EPA, Neurotoxicology Division, RTP, NC 27711.

3-Acetyl pyridine (3AP) produces a characteristic cerebellar ataxia presumably due to the loss of the inferior olivary neurons, the source of cerebellar climbing fibers. In the present experiment we established a profile of the neurobehavioral effects of 3AP using a functional observational battery (FOB). The FOB is a series of observations and measurements that can be rapidly applied to toxicant-treated rats, and includes home-cage and open-field observations, neuromuscular and sensorimotor tests, and physiological measures. Evaluations were made according to U.S. Environmental Protection Agency testing guidelines so as to determine dose-, time-, and sex-related toxicant effects. Long-Evans hooded rats of both sexes (N=10/dose) were dosed i.p. with either saline, 40, 50, or 60 mg/kg 3AP. Testing occurred immediately before dosing and at 4 hr, 1, 4, 10, and 23 days after dosing. Three rats from each dose group were perfused with 4% paraformaldehyde 35 days after dosing. Alternate sections from the region containing the inferior olive were either stained with cresyl violet to estimate cell loss, or prepared for immunohistochemical localization of glial fibrillary acidic protein (GFAP)-like reactivity. In addition, samples of a corresponding region were taken from an additional three rats per dose group and assayed for GFAP by RIA. The effects of 3AP on muscle tone and equilibrium were most outstanding and lasted from 1 day to the last day of testing (23 d). Effects of 3AP included altered gait (ataxia and tip-toe walking), decreased muscle resistance and grip strengths, increased landing foot splay, and catalepsy. Decreased motor activity, general signs of toxicity, and hypothermia were observed only on days 1 and 4 post-dosing. The highest dose was lethal to 2 males and 1 female, between 1 and 7 days after dosing; these rats showed dypsnea, lacrimation, salivation and marked debilitation. Females were much less affected on many of the measures by 3AP than were males. There were fewer neuromuscular effects in females, for example, no changes in grip strengths or landing foot splay were obtained. In addition, a dose of 40 mg/kg was sufficient to produce significant effects on gait in males, but only the highest dose (60 mg/kg) was effective in females. Histological examination revealed a dose-dependent loss of large neurons, and gliosis in the region of the inferior olive of both male and female rats. In summary, 3AP profoundly affected primarily neuromuscular function in rats. In males, but not females, the extent of morphological changes appeared to correspond to the extent of functional deficits.

258.11 POLYCHLORINATED DIBENZOFURANS REDUCE CELLULAR DOPAMINE AND NOREPINEPHRINE CONCENTRATIONS IN PHEOCHROMOCYTOMA (PC-12) CELLS. R.F. Seegal, W. Shain, B. Bush*, P.W. O'Keefe* and K.O. Brosch*. Wadsworth Center, NYS Dept. of Health, Albany, NY 12201.

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants shown to produce neurological dysfunctions in humans and behavioral and neurochemical changes in animals. We have recently demonstrated (NSA, 12, 91, 1986) that PCBs decrease PC-12 cellular concentrations of dopamine (DA) and norepinephrine (NE). However, PCBs are often contaminated during manufacture and use with the more toxic polychlorinated dibenzofurans (PCDFs). In order to determine whether PCDFs are also neurotoxic we studied the effects of PCDFs alone and in combination with PCBs on catecholaminergic function of rat PC-12 cells in culture.

PCDFs were separated from the PCBs (Aroclor 1254) by Florisil column chromatography. Gas chromatography-mass spectrometric analysis showed that the PCB fraction contained PCDFs below the 1 ppb detection limit. The PCDF fraction contained over 95% of the 20 ppm total PCDFs present in the original Aroclor mixture. Nearly confluent cultures of PC-12 cells were exposed to various concentrations of PCDFs, PCBs only, or PCDFs + PCBs (Aroclor 1254) for 24h. These mixtures were dissolved in dimethylsulfoxide (DMSO) and diluted in growth media. The final DMSO concentration was 0.1%. PCB concentrations ranged from 0.1 to 100 ppm. PCDF concentrations were made equivalent to the concentrations in the Aroclor 1254 mixture and ranged from 2 ppt (equivalent to .1 ppm PCB exposure) to 2 ppb (equivalent to 100 ppm PCB exposure). After exposure, cells were washed with HEPES-buffered Hanks and extracted with 0.2N perchloric acid containing 200 mg/l EDTA. Cell supernatants were analyzed by high-performance liquid chromatography with electrochemical detection. Catecholamine content of each well was normalized to protein content by incorporation of radioactive amino acids. Values of amino acid incorporation were converted to mg protein after independently assaying total protein and amino acid incorporation in parallel wells. Changes in catecholamine content are expressed as percent of DMSO-vehicle control values.

Exposure to clean PCBs produced a significant reduction at only the highest exposure level, while exposure to PCBs + PCDFs (the original Aroclor mixture) produced significant reductions of DA and NE concentrations at concentrations ≥ 1 ppm (a 50-65% inhibition at the 100 ppm exposure level). Exposure to PCDFs only at the 200 ppt level (equivalent to 10 ppm of Aroclor 1254) produced a 15-20% reduction in DA and NE concentrations and a 25-35% reduction at the 2 ppb level. These effects on catecholamine function are not due to cell loss since neither PCBs nor PCDFs decreased the protein content of the wells.

These results represent the first experimental evidence that PCDFs are capable of altering catecholaminergic function. The magnitude and direction of change induced by these three mixtures (e.g. PCBs, PCBs + PCDFs, PCDFs) suggests that the effects of PCBs + PCDFs are additive, with PCDFs possessing neurochemical action at concentrations of between 4 and 5 orders of magnitude lower than that shown following exposure to PCBs only.

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258.12 Characterization of Specific [^{3}H]PK 11195 Binding Sites in Rainbow Trout Brain Membranes. A.J. Eshleman* and T.F. Murray. Toxicology Program/College of Pharmacy, Oregon State University, Corvallis, Or. 97331

Peripheral benzodiazepine binding sites (PBS) have been labelled in rat brain, kidney and heart using both [^{3}H]RO5-4864 and [^{3}H]PK 11195, a non-benzodiazepine isoquinoline carboxamide derivative. Previous reports have indicated low or undetectable levels of PBS in non-mammalian vertebrates when labelled with [^{3}H]RO5-4864. We now report measurement of a high affinity binding site for [^{3}H]PK 11195 in rainbow trout (Salmo gairdneri) brain, using a modified P_2 fraction. Equilibrium binding of [^{3}H]PK 11195 to brain membranes was determined in hypotonic buffer at 4°C following 100 min. incubation. Specific binding was defined as the difference between binding in the absence and presence of 10μM PK 11195. Equilibrium binding analysis yielded a K_d of 1.6±0.2 nM for PK 11195, as determined both by competition experiments and by non-linear least squares analysis of saturation isotherm data. The maximal binding capacity in whole trout brain was 1.1±0.05 pmol/mg protein. Regional distribution was not uniform and relative densities of sites were spinal cord > rhombencephalon > optic tectum > olfactory bulb > cerebellum > cerebrum. The range of densities in these brain regions was 5 fold.

Competition experiments revealed that the sites labelled by [^{3}H]PK 11195 in trout brain have a unique pharmacological profile compared to those in murine brain. The K_d for PK 11195 in mouse cerebral cortex was 1.8±0.9 nM, similar to the trout. However, RO5-4864, which has high affinity in the mouse (IC_{50}=10.3±8.8 nM) has low affinity in the trout (IC_{50}=106μM). Furthermore, diazepam was 100 fold, and clonazepam 15 fold, less potent at the trout brain sites. In contrast, RO15-1788, a central benzodiazepine receptor (CBR) antagonist, had a low potency (IC_{50}=44μM) in trout with no effect in the mouse. Other compounds which showed efficacy only in the trout include CGS 9896 and CGS 8216, pyrazoloquinolines with agonist and antagonist activity, respectively, at the CBR in rat brain, with IC_{50}'s of 1.1±0.4μM and 2.3±0.4μM for [^{3}H]PK 11195; and diltiazem, a benzothiazepine Ca^{2+} channel antagonist, with low μM affinity. However, two structurally dissimilar Ca^{2+} channel antagonists, verapamil, a phenylalkylamine, and nifedipine, a 1,4-dihydropyridine, had less interaction with this site, giving no effect in either species or high μM displacement in the trout only. The Type I pyrethroid, permethrin, had low μM potency in both species, while deltamethrin, a Type II pyrethroid, had high nM potency in the mouse and no effect in the trout. The physiological significance of this unique binding site in Salmo gairdneri brain membrane remains to be determined. (Supported by NIEHS Center grant ESO3850).

258.13 AMIODARONE-INDUCED LAMELLAR INCLUSION BODIES ARE A MARKER OF DRUG USE RATHER THAN CELL DAMAGE.
A.J. Windebank, C. Schreiber*, and M. Blexrud*(SPON: J. Whisnant). Department of Neurology, Mayo Clinic, Rochester, MN 55905.

Amiodarone is an anti-arrhythmic drug which may produce peripheral neuropathy. Because it intereferes with lipid metabolism, it has been postulated that the drug disrupts myelination. However, clinical and biopsy studies suggest a pathologic process primarily involving the axon. We have directly investigated the effect of amiodarone on dorsal root ganglion (DRG) neurons in vitro. Three aspects of cell function were studied quantitatively; cell survival, neurite extension, and accumulation of cytoplasmic inclusion bodies (IB). All studies were carried out using E15 DRG in medium supplemented with 15% calf serum and 100 ng/ml 7S NGF. Amiodarone was added to media and actual concentration measured by HPLC.

Cell Survival. Dissociated neurons were plated into dishes with a grid pattern etched on the surface. Neurons were counted for 2 days in control medium and then sequentially for 12 days following addition of amiodarone (0-10 µg/ml) containing medium. There was no significant effect of drug on cell survival.

Neurite Extension. DRG explants were placed on a collagen surface. Incubation was carried out in control medium or the same medium with amiodarone (0.3, 2.2, 3.9, 16.7, and 32 µg/ml). Radial neurite outgrowth from individual explants was measured sequentially for 48 hours and then DRG were fixed and embedded for quantitative microscopy. Rate of neurite outgrowth was significantly ($p < .02$) affected at the highest concentration (control: .38 ± .02 mm/day; .39 ± .02 at 0.3 µg/ml; .36 ± .02 at 2.2 µg/ml; .31 ± .01 at 3.9 µg/ml; .33 ± .02 at 16.7 µg/ml; and .29 ± .02 at 32 µg/ml).

Accumulation of IB. Typical lamellar IB were identified by electron microscopy and counted in transverse sections of DRG explants. They were never seen in control neurons but appeared at the lowest concentration of drug used (0.2 µg/ml) within 48 hours. The number of IB increased with increasing drug concentration at low levels and then reached a plateau (IB/cell profile: 0.0 ± 0.1 in controls; 4.0 ± 0.6 at 0.2 µg/ml; 6.7 ± 1.4 at 1.0 µg/ml; 6.9 ± 1.2 at 2.0 µg/ml; 8.6 ± 0.7 at 3.9 µg/ml; 8.8 ± 1.4 at 5.1 µg/ml; 8.6 ± 1.5 at 9.8 µg/ml; 8.8 ± 1.0 at 16.7 µg/ml).

We conclude that the appearance of IB occurs rapidly at concentrations below the lower end of therapeutic levels (1-3 µg/ml in serum). However, cell damage measured by survival or axonal extension is not affected until higher concentrations. This would lead to the hypothesis that lamellar IB seen in tissue are a marker of drug use rather than of drug induced cell damage.

258.14 EFFECT OF GAMMA RADIATION ON AGGRESSIVE BEHAVIOR IN MALE SWISS WEBSTER MICE. D.M. Maier* and M.R. Landauer* (SPON: L. Cockerham). Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

The resident-intruder paradigm was used to assess the effects of gamma radiation on aggressive behavior in male Swiss Webster albino mice. Resident mice (individually housed) were exposed either to 10 Gy (1000 rads) cobalt-60 radiation (N=13) or were sham-irradiated (N=13). A weight-matched isolated male mouse of the same strain was designated as the intruder and was not exposed to radiation. Aggressive behavior was assessed 2 days prior to irradiation and during the first week postirradiation by placing intruders into the resident's home cage for 5 min. Immediately prior to this test, resident mice were given a preference test in which they could investigate a chamber housing their weight-matched intruder or an uninhabited chamber which served as a control for non-social investigation (Landauer, M.R. and Balster, R.L., Psychopharmacology, 78:322, 1982). Locomotor activity, assessed as the number of crossovers between the two stimulus compartments, was concurrently recorded over the 15 min preference test.

Over the course of resident-intruder testing, aggressive behavior was greatest in both groups of mice on the first test session (2 days prior to treatment). By 7 days postirradiation, the number of attacks and total attack time were significantly decreased in the mice exposed to 10 Gy radiation compared to sham-irradiated animals. Irradiated mice also exhibited an increase in the latency to fight on this day. Locomotor activity between the two groups did not differ until day 7 post-irradiation when the irradiated animals showed a significant decrease in the number of crossovers. On all test days, investigation of the conspecific was greater than investigation of an uninhabited compartment for sham-treated mice. However, in the irradiated mice, the preference for the conspecific decreased after irradiation and had completely disappeared by 7 days postirradiation.

The results of this experiment indicate that a dose of 10 Gy gamma radiation did not significantly decrease aggressive behavior, locomotor activity, or social investigation until 7 days postirradiation. All animals exposed to 10 Gy died 8 to 9 days postirradiation. A dose-response study is now in progress to determine the effects of sublethal doses of gamma radiation on social behavior.

258PO PERINATAL UPREGULATION OF BENZODIAZEPINE RECEPTOR ONTOGENY: "FEARLESS" AND MORE EFFICIENT GOAL-DIRECTED BEHAVIOR OF MATURE, FOUR MONTHS OLD RAT PROGENIES. T.J. Marczynski, M.C. Hawkins*, P.G. Swann*, A.F. Krivograd* and M. Dugich*. Department of Pharmacology, University of Illinois College of Med., Chicago, IL 60612.

For 3 weeks, between gestation day 14 and postpartum day 14, pregnant and subsequently lactating rats had ad libitum access to Purina Chow and drinking water. In the 1st group of 10 rats, water contained a benzodiazepine antagonist, RO 15-1788 (17.5 mg dissolved in 3 ml of the vehicle and added to 1 L of tap water); in the 2nd group, water contained diazepam (DZ; 35 mg/L), and in the 3rd group water contained a comparable volume of the vehicle (LaRoche Co.). On the average, the pregnant and subsequently lactating dams consumed 2.9 mg/kg/day of RO 15-1788, or 5.3 mg/kg/day of DZ. On the postpartum day 15, the lactating dams regained access to plain tap water. The litters were reduced to 6-7 pups per dam by removing the females on postnatal day 2.

The gross development of the male offsprings was comparable in all three groups, as judged by their viability, weight gain and motor skills. However, the mature 4 months old offsprings (N=9) that had been perinatally exposed to RO 15-1788 displayed a significantly different behavioral profile than the control (N=9) or the DZ-exposed progenies (N=9): a) During 13 daily trials in the Radial Arm Maze, they readily habituated to novel environment, their exploratory activity was uninhibited by "intimidating" visual and auditory stimuli (a pendulum with a ticking clock that swung 1 ft over the center platform of the radial maze), whereas both the control and the DZ-exposed offsprings tended to "hide" in the least illuminated part of the maze; b) their mean latencies to explore all 8 alleys and to collect all baits were significantly shorter (2.4 min; $p<0.0002$) than for the control group (3.5 min) or the DZ group (5.3 min); c) they made much fewer errors in the maze, as defined by Olton & Samuelson (J. exptl. Psychology, Animal Beh. Processes, 1:97, 1976); their ratio between the sum total of errors and the number of collected baits over thirteen 20 min daily trials equaled 212/936, and was smaller ($p<0.0001$) than the ratios of 305/929 and 322/913 for the control and the DZ-exposed group, respectively; d) they had a much better control over their bowels and urinary bladders than either the control or the DZ-exposed animals, as judged by the urination/defecation scores ($p<0.0003$); and e) at the age of 5 months, they had an approximate 66% increase in the density of benzodiazepine binding sites in the hippocampal formation, as compared to the control or the DZ-exposed offsprings [$p<0.02$; the Peritz' multiple comparison test based on Scatchard analysis of binding assays described by Medina et al. (Europ. J. Pharmacol. 90:125, 1983)].

The results indicate that: 1) perinatal exposure to RO 15-1788 stimulates the ontogeny of benzodiazepine bindings sites in the hippocampus; 2) the increased density of these binding sites is retained in adult animals and confers to them "immunity" to emotional stress normally caused by novel and "threatening" environmental stimuli; and 3) the emotional stability apparently allows the animals to perform more efficiently in the Radial Arm Maze.

258PO FURTHER EVIDENCE THAT 5,6-DIHYDROXYTRYPTAMINE MEDIATES SOME OF THE NEUROTOXIC EFFECTS OF METHYLAMPHETAMINE: 5,6-DHT-INDUCED NEURONAL DEGENERATION IN THE SOMATOSENSORY CORTEX. L.S. Seiden and D. Commins*. Department of Pharmacol. Physiol. Sciences, The University of Chicago, Chicago, IL 60637.

Methylamphetamine (MA) and some related drugs including methylenedioxyamphetamine, methylenedioxymethylamphetamine (MDMA), and parachloramphetamine (PCA) are toxic to serotonergic neurons. Administration of these drugs to rats produces long-term neurochemical deficits indicative of nerve terminal destruction, including depletions of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid, and a reduction in the number of high affinity uptake sites for 5-HT. 5,6-Dihydroxytryptamine (5,6-DHT), a serotonergic neurotoxin, has recently been detected in the rat brain following administration of MA or PCA leading us to speculate that the endogenous formation of 5,6-DHT from 5-HT may mediate the neurotoxic effects of MA and related drugs on the serotonergic system.

In addition to their toxic effects on serotonergic neurons, MA, PCA and MDMA cause degeneration of neurons in a restricted area of laminae III and IV of primary somatosensory cortex. We hypothesize that two factors may account for the selective degeneration of these particular non-serotonergic neurons. First, these neurons may receive a particularly dense serotonergic projection so that the local concentration of 5,6-DHT formed from 5-HT following administration of MA or related drugs is high enough to damage the somatosensory cortical neurons despite the fact that they are not serotonergic. Secondly, these neurons may themselves possess some property that makes them more susceptible than other non-serotonergic neurons to the toxic effects of 5,6-DHT.

In the present experiment we tested the latter hypothesis by infusing 125 µg of 5,6-DHT or vehicle unilaterally into the lateral ventricle of rats. The rats were perfused 1-2 days after surgery with 10% formal saline and their brains were processed according to method I of Fink and Heimer. Preliminary results indicate that the distribution of degenerating neurons in the rat somatosensory cortex following infusion of these high doses of 5,6-DHT is similar to that seen after administration of MA, PCA and MDMA, supporting our hypothesis that: 1) endogenously produced 5,6-DHT mediates the neurotoxic effects of MA and related drugs and 2) neurons in laminae III and IV of the somatosensory cortex are selectively affected by MA and related drugs because they are more sensitive to the toxic effects of 5,6-DHT than other neocortical neurons. This research is supported by NIDA DA-00085; USPHS MH-14274 Training Grant. L. Seiden is the recipient of RSA MH-10562.

259.1 NEUROTENSIN SELECTIVELY ATTENUATE DOPAMINE INHIBITION OF MIDBRAIN DOPAMINERGIC NEURONS. W.-X. Shi* and B.S. Bunney (SPON: S.H. Koslow). Depts. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT 06510.

Many lines of evidence suggest an interaction between neurotensin (NT), a tridecapeptide, and dopamine (DA) in the mammalian central nervous system. A number of reports have indicated that NT has physiological and behavioral effects that resemble those of DA antagonist. These observations have led Nemeroff to postulate that NT could be an endogenous neuroleptic (Nemeroff, C.B., Biol. Psychiatry, 15:283,1980). We report here that NT can selectively attenuate the response of midbrain DA neurons to microiontophoretically applied DA.

Male rats weighing between 280-320 g were used. The animals were anesthetized with chloral hydrate. Extracellular neuronal signals were recorded through the central barrel of a seven-barrel micropipette. One of the outer barrels was filled with 0.5 m NaCl and used for the automatic neutralization of tip current. Others were filled with either NT (0.5 mM in 5 mM NaCl, pH 6.0), DA (0.1 M, pH 4.0), GABA (0.05 M, pH 4.0), glutamate (0.01 M, pH 8.0) or NaCl (5 mM, pH 6.0). DA neurons were identified on the basis of established extracellular electrophysiological criteria (Grace, A.A., and Bunney, B.S., Neurosci., 10:333,1983).

As previously reported for DA neurons in the substantia nigra (SN) (Andrade, R. and Aghajanian, G.K., Soc. Neurosci. Abstr., 7: 573,1981), we found that NT could produce dose-dependent significant increases in the firing rate of DA neurons in both the ventral tegmental area (VTA) and the SN. However, in most cases, NT either had no effect or only produced small to moderate increases in firing rate. On the other hand, NT significantly antagonized the inhibition of SN and VTA DA cells induced by local application of DA. The specificity of this action of NT is suggested by the finding that NT had no measurable effects on either GABA-induced inhibition or glutamate-induced excitation of these same DA neurons. The effect of NT on DA-induced inhibition also was dose-dependent and without a change in baseline activity.

NT-containing neurons and fibers are found in both the VTA and the SN. High densities of NT binding sites are also found in these areas (Lazarus, L.H., et al., Neuropharmacol., 16:625,1977; Young, W.S., III, and Kuhar, M.J., Brain Res., 203:273,1981). The striking depletion of such sites following experimental (6-OHDA) lesion of DA neurons suggests their location is on DA cells (Quirion, R., et al., Brain Res., 327:385,1985, Palacios, J.M. and Kuhar, M.J., Nature, 294:587,1981). These facts support the hypothesis that the observed modulatory effects of NT on DA cell activity may reflect an underlying physiological action of endogenous NT.

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259.2 EFFECTS OF CHOLECYSTOKININ AND DOPAMINE ON THE MEMBRANE PROPERTIES OF MESENCEPHALIC DOPAMINE CONTAINING NEURONS STUDIED IN VITRO. M.S. Brodie and A.L. Mueller, Abbott Laboratories, Abbott Park, IL 60064.

Neurons of the substantia nigra (SN) and ventral tegmental area (VTA) which are presumed to contain dopamine have been demonstrated to have unique electrophysiological characteristics in vivo. These include a low firing rate (0.5 to 5 Hz), a broad action potential (2.5 to 5 msec) and an obvious inflection on the rising phase of the extracellularly recorded action potential. These cells are inhibited by local application of dopamine and by the systemic administration of the dopamine agonist apomorphine. We have previously reported that presumed dopamine-containing neurons of the VTA recorded in vitro have characteristics similar to those observed in vivo. In addition, it has been reported that administration of cholecystokinin octapeptide (CCK-8), which is colocalized with dopamine in some neurons of the VTA, potentiates the inhibitory action of dopamine on the firing rate of these cells in vivo. We also observed this action with extracellular recordings of neuronal activity of these cells maintained in vitro. To extend our earlier observations, we have used intracellular recording techniques in attempts to uncover the specific membrane effects of dopamine and CCK-8. Sprague-Dawley rats (100-200 gm) were sacrificed and coronal slices (300 uM) containing the ventral tegmental area and substantia nigra were prepared. A rostral section of the VTA was chosen for study, since a larger percentage of neurons which contain both CCK-8 and dopamine are found there. Intracellular recordings were made using glass microelectrodes containing 2M KCl (40-80 MOhm). Stable recordings were obtained which could be maintained for up to four hours. Dopamine, in concentrations of from 10 to 50 uM, reduced the firing rate of those cells which exhibited spontaneous activity. In agreement with observations reported by others, dopamine decreased the input resistance of cells of the SN and some cells of the VTA. There was, however, a distinct population in which the dopamine-induced reduction in spontaneous activity was accompanied by a resistance increase. Addition of CCK-8 (5-10nM) to the superfusion medium depolarized the cell membrane; this depolarization was not accompanied by an increase in firing rate. Consistent with observations made extracellularly, prior administration of CCK-8 produced a potentiation of the inhibitory effect of dopamine on firing rate, but no clear potentiation of the effect on membrane potential or resistance was seen. While cells of the VTA exhibit a large degree of heterogeneity, those cells in which dopamine inhibition is potentiated by CCK-8 may be a select, more homogeneous population useful for studying the neuromodulatory role of CCK-8 in the central nervous system.

259.3 CHOLECYSTOKININ OCTAPEPTIDE (CCK8) POTENTIATES GABA-INDUCED INHIBITION OF DOPAMINE NEURONS IN THE RAT VENTRAL TEGMENTAL AREA IN VITRO. J.D. Stittsworth, Jr., A.L. Mueller, and M.S. Brodie. Dept. of Neurosci., Abbott Laboratories, Abbott Park, IL 60064.

Previous studies have suggested the presence of a GABA-ergic input to the ventral tegmental area (VTA). It has been proposed that this GABA-ergic input modulates DA function and alterations of this modulation may play a role in the etiology of schizophrenia. In vivo administration of either GABA or baclofen usually inhibits the firing of DA neurons; however, excitation is sometimes seen. We have previously demonstrated that CCK8 applied to DA-containing neurons in the VTA excites these neurons and potentiates the DA-induced inhibition of these neurons. Previous in vivo work suggests CCK8 potentiates GABA effects (Bunney et al., 1975, Neuronal CCK). The purpose of this study was to determine if CCK_8 might also affect GABA-induced actions, and to determine the specificity of these interactions using a VTA brain slice preparation.

Coronal brain slices, containing the VTA and SN, were prepared from male Sprague-Dawley rats (75-150 gm). Extracellular recordings were obtained from DA-containing neurons. Neurons were presumed to be DA-containing based on a spontaneous firing rate of 0.5-4.0 Hz, an action potential duration >2.5 msec, and a characteristic spike waveform. Drugs were added to the recording chamber dropwise in known concentrations and changes in the spontaneous firing rates were measured. GABA (1 uM - 100 uM), bicuculline methiodide (BMI, 10 nM - 10 uM) and baclofen (3 nM - 300 nM) were used for this study. Before a GABA agonist or antagonist was used, the neuron was tested for responsiveness to DA; only neurons responsive to DA were used in this study. Application of GABA to the recording chamber produced a dose-dependent inhibition of neuronal firing. When CCK_8 preceded the GABA-induced inhibition, potentiation of inhibition was observed in 56% of the neurons tested. Application of BMI, a GABA-A receptor antagonist, to the recording chamber resulted in a dose-dependent increase in firing of the DA neuron. BMI blocked the GABA-induced inhibition in 63% of the neurons tested. When it was possible, CCK8/GABA interactions were studied following the application of BMI. It was observed that CCK8 still potentiated GABA-induced inhibition of those neurons not affected by BMI. In another series of experiments, baclofen, a GABA-B agonist, was tested on DA neurons. Baclofen application to the bath resulted in a dose-dependent reduction of DA neuronal firing. This baclofen-induced inhibition of neuronal firing was potentiated following CCK8 application. These data suggest that both GABA-A and GABA-B receptors are present in the VTA, and that inhibition of DA neuronal firing by GABA via a GABA-B type receptor is potentiated by CCK8.

259.4 CHOLECYSTOKININ OCTAPEPTIDE POTENTIATES THE INHIBITORY RESPONSE MEDIATED BY A D-2 DOPAMINE RECEPTOR IN THE RAT VENTRAL TEGMENTAL AREA IN VITRO. A.L. Mueller, J.D. Stittsworth, Jr. and M.S. Brodie. Abbott Laboratories, Abbott Park, IL 60064.

We have reported previously that exogenously applied cholecystokinin octapeptide (CCK8) elicits an excitation of midbrain dopamine (DA)-containing neurons in the rat ventral tegmental area (VTA). In addition, CCK8 potentiates the DA-induced inhibition of these same neurons (Mueller et al., Soc. Nsci. Abst., 1986). Biochemical and pharmacological data suggest the existence of two subtypes of DA receptors, D-1 and D-2, within the CNS (Stoof and Kebabian, Life Sci., 1984). The purpose of the present study was to utilize agonists and antagonists relatively selective for these receptor subtypes (Stoof and Kebabian, 1984) in order to determine whether the DA-induced inhibition of VTA neurons which is potentiated by CCK8 is mediated via an action of DA at a D-1 or D-2 receptor.

Coronal slices of mesencephalon were prepared from male Sprague-Dawley rats (75-150 gm) as described previously (Brodie and Dunwiddie, Soc. Nsci. Abst., 1985). Extracellular recordings were obtained from presumed DA-containing neurons identified on the basis of their spontaneous firing rate (0.5-4.0 Hz), and action potential duration (>2.5 msec) and waveform. Drugs were added directly to the recording chamber in known concentrations, and changes in spontaneous firing rate were measured. All neurons were initially tested for their inhibitory response to exogenously applied DA (3-50 uM). DA-induced inhibition was blocked totally by the D-2 antagonist, 1-sulpiride (1-10 uM). CCK8 administration did not restore a DA-induced inhibition in the presence of 1-sulpiride. The D-2 agonist, LY-171555, produced a dose-dependent inhibition of DA neuronal firing (1-300 nM). In addition, this LY-induced inhibition was potentiated by CCK8 in 7/10 neurons. On the other hand, the D-1 agonist, SKF-38393 (300 nM - 300 uM), elicited no changes in firing rate, either alone or following CCK8 administration. Neither did the D-1 antagonist, SCH-23390 (500 nM - 10 uM), produce any measurable alterations in spontaneous firing rate. Taken together, these findings suggest that CCK8 modulates the inhibitory action of DA at a D-2 receptor subtype on midbrain DA-containing neurons.

259.5 DOPAMINE-GABA INTERACTIONS : EVIDENCE THAT ONLY D2 RECEPTORS CONTROL THE ACTIVITY OF THE GABA SYSTEM. R. Bernasconi*, T. Leonhardt*, D. Aryee*, A. Steulet*, P. Martin* M. Williams and S. Bischoff* (SPON: W. Haefely). Biol. Res. Lab., Pharmaceutical Div., Ciba-Geigy Ltd., CH-4002 Basle, Switzerland.

Anatomical, physiological and pharmacological data indicate the existence of important interactions between the GABA and dopamine (DA) neuronal systems. We have studied the effects of specific D1 and D2 agonists and antagonists on cortical and hippocampal GABA turnover in mice. GABA turnover was estimated by measuring the accumulation of GABA after GABA-T inhibition with gabaculine This GABA accumulation seems to be nerve-impulse dependent, and may reflect GABAergic neuronal activity (Perez de la Mora, M., Fuxe, K., Hoekfelt, T. and Ljungdahl, A. Neurosci. Lett. 5:75, 1977).

Stimulation of DA receptors by apomorphine, a mixed D1/D2 agonist, dose-dependently reduced GABA turnover, the threshold dose being between 0.25 and 0.5 mg/kg s.c. In contrast, the selective D2 antagonist sulpiride (100 mg/kg i.p. or 300 mg/kg p.o.) had no effect on either GABA levels or on GABA turnover. However, sulpiride antagonized the reduction of GABA turnover rate produced by apomorphine. Since apomorphine alone did not modify either GABA levels or GABA-T activity (Perez de la Mora, M., Fuxe, K., Hoekfelt, T. and Ljungdahl, A., Neurosci. Lett. 1:109, 1975) the above results suggest the existence of important GABA-DA interactions within the cortex and the hippocampus. They also indicate that the reduction in GABA turnover induced by apomorphine is dependent only on the occupancy of the D2 receptors. This hypothesis was supported by the fact that SCH 23390, a D1 antagonist, which by itself failed to change the concentration of endogenous GABA and only slightly decreased GABA turnover, did not antagonize the effect of apomorphine. On the contrary, SCH 23390 (0.2, 0.6 and 1.0 mg/kg i.p.) slightly, but significantly enhanced the reduction in the rate of GABA synthesis produced by apomorphine.

Since clinical experiments indicate that there is only a correlation between the clinical antipsychotic doses of various neuroleptics and their potencies at the D2 receptor, these results suggest that GABAergic neurotransmission may be involved in the mechanism of action of neuroleptics.

259.6 STRIATONIGRAL SUBSTANCE P NEURONS RESPOND NONUNIFORMLY TO LOSS OF DOPAMINERGIC INPUT IN STRIATUM. C.J. Cruz* and R.M. Beckstead (SPON: L.D. Middaugh). Department of Neuroscience, University of Virginia, Charlottesville, VA 22908.

Removal of dopaminergic input to the striatum by either pharmacological manipulations (chronic administration of dopamine antagonists) or by a 6-hydroxydopamine (6-OH-DA) lesion of the nigrostriatal dopamine projection has been repeatedly demonstrated to affect peptide neurotransmitter dynamics in striatal neurons. Levels of opioid and tachykinin peptides in terminals of striatal efferent neurons are changed by these treatments, for example, as are the amounts of mRNAs encoding their precursors in the striatum. Following removal of DA input to striatum, depletion of substance P (SP) (a tachykinin peptide synthesized in the striatum and found in high quantities in striatonigral axon terminals) in whole substantia nigra can be detected by microdissection and RIA. We sought to determine whether in fact this loss represents a uniform depletion of SP in all striatonigral neurons or whether there are differences among striatonigral SP neurons in their responsivity to loss of dopamine. We present here evidence obtained by SP radioimmunocytochemistry and quantitative autoradiography for a subpopulation of striatonigral SP neurons which maintain near normal SP levels in their terminals following chronic haloperidol or a unilateral 6-OH-DA lesion despite a loss of total nigral SP.

Adult rats were either subjected to a unilateral nigral 6-OH-DA lesion or begun on a regimen of the dopamine antagonist haloperidol (2 mg/kg s.c. daily). Animals were perfused 3-28 days later with buffered formalin, and the brains were processed for SP radioimmunocytochemistry using [^{3}H]-biotin. The biotin bound to sections using this procedure correlates well ($r > 0.95$) with the amount of radioimmunoassayable SP present. Midbrain images generated by apposition of labeled sections to LKB Ultrofilm were analyzed by quantitative autoradiography and comparisons of the nigral distribution of biotin were made between 1) the lesioned and control sides of 6-OH-DA animals and 2) drug and vehicle rats.

A 20-30% loss ($p < 0.01$) of SP in whole SN was detected after both treatments. Careful examination of the regional distribution of SP in treated animals revealed, however, that the content of SP in afferents impinging on the middle, medial pole of SN is virtually unaffected by a 6-OH-DA lesion and only slightly decreased (12%) by chronic haloperidol. We conclude that 1) there exists a subpopulation of striatal SP neurons projecting to middle, medial SN which are resistant to the SP-depleting effects of striatal DA denervation and 2) when examining brain antigen levels, radioimmunocytochemistry and quantitative autoradiography together allow the determination of small regional differences which would go undetected by conventional microdissection/RIA methodology.

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259.7 DOPAMINE RECEPTORS MEDIATE ALTERATIONS IN STRIATO-NIGRAL DYNORPHIN AND SUBSTANCE P PATHWAYS. I. Nylander*, L. Terenius* (SPON: L. A. Bettinger) Department of Pharmacology, Uppsala University, Uppsala, Sweden.

Dynorphin peptides are present in a striato-nigral pathway parallel to the substance P pathway, and evidence for an interaction between nigro-striatal dopamine neurons and peptidergic neurons has been presented (Christensson-Nylander et al. Exp. Brain Res. 64: 169-192, 1986; Herrera-Marschitz et al. Exp. Brain Res. 64: 193-207, 1986). This study investigated the effect of different dopamine receptor acting substances on opioid peptides and substance P.

Substance P and the proposed prodynorphin (proenkephalin B) derived peptides: dynorphin A, dynorphin B, (Leu)enkephalin-Arg^6 and (Leu)enkephalin were measured in substantia nigra, globus pallidus and striatum after a subacute (5 doses every 6h) treatment of rats with a number of dopamine agonists and antagonists. Drugs selective for the dopamine D1 and D2 receptors, respectively, as well as unselective drugs were used. After decapitation and dissection the tissues were extracted with 1 M acetic acid and the extracts were applied to ion exchange columns to separate and purify the peptides. Each peptide was measured using a radioimmunoassay procedure.

In substantia nigra, levels of immunoreactive dynorphin A and dynorphin B were increased after treatment with a D2-antagonist (sulpiride, 100 mg/kg) and a D1-agonist (SKF 38393, 15 mg/kg), while a D1-antagonist (SCH 23390, 0.1 mg/kg) reduced levels. A corresponding increase of nigral (Leu)enkephalin levels were found after sulpiride treatment. In contrast to dynorphin peptides, (Leu)enkephalin-Arg^6 levels were markedly increased after both D1- and D2-(LY 171555, 0.5 mg/kg)-stimulation. Substance P tended to be reduced after D1-stimulation and treatment with all the dopamine antagonists; the reduction was significant with sulpiride and cis-flu-penthixol (0.1 mg/kg).

Peptide levels were also measured in striatum and globus pallidus, and were generally affected as in substantia nigra.

The results in this study present further evidence for different effects of dopamine receptor agents on dynorphin levels compared to substance P. Effects on the proposed prodynorphin derived peptides (Leu)enkephalin and (Leu)enkephalin-Arg^6, only partly paralleled the effects on dynorphin levels, suggesting the D1 and D2 receptors elicit different responses on different products from prodynorphin, that is, differentially affect processing.

259.8 IN VITRO EXPERIMENTS ON INTERACTIONS BETWEEN DOPAMINE AND NEUROTENSIN RECEPTORS IN THE RAT STRIATUM. H. Ishida*, S. Kito and R. Miyoshi (SPON: M.Shimoyama). Third Department of Internal Medicine, Hiroshima University School of Medicine, 1-2-3 Kasumi, Minami-ku, Hiroshima 734, Japan.

Functional relationship between neuropeptides and conventional neurotransmitters is one of the most up-to-date themes in field of neuroscience. In the present study, we investigated effects of neurotensin (NT) on dopamine (DA) receptor binding in relation with DA receptor subtypes from viewpoints of receptor binding and release experiments using brain slices.

Wistar strain male rats weighing 200g were decapitated. After rapid removal of the striatum, P_2 fractions of the tissue were prepared by the method of De Robertis et al. The resulting pellets were resuspended in 50mM Tris/HCl buffer (pH7.4) and used for binding experiments. To examine NT's effect on the striatal DA D1 receptor, either NT or its analog, NT(1-8), was added in the DA/^{3}H-SCH23390 inhibition experimental system. As the results, both NT and NT(1-8) had effects of converting a part of D1 high affinity binding sites to low affinity ones, when analysed by our computer system. Such effects of NT on D1 receptor were equally obtained by adding GTP instead of NT. When both NT and GTP were added in this binding system, the effects were not additive and results were exactly the same to the cases in which either NT or GTP alone was used. NT and its analogs' effects on DA/^{3}H-spiperone inhibition, that is D2 agonist receptor, were also examined and similar effects of NT on D2 agonist binding were confirmed. From a viewpoint of neuropeptide-neurotransmitter interaction, it is of interest to examine DA receptor agonists' effects on ^{3}H-NT binding or NT-induced accumulation of phosphatidylinositol (PI) metabolites. In this study, striatal slices were incubated with ^{3}H-myo-inositol. After washing, they were incubated with NT and/or DA in presence of 10mM LiCl and released PI metabolites were assayed following the Berridge's method. It was confirmed that DA also exerted some effects on the NT-induced PI turnover showing reciprocal relations between NT and DA systems.

259.9 INTERACTIONS BETWEEN DOPAMINE AND ACETYLCHOLINE IN THE OLFACTORY TUBERCLE AND NUCLEUS CAUDATE. H. Suarez-Roca* and L.X. Cubeddu. Div. Clin. Pharmacol., Univ. North Carolina, Chapel Hill, NC 27514.

The olfactory tubercle (OT) is a limbic structure which receives a dense dopaminergic innervation from the ventral tegmental area and also contains the most rostrally located cholinergic cells in the brain. It has been suggested that there is a lack of interaction between the dopamine (DA) and acetylcholine (ACh) neurons in the limbic structures (Consolo et al., 1977; Bluth et al., 1985; Salama et al., 1986). We conducted a comparative study on the electrically-evoked release of DA and ACh from OT and nucleus caudate (NC) and its modulation by DA receptors. Slices were labelled with 3H-DA and 14C-choline and then superfused. Comparable magnitude of DA and ACh release was evoked by electrical stimulation from both regions. Apomorphine (APO), a D1-D2 agonist, and LY-171555 (LY), a D2 agonist, inhibited DA and ACh release from OT and striatum with similar EC50 and Emax. However, the maximal inhibition of ACh release achieved with APO, LY or DA in the OT was only half of that observed in the NC. In both regions, the inhibitory effects of DA agonists on DA and ACh release were markedly reduced when the number of electrical pulses and/or the frequency of stimulation were increased. l-Sulpiride, a DA D2 antagonist, increased the release of DA and ACh from OT in direct relationship with the frequency of stimulation. In the OT increases in synaptic DA achieved by administration of amphetamine or by blockade of the neuronal uptake pump with nomifensine inhibited the release of ACh. Again these drug treatments produced only a 40-50% inhibition of ACh release. SKF 38393, a D1 agonist, had no effect per se on DA or ACh release in OT slices from control or from reserpine-treated animals (2 mg/Kg, s.c. for 3 or 7 days). With exception of one specific dose combination, coadministration of SKF 38393 and LY produced no additive or synergistic effects on DA or ACh release from OT. APO and LY-induced inhibition of DA and ACh were antagonized by l-sulpiride. Although 30 nM SCH 23390 had no effect, a higher concentration (300 nM) of the D1 antagonist, reduced APO-inhibition of DA and ACh release, without affecting the inhibitory action of LY on DA and ACh release. These observations suggest: 1. The efficacy and potency of drugs acting on striatal and limbic DA receptors modulating DA or ACh release is strongly dependent on the basal activity of the neurons. 2. Ventral tegmental and nigro-striatal DA neurons projecting to the OT and striatum, respectively, are under efficient autoreceptor control. The DA autoreceptors are of the D2 subtype. 3. Inhibition of cholinergic function by DA is less efficient in OT than in the striatum. 4. A possible synergistic interaction between DA D2 and D1 receptors on DA and ACh release from OT, cannot be ruled out. (Supported by NIH Grant NS 21645-02)

259.10 EFFECTS OF SELECTIVE D1 AND D2 AGONISTS ON REGIONAL SEROTONIN AND DOPAMINE METABOLISM IN THE RAT. R. Trifunovich* and D. Wirtshafter (Spon: P. Tueting). Dept. Psychology, Univ. Ill. at Chicago, Chicago, Ill., 60680.

Several workers have reported that systemic administration of the mixed D1/D2 agonist apomorphine leads to an increase in serotonin levels in the striatum, suggesting an interaction between central dopamine and serotonin systems. In the current study we attempted to replicate these results and extend them by examining the effects of selective D1 and D2 agonists.

Apomorphine (3 mg/kg) produced a large decrease in the concentrations of DOPAC and HVA in both the striatum and nucleus accumbens consistent with the notion that this drug inhibits the activity of dopaminergic neurons. Apomorphine also produced a 37% increase in serotonin concentration in the striatum, without influencing serotonin levels in the nucleus accumbens. Identical results were observed after combined injections of the D1 agonist SKF-38393 (20 mg/kg/ and the D2 agonist quinpirole (3 mg/kg).

Injections of SKF-38393 (20 mg/kg), a selective D1 agonist, resulted in a small, but significant, decrease in striatal levels of DOPAC and HVA 30, but not 60 min., following injection. These treatments, however, were without any effect on striatal serotonin levels. In contrast, treatment with the selective D2 agonist quinpirole (3 mg/kg) produced a large decrease in the levels of striatal dopamine metabolites and a 33% increase in striatal serotonin levels. The concentration of 5H1AA was, however, unchanged resulting in a significant decrease in the ratio of 5H1AA to serotonin.

The results of these studies suggest that dopamine D2, but not D1, receptors modulate the activity of serotonergic neurons projecting to the striatum. In contrast, the activity of serotonergic cells projecting to the nucleus accumbens does not appear to be modulated by dopamine.

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259.11 λ-AMINOBUTYRIC ACID (GABA) TRANSAMINASE INHIBITION: EFFECTS ON STRIATAL DOPAMINE (DA) METABOLISM. C.F. Saller, M.J. Czupryna*, and A.I. Salama. Department of Pharmacology, Stuart Pharmaceuticals, A Division of ICI Americas Inc., Wilmington, DE 19897.

The accumulation of GABA, following the inhibition of GABA transaminase, in striatonigral and substantia nigra (SN) neurons has been used as a measure of GABA turnover. However, in attempting to assess the effects of haloperidol, a DA receptor antagonist, on GABA accumulation in the SN and in the superior colliculus (SC), which receives a GABAergic input from the SN, we found that the inhibition of GABA transaminase appeared to profoundly alter the response to haloperidol.

GABA concentrations in rat SN and SC were measured using a sensitive automated HPLC assay, which permits the quantitation of the dansyl chloride derivatives of GABA and its precursor glutamate (Saller and Salama, in preparation). DA and its two major metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were measured using an automated HPLC system with electrochemical detection (Saller and Salama, J. Chromatog. 309:287, 1984). All animals were killed by microwave irradiation, focused on their heads, to avoid postmortem increases in GABA concentrations.

In agreement with Walters et al. (J. Neurochem. 30:759, 1978), the accumulation of GABA was linear as a function of time for at least 75 min in both the SN and SC following the inhibition of GABA transaminase with aminooxyacetic acid (AOAA, 25 mg/kg, i.p.). By itself, AOAA also produced small, but significant, decreases in striatal DOPAC and HVA concentrations. More importantly, when AOAA was given 15 min prior to haloperidol (1 mg/kg, i.p.), the ability of haloperidol to elevate striatal DOPAC and HVA concentrations, at either 30 or 60 min after haloperidol, was greatly reduced. These observations are in accord with the well known inhibitory influence of GABAergic neurons in the SN on nigrostriatal dopaminergic transmission. Thus, the data indicate that the accumulation of GABA following GABA transaminase inhibition is apparently sufficient to dramatically alter dopaminergic transmission, particularly in response to the DA receptor antagonist haloperidol. Moreover, these findings suggest that the elevations in GABA produced by GABA transaminase inhibition, by altering dopaminergic activity, may also alter the activity of GABAergic neurons that are innervated by DA-containing neurons, confounding measurements of GABA turnover.

259.12 **COMPARISON OF CHOLINESTERASE ACTIVITIES AND MUSCARINIC AND DOPAMINERGIC RECEPTORS AMONG THREE DIFFERENT RAT STRAINS: S.D., WKY AND SHR.** D. K. Lim, Y. Ito, A. B. Porter, B. Hoskins, I. K. Ho and R. W. Rockhold. Dept. Pharmacol. and Toxicol., Univ. MS. Med. Ctr., 2500 North State Street, Jackson, MS 39216, USA

The cholinesterase activities in liver, blood and brain and the dopaminergic and muscarinic receptors in specific brain areas from three rat strains were studied.

Male Sprague-Dawley (S.D.), Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats were used at 9 weeks of age.

The blood pressures in S.D., WKY and SHR rats were: 117±4, 120±6 and 169±7 mmHg, respectively, measured by a tail-cuff method.

Serum cholinesterase activity in WKY was significantly lower (50%) than activity in other strains. However, the acetylcholinesterase and butyrylcholinesterase activities in whole blood, liver and brain (striatum) were not significantly different among the three strains.

Studies of dopaminergic receptors revealed that the densities (B_{max}) of both D-1 ([^{3}H]-SCH 23390 binding sites) and D-2 ([^{3}H]-spiperone binding sites) receptors in striatum were significantly higher in SHR rats than in other strains. However, there were no differences in the affinity constant (K_d). Neither B_{max} nor K_d was different between S.D. and WKY. The receptor densities of D-1 and D-2 in striata from SHR rats were 34% and 18% higher than densities in striata from S.D. and WKY; respectively. The receptor density of D-2 in hypothalami from SHR rats was also higher than other strains (32%). Furthermore, the higher density in hypothalami from SHR rats was mainly due to the high population of D-2 receptors in posterior hypothalamus. In the anterior hypothalamus, the D-2 receptor density in SHR rats was slightly lower than that in WKY rats. In substantia nigra, the density of D-1 receptor was highest in SHR rats followed by WKY and S.D. rats.

In studies of muscarinic receptors, the number of [^{3}H]-QNB binding sites in striata from S.D. rats was lower (18%) than those from WKY and SHR rats. However, muscarinic receptor properties (K_d and B_{max}) were the same in hypothalami.

These results suggest that the metabolism of certain substances are different among these strains due to the different enzyme activities. Also, the different activities of dopaminergic and cholinergic neurons among these strains may alter the response to different drugs. Finally, the results also suggest that altered dopaminergic nerve activity plays an important role in the development of hypertension. (Supported by National Institute on Drug Abuse, DAO4264.)

259.13 THE EFFECT OF NICOTINIC STIMULATION ON DOPAMINE RELEASE FROM DISSOCIATED MESENCEPHALIC CELLS IN CULTURE. S. Welner*, H. Mount, P. Boksa, R. Quirion and R. Wise (Spon: J.V. Milligan). Douglas Hospital Research Centre, Verdun, Québec. and Centre for Studies in Behavioral Neurobiology, Concordia University, Montreal, Québec.

Recent behavioral evidence indicates that, in rats, nicotinic stimulation of cells in the ventral tegmental area (VTA) results in an increase in locomotor activity, an effect said to be mediated by the release of dopamine (DA). As well, electrophysiological studies show modulation of firing of VTA-DA neurons by systemic nicotine administration. Whether nicotinic stimulation in this area acts directly on dopamine neurons or whether it acts through another system to disinhibit or modulate the dopaminergic pathway, however, is not known. We investigated this question using a system of dissociated cells where the normal neuronal organization between DA neurons and other neurons which modulate these DA cells is likely disrupted. Therefore, one is able to examine direct effects of agonists on DA cells.

Suspensions of mesencephalic cells obtained from day 15 embryonic rat brain were cultured in a serum-containing medium for 5 to 7 days. Initial studies indicated that the cells are capable of taking up [^{3}H]DA (50 nM) and releasing it in response to high concentrations of K^+ (56 mM). This release is blocked in the presence of an inhibitor of DA uptake into dopaminergic neurons (benztropine, 5 μM) but not by an inhibitor of amine uptake into noradrenergic neurons (desipramine, 5 μM) or into serotonergic neurons (fluoxetine, 1 μM).

We found that cytisine, a potent nicotinic agonist, at nanomolar concentrations, induces the release of labelled DA from these cultures. This effect can be blocked by administration of the nicotinic antagonist mecamylamine (1 μM). Cytisine also induces the uptake of $^{45}Ca^{++}$ into these cultures. We studied the localization of nicotinic receptor sites using a new ligand, [^{3}H] methyl carbachol, which appears to be more selective for high affinity nicotinic sites and found that these sites are present in the VTA. Therefore, the present findings indicate that nicotinic stimulation of DA neurons in the VTA appears to be a direct rather than an indirect effect. Since DA pathways projecting from the VTA have been implicated in other systems of reward, such as that involving the opiates, the present result may also have some importance in understanding the central mechanism by which tobacco smoking produces its rewarding effect. This work is supported by MRC Canada.

259.14 GLUTAMATERGIC STIMULATION OF DOPAMINE RELEASE FROM DISSOCIATED MESENCEPHALIC CELLS IN CULTURE. H. Mount, S. Welner*, P. Boksa and R. Quirion. Depts. of Pharmacology and Psychiatry and Douglas Hospital Research Centre, McGill University, Verdun, Qué. H4H 1R3 Canada.

The ventral mesencephalon contains neurons involved in the formation of nigrostriatal, mesolimbic and mesocortical dopaminergic pathways. It is also known to receive excitatory inputs from cortex and striatum. Dissociated primary cell cultures from fetal rat mesencephalon (Welner et al. this meeting) were used to demonstrate and characterize receptor-mediated stimulation of dopamine (DA) release from these neurons by excitatory amino acids.

Embryonic cells were taken at day 14 of gestation, mechanically dissociated and grown in medium containing 10% serum. On the sixth day in culture, cells were loaded with [^{3}H]-DA (50 nM) in the presence of desipramine (0.5 μM). Five min exposure to L-glutamate (GLU) resulted in Ca^{2+}-dependent release of loaded DA. In separate experiments, at 0.1 mM and 1.0 mM, GLU also stimulated uptake of $^{45}Ca^{2+}$ into cells. N-methyl-D-aspartate (NMDA), kainate (KAIN) or quisqualate (QUIS) also produced concentration-dependent release of [^{3}H]-DA. Maximal GLU- and NMDA-stimulated release were observed at 0.1 mM. KAIN was equipotent with GLU at concentrations up to 0.1 mM, but was more than twice as potent at 1.0 mM. The concentration-response curve for QUIS showed lower potency and was biphasic, reaching a maximum at 10 uM. Cis-2,3-piperidine dicarboxylic acid (<5 mM), a glutamate antagonist, produced concentration-dependent inhibition of GLU- and KAIN-stimulated DA release.

Three lines of evidence suggest that GLU-induced acute excitotoxicity cannot account for the release of [^{3}H]-DA. First, normal DA uptake and stimulated release were observed in cells preincubated for 10 min with GLU, NMDA or KAIN (0.1 mM) before loading of [^{3}H]-DA. Second, GLU-stimulated release was not dependent on extracellular Cl^- or Na^+. Third, 30 min exposure to GLU, NMDA, or KAIN (1.0 mM) did not cause a measurable increase in leakage of a cytosolic marker (lactate dehydrogenase) from cells into the medium.

These results suggest that dopaminergic neurons in the mesencephalon are directly stimulated by excitatory amino acids.

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CHARACTERIZATION OF NEURONAL NICOTINIC CHOLINERGIC RECEPTORS

260.1 MOLECULAR MECHANISMS MEDIATING THE MARKED NICOTINIC ANTAGONIST INDUCED UP-REGULATION OF α-BUNGAROTOXIN SITES IN ADRENAL CHROMAFFIN CELLS IN CULTURE. M. Quik, S. Geertsen* and J.M. Trifaró*. Depts. of Pharmacology, McGill University, Montreal, Que. and Univ. of Ottawa, Ottawa, Ont., CAN.

Previous work had shown that a 1 to 3 day preincubation of adrenal chromaffin cells in culture with the nicotinic antagonist d-tubocurarine resulted in a 5 to 10 fold increase in α-bungarotoxin (α-BGT) binding to the cells (Quik et al., 1987, Molec. Pharmacol. 31). Because the increase in α-BGT sites in chromaffin cells is so marked as compared to the size of the antagonist induced increase (~25%) in other neuronal cells in culture, this could infer that the α-BGT sites have a role in these cells. The molecular mechanisms responsible for the induced increase in the α-BGT sites were therefore studied. To determine whether the antagonist induced increase in the toxin binding sites can be modified by changes in the extracellular K^+ concentration, the chromaffin cells were incubated in the presence of varying KCl (2-50 mM); this treatment resulted in an increase in the α-BGT sites of similar magnitude as the nicotinic antagonist induced increase. D-tubocurarine in combination with high K^+ resulted in an increase in the sites only slightly greater than that with either agent alone; this suggests that the d-tubocurarine induced increase may occur at least in part by alterations in K^+. Further experiments with the phorbol ester 4β-phorbol 12-myristate 13-acetate (PMA) showed that this agent also enhanced α-BGT binding to the cells (10 fold); this increase was partially additive with the d-tubocurarine induced increase in the binding of α-BGT to the cells in culture. On the other hand, high K^+ together with PMA resulted in an increase in the toxin binding sites equivalent to that observed with either agent alone. These data suggest that one mechanism whereby nicotinic antagonists result in an increase in α-BGT sites involves activation of protein kinase C, possibly in response to changes in the K^+ concentration; however, alternate pathways must also exist as evidenced by the finding that the increase in the presence of d-tubocurarine plus PMA was greater than with either agent alone but less than additive. Regulation of the α-BGT sites by cAMP was also observed. Addition of dibutyryl cAMP to the cells in culture for 3 days led to a dose dependent reduction in both the d-tubocurarine induced and high K^+-induced increases in α-BGT binding to the cells.

Thus, these results show that various molecular mechanisms are available to allow for a finely regulated control of the α-BGT binding sites in adrenal medullary chromaffin cells. Supported by the MRC (Canada).

260.2 NICOTINIC-INDUCED TRANSLOCATION OF PROTEIN KINASE C IN PC12 CELLS DIFFERENTIATED WITH NERVE GROWTH FACTOR. R.O. Messing and A.M. Stevens*. Dept. of Neurology and Gallo Research Center, San Francisco General Hospital, University of California, San Francisco, CA 94110.

Activation of protein kinase C (PKC) is associated with increased PKC activity in the particulate fraction of cells. We studied activation of PKC in the neural cell line, PC12, by examining changes in the subcellular distribution of the enzyme in response to cholinergic agents. Cells grown for seven days with 2.5 S NGF were incubated in medium with indicated drugs at 37°C, and incubations were terminated by aspiration and one wash with ice-cold 250 mM sucrose, 1 mM EDTA, 1 mM EGTA, and 20 mM Tris-HCl (pH 7.5). Cells were homogenized in 20 mM Tris-HCl (pH 7.5, 4°C), 50 ug/ml leupeptin, and 1 mM PMSF, and sucrose was added to a final concentration of 250 mM just prior to centrifugation at 100,000 x g for 1 hr at 4°C. The supernatant (soluble fraction) and the Triton X-100 solubilized pellet (particulate fraction) were subjected to DEAE chromatography, and PKC in the eluates was measured by phosphorylation of H_1 histone in the presence of Mg^{2+}, ATP, phosphatidylserine, 1,2 diolein, and Ca^{2+}. In the absence of drugs, 13 ± 2% of total cellular PKC activity was found in the particulate fraction. Exposure to 1 mM carbachol increased particulate PKC activity to 50% of the total within five seconds. Carbachol's effect was concentration-dependent with a biphasic dose-response curve yielding approximate EC_{50} values of 10^{-7} and 10^{-4} M for the high and low affinity components respectively. In cells incubated with 100 uM 1,1-dimethyl- 4-phenylpiperazinium (DMPP), a selective nicotinic agonist, 39 ± 3% of total PKC activity was found in the particulate fraction whereas only 24 ± 1% was present in the particulate fraction of cells incubated with 300 uM muscarine. Since nicotinic stimulation of PC12 cells activates voltage-dependent Ca^{2+} channels, we examined the effect of Ca^{2+} channel blockade on PKC translocation. Preincubation for five minutes with the dihydropyridine Ca^{2+} channel antagonist nifedipine (1 uM) reduced PKC activity in the particulate fraction of DMPP-treated cells from 45 ± 1% to 32 ± 3% of total activity ($p < 0.03$). Thus, cholinergic-induced PKC translocation in PC12 cells is predominantly due to activation of nicotinic receptors and is partly dependent on Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels.

260.3 **PURIFICATION AND STRUCTURAL CHARACTERIZATION OF A HIGH MOLECULAR WEIGHT α -BUNGAROTOXIN BINDING PROTEIN FROM *APLYSIA CALIFORNICA*.** J. T. McLaughlin* and E. Hawrot.(Spon:R.G. Pellegrino) Dept. of Pharmacology, Yale Univ. School of Medicine. New Haven CT 06510.

An α-Bungarotoxin(Btx) binding protein was identified in membranes prepared from *Aplysia californica* cerebral, buccal, and pleural ganglia. The protein was initially characterized with a combination of SDS gel electrophoresis and electroblotting, using ^{125}I-α-Btx as a probe. The *Aplysia* toxin binding activity migrated with an apparent molecular weight of approximately 240 kD, similar to binding activity previously described in lower vertebrate CNS (Hawrot et al., 1986 Brain Res. 373:227-234); this observation distinguishes these high molecular weight (HMW) activities from the toxin binding α-subunit of the *Torpedo* nicotinic acetylcholine receptor (AChR), and other, previously characterized neuronal toxin binding proteins. A number of other unique physical characteristics of the *Aplysia* binding activity were revealed using the combination of SDS-PAGE and electroblotting. Unlike *Torpedo* binding, the *Aplysia* HMW binding activity is unaffected by alkylation, and is abolished by boiling, low pH, or exposure to 6 M Urea.

An important question that could not be addressed with the combination of electrophoresis and electroblotting was whether the mobility on SDS-PAGE was due to a multimeric protein resistant to SDS dissociation, or simply a reflection of monomeric structure.To distinguish between these two possibilities, the toxin binding protein was purified using α-bungarotoxin-coupled Sepharose 4B affinity chromatography. Purification was confirmed by silver staining of SDS gels,and the purified protein was then iodinated and subjected to treatments previously shown to abolish toxin binding activity. None of these treatments altered the mobility of the iodinated HMW protein. These results indicate that the binding activity in *Aplysia* resides on a HMW monomer which represents a previously uncharacterized α-bungarotoxin binding protein.

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260.4 THE NICOTINIC ACETYLCHOLINE RECEPTOR IN THE CENTRAL NERVOUS SYSTEM OF MANDUCA SEXTA M. L. Perez and D. J. Prescott*. Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

In addition to efficient modes of excretion and/or detoxification of ingested nicotine, tobacco feeding insects may possess a variant of the cholinergic receptor which is less susceptible to this alkaloid (Morris, C., J. Exp. Zool. 229: 361-374, 1984). A receptor variant was isolated from nicotine fed Drosophila which differs in isoelectric point from that found in wild-type (Hall, L. M. et al., FEBS Lett. 95: 243-246, 1978).

We are characterizing the putative nicotinic acetylcholine receptor in Manduca sexta, the tobacco hornworm. This protein is solubilized from cerebral ganglia by Triton X-100. Binding is saturable and picomoles of alpha bungarotoxin binding sites per mg soluble protein are obtained. Both equilibrium and kinetic binding studies demonstrate a K_d of 0.5 nanomoles. The most efficient inhibitors of toxin binding are methyllycaconitine, lobeline, nicotine and curare. Atropine, muscarine, scopolamine and dopamine are ineffective as competitors. Studies on the structure of the multimer and its subunits are currently underway and show similarities to other neuronal nicotinic acetylcholine receptors.

260.5 CHARACTERIZATION OF AN ACETYLCHOLINE (ACh)-ACTIVATED CHANNEL IN LARVAL Drosophila NEURONS. J.L. Albert* and C.J. Lingle. Dept. Biol. Sci., Florida State Univ., Tallahassee, FL 32306

Recent isolation and functional incorporation into bilayers of a putative nicotinic ACh receptor (nAChR) protein from insects (Hanke & Breer, 1986,Nature) has led to the suggestion that the insect nAChR is a primitive form of vertebrate (v.) nAChRs (Breer et al, 1985,J.Neurosci 5). Proper evaluation of isolated, putative nAChRs requires an understanding of the behavior of nAChR channels in native insect neuronal cell membranes. As yet little is known about the properties of single AChR channels in insects. Using patch-clamp techniques we report here the properties of an ACh-activated channel from third instar Drosophila (D.) larval neurons in primary culture.

Using high K saline (140 mM) to short-circuit the high input resistance of these cells we observe two major classes of channel using 1-200 uM ACh in the pipette in cell-attached recordings. At 23-27 C, an infrequently occurring large amplitude channel has a conductance of 63.7+3.1 pS (n=3,+/-SD) and a single exponential on-time distribution with a mean open time of 0.35+0.09 ms (n=4). This channel will not be discussed further here. The small amplitude channel has a conductance of 34.9+8.1 pS (n=6,+/-SD) and a two component on-time distribution: Tfast=0.25+0.11 ms (n=5), Tslow=1.34+0.22 ms (n=5), when patches are compared at a similar single channel current amplitude.

We have explored the effects of [ACh] from 1 to 200 uM on the small amplitude channel. Above 10 uM, the grouping of repeated openings of a single channel into bursts can be observed. Unlike v. muscle nAChRs (max. P(o)>0.9), the probability of being open within a burst (P(o)) for the D. channel remains around 0.2-0.3 from 10-200 uM ACh. However, substantial heterogeneity among bursts is evident and limits our attempts to evaluate specific off-time components. Although other explanations are possible, we interpret the lack of dependence of P(o) on [ACh] over this range as indicative that at these [ACh] off-times are dominated by closed states other than unliganded and monoliganded closed states preceding channel opening. Although little is known about v. neuronal nAChRs, it appears that a low P(o) may also be characteristic of them (Takeda et al, 1986, Br. Res 378). Based on the biophysical characteristics of the D. channel we suggest that it is nicotinic and functionally resembles the v. ganglionic nAChR channel.

260.6 NICOTINE AND MUSCARINE EVOKE DIFFERENT RESPONSES IN ISOLATED, NEURONAL SOMATA FROM LOCUST THORACIC GANGLIA. J.A. Benson and R. Neumann*, Entomology Basic Research, Agricultural Division, CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland.

The mechanically-separated neurones from the thoracic ganglia of Locusta migratoria remain electrophysiologically viable in vitro for many hours (Holden et al., J. Physiol. 276:4P, 1978; Lees et al., Brain Res. 401:267, 1987). These isolated somata respond to acetylcholine with a membrane potential depolarisation accompanied by a decrease in input resistance (Suter & Usherwood, Comp. Biochem. Physiol. 80C:221, 1985). Under voltage-clamp, pressure micro-application of 10^{-4}M nicotine for 10 to 100 ms evokes an inward current that decreases with membrane depolarisation and has a projected reversal potential of 0 to 20mV. Muscarine, applied at 10^{-3}M for 500 ms to 2 s, evokes a smaller, slower, inward current that increases with membrane depolarisation over the potential range -60 to -30mV. The receptors mediating these two distinct responses are referred to here as acetylcholine-1 (ACh1) and acetylcholine-2 (ACh2) receptors respectively. The response to 100 ms pulses of 10^{-4}M acetylcholine is dominated by the current activated via ACh1 receptors.

The ACh1 receptors are blocked in a dose-dependent manner by bath application of the vertebrate nicotinic receptor antagonists α-bungarotoxin (EC_{95} ca. 10^{-7}M), mecamylamine, hexamethonium, gallamine, chlorisondamine, lobeline and d-tubocurarine. All of these antagonistic effects are at least partially reversible, with α-bungarotoxin being slowest (total block 60% reversed in 2 hours). However, the ACh1 receptors are also blocked by some vertebrate muscarinic receptor antagonists such as atropine, scopolamine and benactyzine. In addition, these receptors are blocked by the vertebrate $GABA_A$ receptor antagonists, bicuculline and its methiodide salt (EC_{50} ca. 10^{-5}M), which do not affect the locust somal GABA response at concentrations up to 10^{-4}M (Lees et al., 1987).

The ACh2 receptors are activated by oxotremorine and blocked by atropine but not by α-bungarotoxin (10^{-7}M) or hexamethonium (10^{-4}M). The response mediated by these receptors resembles the effect of muscarine on crab stomatogastric neurones (Marder & Paupardin-Tritsch, J. Physiol. 280:213, 1978).

The data obtained so far suggest that the ACh1 and ACh2 receptors may correspond respectively to the "mixed" nicotinic/muscarinic and muscarinic receptors reported from numerous ligand-binding studies on insect neural tissue homogenates.

260.7 **NICOTINIC ACETYLCHOLINE RECEPTORS FROM BOVINE AND HUMAN BRAIN CHARACTERIZED USING MONOCLONAL ANTIBODIES.** P.J. Whiting* and J.M. Lindstrom. Receptor Biology Lab., The Salk Institute for Biological Studies, P.O. Box 85800, San Diego, CA 92138.

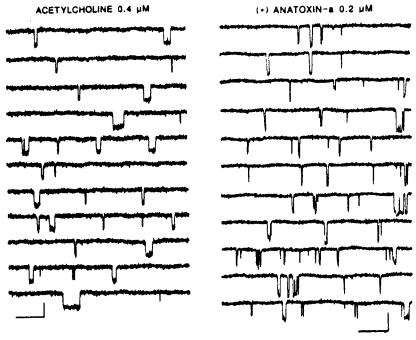
Using monoclonal antibodies (mAbs) prepared to nicotinic acetylcholine receptors (AChRs) from Torpedo electric organ AChR, we immunoaffinity purified nicotinic AChRs from chicken brain (Whiting and Lindstrom, Biochem. 25:2082, 1986). Subsequently, we prepared mAbs to AChRs from chicken brain and used them to immunoaffinity purify AChRs from rat brain (Whiting and Lindstrom, PNAS 84:595, 1987). Two subtypes of AChRs were identified in chicken brain, which apparently had the same subunit (Mr 49,000) but different β subunits (β=Mr 59,000 and β'=Mr 75,000). Rat brain AChR contained α (Mr 51,000) and β' subunits (Mr 79,000). N-terminal amino acid analysis showed that β' corresponded to the cDNA α_4 described by Goldman et al. (Cell 48:965, 1987). These AChRs exhibit nM binding affinities for nicotine and acetylcholine, but do not bind αbungarotoxin (αBgt). Their β or β' subunits are affinity labelled by the ACh binding site reagents MBTA and BAC, indicating that these subunits form the ACh binding site and contain the functional equivalent of cysteines α192, 193 of electric organ AChRs.

To obtain probes which crossreacted with human AChRs, we prepared a library of mAbs to AChRs from rat brain. Western blot analysis indicated that three of these rat mAbs bound to the β' subunit of rat AChRs. The other six mAbs were conformation-dependent, failing to bind to denatured AChRs. Several of the mAbs exhibited binding to the nicotinic AChR from both bovine and human brain, and were used as biochemical probes to characterize these AChRs. Sucrose gradient analysis indicated that these AChRs are approximately 10S in size, slightly larger than muscle AChRs. AChR was immunoaffinity purified from bovine brain AChR and found to consist of two subunits, Mr 50,000 and Mr 75,000, homologous to the α and β' subunits of chicken and rat brain AChR. Both bovine and human brain AChR exhibited high affinity binding for nicotine (K_I=15.8 nM and 5.4 nM, respectively) and for ACh (K_I=43 nM and 2.7 nM, respectively) as determined by inhibition of DL[^{3}H]nicotine binding, but neither AChR bound αBgt. The binding of [^{3}H]nicotine could be inhibited by reduction of these AChRs with dithiothreitol, and subsequent labelling with BAC and MBTA. The β' subunit was affinity labelled with [^{3}H]MBTA, indicating that it contains the ACh binding site.

Thus, the AChR from brain with high affinity for nicotine but no affinity for αBgt is formed from two types of subunits in brains of chickens, rats, cattle, and probably humans. In adult chickens, half of the AChRs are of the αβ subtype and half are of the αβ' subtype. In the mammalian species, greater than 90% appeared to be of the αβ' subtype.

260.8 PRESENCE OF NICOTINIC ACETYLCHOLINE RECEPTOR-ION CHANNEL (AChR) ON CULTURED RAT HIPPOCAMPAL CELLS. Y. Aracava*[1], H. Rapoport*[2], G. Lunt*[3], S. Wonnacott*[3], and E.X. Albuquerque*[1]. (SPON: T. Rogers). [1]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med. Baltimore, MD 21201; [2]Dept. Chem. Univ. California, Berkeley, CA 94720 & [3]Dept. Biochem. Univ. Bath, Bath BA2, U.K.

Using (+)anatoxin-a (*Mol. Pharmacol.* **29**: 250, 1986) and acetylcholine (ACh) as probes of AChR and employing the patch-clamp technique, we recorded channel activation in hippocampal neurons cultured from fetal rats (10 to 20-day-old culture). Both ACh (0.4 μM) and (+)anatoxin-a (0.1-0.4 μM) activated single channel currents whose amplitudes and durations were quite similar to those recorded at the perijunctional region of the muscle endplate. Figs. a and b show typical channel activity recorded from an area close to the axon hillock of the hippocampal neurons. Although some differences in agonist effects at central and peripheral receptors have been encountered (see abstracts of Rozental *et al.* and Lunt *et al.* at this meeting), the present study, still preliminary, disclosed marked homology between the putative hippocampal AChRs and those on the muscle. The hippocampal AChRs showed a similar sensitivity to histrionicotoxin, a probe for ion channel sites, as the muscle AChRs (*FEBS Lett.* 212:292, 1987). Additionally, AChRs activated by either ACh or (+)anatoxin-a were sensitive to α-bungarotoxin and dihydro-β-erythroidine. These studies suggest the presence of functional nicotinic AChRs on the neurons of rat hippocampus. (Support: NIDA Grant DA 02804 & U.S.Army Med. Res. & Devel. Comm. Contract DAMD17-84-C-4219).



Legend: Single channel currents activated by acetylcholine (a) and (+) anatoxin (b) recorded from cultured hippocampal neurons in the cell-attached configuration. Holding potential: -80(a) and -140 mV (b). Cal.: vert. 3 (a) and 8 pA (b); hor. 25 msec (a & b).

260.9 THE EFFECTS OF α-BUNGAROTOXIN AND MAGNESIUM ON THE ELECTROPHYSIOLOGICAL ACTIONS OF NICOTINE IN THE RAT CEREBELLUM. R. de la Garza, T.J. MCGUIRE,* B.J. Hoffer, R. Freedman. Univ. of Colo., Health Sci. Ctr., Dept. of Pharmacol. C236, 4200 E. 9th St., Denver, CO 80262 and Denver VAMC.

A recent study from our laboratory described the heterogenous electrophysiological actions of nicotine in the rat cerbellum. A curare-sensitive site was found to mediate the excitatory effects of nicotine on identified cerebellar inhibitory interneurons and a hexamethonium-sensitive site was found to mediate the inhibitory effects of nicotine on identified Purkinje cells. The experiments reported here investigated whether another more selective nicotine blocker, α-Bungarotoxin (α-BTX), would also antagonize the actions of nicotine on these neuronal populations. Additionally, we also investigated whether the actions of nicotine on either cell type are dependent on direct postsynaptic or indirect presynaptic actions by examing its effects in the presence of elevated concentrations of magnesium, ion which would reduce transmitter release.

Nicotine elicited reversible and dose-dependent effects on both cell types. The excitatory actions of nicotine on cerebellar interneurons were blocked by the simultaneous application of α-BTX in 17 of 18 cells tested. In some experiments, the antagonistic actions of α-BTX showed no recovery an hour after the removal of the toxin. In contrast, the inhibitory actions of nicotine on Purkinje cells were not blocked by similar applications of α-BTX in 17 of the 20 cells tested. A chi-square analysis of the data showed that the antagonistic effects of α-BTX depended on the type of cell studied in a statistically significant manner ($p < .0001$).

Additionally, the microiontophoretic application of magnesium ion failed to block the actions of nicotine on any of the 15 Purkinje cells and 10 interneurons tested. Student's *t*-tests for correlated samples showed that there were no statistically significant differences in Purkinje cell [$t(14) = -0.87$, $p > 0.05$] or interneuronal nicotine-induced responses [$t(9) = -1.67$, $p > 0.05$] in the presence and absence of magnesium ion.

The present data support the hypothesis of multiple nicotinic sites of action in mammalian brain (de la Garza et al., J. Pharmacol. Exp. Ther. 240: 689-695, 1987). Our findings also suggest that the pharmacological actions of nicotine on cerebellar cortex neurons are due to direct postsynaptic mechanisms (Supported by USPHS grant DA 07043 and the Veterans Administration).

260.10 **PURIFICATION AND CLONING OF THE MUSCLE-LIKE NICOTINIC ACETYLCHOLINE RECEPTOR FROM THE HUMAN MEDULLOBLASTOMA CELL LINE TE671.** M. Luther*, R. Schoepfer*, P. Whiting*, M. Montal+, and J. Lindstrom (SPON: D. Sakaguchi), Receptor Biol. Lab., The Salk Institute for Biol. Studies, P.O. Box 85800, San Diego, CA 92138 and +The Roche Institute of Molecular Biology, Nutley, NJ 07110.

Studies on the human muscle nicotinic acetylcholine receptor (AChR) have been difficult due to the low number of AChRs present on striated muscle, proteolysis, and the limited availability of tissue. Surprisingly, the AChR from the human medulloblastoma cell line TE671 exhibits electrophysiological, immunological, and pharmacological properties similar to those of AChRs from human muscle. Unlike AChRs from TE671 cells, the α bungarotoxin (αBgt) binding components from human brain do not bind monoclonal antibodies (mAbs) to AChRs from human muscle or autoantibodies to these AChRs from myasthenia gravis patients (Whiting and Lindstrom, J. Neuroimmunol., in press). Neither AChRs from TE671 cells nor brain αBgt binding components are bound by mAbs to brain AChRs which have high affinity for nicotine but do not bind α Bgt. Evidence suggests that AChRs from muscle and neurons and neuronal αBgt binding components are all members of a gene family. TE671 cells may derive from a precursor cell type whose mature form does not express muscle-like AChRs.

AChR protein from TE671 was purified 2,000-fold by toxin-agarose affinity chromatography, and its subunits were characterized by polyacrylamide gel electrophoresis, N-terminal amino acid analysis, and western blotting with subunit-specific mAbs to AChRs from fish electric organs or mammalian muscle. Sucrose gradients show that TE671 AChRs exist as both monomers and dimers. cDNA probes for all four subunits of mouse muscle AChR (a gift from J. Boulter) detect corresponding mRNA species in TE671 at high stringency. To determine the primary structure of the AChR, a cDNA library was made in λ-ZAP, a cloning vector furnishing an automatic excision procedure for the insert. Using a mouse muscle α subunit cDNA probe, several clones were obtained at high stringency, and sequence analysis was performed.

The expression of this AChR is enhanced by both nicotine (as previously reported by Siegel and Lukas, Neurosci. Abstr. 11.9, 1986) and calcitonin gene-related peptide (a putative neurotrophic factor reported to increase AChR synthesis in muscle by Fontaine et al., Neurosci. Letts. 71:59, 1986), while forskolin (which stimulates adenylate cyclase, increases phosphorylation of muscle AChR subunits and thereby causes desensitization as reported by Anthony et al., Neurosci. Abstr. 40.1, 1986) decreases both the expression and the level of mRNA for α subunits.

Thus, TE671 provides an excellent source for the study of human muscle AChRs at the protein and cDNA level.

260.11 IDENTIFICATION OF A GENE PROPOSED TO ENCODE A NON-ALPHA SUBUNIT OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS. E.S. Deneris*, J. Boulter*, J. Connolly*, K. Wada*, J. Patrick, and S. Heinemann. Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138.

We have taken a molecular genetic approach to isolate and characterize nicotinic acetylcholine receptors (nAChR) expressed in the brain. Through the isolation of rat cDNA clones we have identified three genes that are expressed in the nervous system and encode proteins with sequence and structural homology to the alpha subunit of the nAChR expressed at the mammalian neuromuscular junction. We have proposed that these genes encode alpha subunits (or ligand binding subunits) because they contain two adjacent cysteine residues that are the homologues of CYS 192 and CYS 193 of the *Torpedo* alpha-subunit. CYS 192 and possibly CYS 193 of the *Torpedo* alpha-subunit have been shown to be labeled with the affinity alkylating reagent 4-(N-maleimido) benzyltrimethylammonium iodide (Kao, P.N. et al., 1984, J. Biol. Chem. 259:11662) and therefore lie close to the binding site for acetylcholine. We have thus named these neuronally expressed genes: alpha2, alpha3 and alpha4.

Using the nAChR expressed at the neuromuscular junction as a model, we have hypothesized that the neuronal receptors contain non-alpha subunits as well. To test this hypothesis, we have screened cDNA libraries prepared from mRNA extracted from rat brain and the PC12 cell line. Screening with a radiolabeled alpha3 cDNA has resulted in the isolation of a novel set of cDNAs; one of which, PCX49 isolated from a PC12 library, encodes a full length protein. Nucleotide sequence analysis reveals that these cDNAs encode a protein with sequence and structural homology to the proposed neuronal alpha-subunits. However, the PCX49 encoded protein lacks the two adjacent cysteine residues found at position 192 and 193 in the *Torpedo* and muscle alpha-subunits. In this respect, this protein is similar to the non-alpha subunits found in the *Torpedo* and muscle nAChRs. Based upon this fact, we have hypothesized that the PCX49 encoded protein is a non-alpha subunit of a neuronal nAChR and have therefore named its gene beta2.

In situ hybridization histochemistry is being used to determine where the beta2 gene is expressed in the rat central nervous system. Preliminary results indicate a wide distribution of expression that includes the thalamus, medial habenula, the geniculate nuclei, and hypothalamus. These regions have been shown to express collectively, the alpha3 and alpha4 genes and raises the possibility that the beta2 gene product is assembled with either the alpha3 or alpha4 gene product at different synapses to form distinct nAChRs in the nervous system.

A *Xenopus* oocyte expression system has been used to determine if the beta2 gene encodes a functional nAChR subunit. We have found that acetylcholine and nicotine will elicit a depolarizing response in oocytes that have been injected with beta2 mRNA and either alpha3 or alpha4 mRNA. This response is blocked by toxin 3.1, but not alpha-bungarotoxin indicating that these genes encode proteins that produce a functional nAChR with the pharmacology of a ganglionic or neuronal nAChR.

260.12 ISOLATION AND CHARACTERIZATION OF A GENE CODING FOR A RAT BRAIN NICOTINIC ACETYLCHOLINE RECEPTOR α-SUBUNIT. K. Wada[1*], M. Ballivet[2*], J. Boulter[1*], J. Connolly[1*], E. Deneris[1*], E. Wada[3*], L. Swanson[3,4], S. Heinemann[1] and J. Patrick[1]. (SPON: G. Lemke) [1]Molecular Neurobiology Laboratory., [3]Neural Systems Laboratory. and [4]Howard Hughes Medical Institute, The Salk Institute, La Jolla, CA 92037, [2]Dept. of Biochemistry, University of Geneva, CH-1211, Geneva, Switzerland.

The molecular genetic approach we have employed has led to the identification of a family of nicotinic acetylcholine receptor α-subunit genes in rat. The genes thus far identified have been designated alpha1 through alpha4. Partial sequence data of the alpha2 gene were reported previously (P.Nef et al., in Nicotinic Acetylcholine Receptor:Structure and Function, Ed. A.Maelicke, Springer-Verlag, p417, 1986). Recently we have isolated rat genomic and cDNA clones encoding the mature alpha2 gene product. Partial sequencing of the genomic clones has shown that the alpha2 gene is composed of at least 6 exons and encodes a mature protein of 481 amino acids having characteristic features of nicotinic acetylcholine receptor α-subunits. Clones derived from the alpha2 gene were isolated from rat brain cDNA libraries. mRNA transcribed from one of the longest alpha2 cDNA clones was injected into *Xenopus* oocytes in combination with mRNA derived from a cDNA clone, PCX49. PCX49 was isolated from PC12 cell libraries and is encoded by the beta2 gene(E.Deneris et al., this meeting). The injection resulted in the synthesis of functional neuronal nicotinic acetylcholine receptors. Depolarising responses were recorded to perfused acetylcholine(1-5μM). The responses were blocked by d-tubocurarine and hexamethonium(0.1mM) but not by α-bungarotoxin(0.1μM, 30 minutes incubation) or atropine(1μM). These are the properties expected of neuronal nicotinic acetylcholine receptors. The localization of alpha2 gene transcripts in the rat brain was determined by *in situ* hybridization histochemistry using an ^{35}S-RNA probe encoding the 3'-untranslated sequence of the alpha2 gene. Transcripts were found in the interpeduncular nucleus and the hippocampus. Little or no signal was detected in other brain regions by this method.

260.13 ANATOXIN-a ACTS AT CENTRAL NICOTINIC ACETYLCHOLINE RECEPTORS. G. Lunt*[1], S. Wonnacott*[1], B. Thorne*[1], H. Rapoport*[2], Y. Aracava*[3], and E.X. Albuquerque*[3]. (Spon: L. Goldman) [1]Dept. Biochem., Univ. Bath, Bath BA2 7AY, U.K., [2]Dept. Chem., Univ. California, Berkeley, CA 94720 and [3]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Anatoxin-a, a neurotoxin produced by the algae *Anabaena flos-aquae*, is a potent and stereospecific agonist at the vertebrate neuromuscular juction, closely resembling acetylcholine in its channel conductance properties. We have investigated the effects of anatoxin-a isomers at nicotinic sites in the CNS. In binding experiments, (+)anatoxin-a was a potent competitor of [^{3}H](-)nicotine binding to rat brain membranes (K_i = 3 x 10^{-10}M); the (-)anatoxin enantiomer was two orders of magnitude less effective. To see if (+)anatoxin-a also acts as an agonist in the CNS, the toxin was tested in a model system based on the presynaptic nicotinic facilitation of transmitter release. Synaptosomes were prepared from rat hippocampus, and the intraterminal pool of acetylcholine was labelled by incubation with [^{3}H]choline. Perfusion of the labelled synaptosomes with Kreb's bicarbonate buffer into which pulses (100 μl; 50 μM) of (-)nicotine were introduced resulted in the release of discrete peaks of [^{3}H]acetylcholine, and this response could be partially blocked by the nicotinic antagonist dihydroβerythroidine (10 μM). Similarly, hippocampal synaptosomes loaded with [^{3}H]GABA responded to pulses of nicotinic agonist by releasing radiolabelled transmitter. The higher labelling [^{3}H]GABA allows lower agonist concentrations to be tested. In this case, 10 μM (-)nicotine and 1 μM (+)anatoxin-a released comparable amounts of [^{3}H]GABA. The results suggest that (+)anatoxin-a is at least 5 times more effective than (-)nicotine at presynaptic nicotinic receptors in mammalian brain. Additional evidence of the existence of nicotinic receptors in the CNS has been provided by electrophysiological studies (see abstract of Aracava *et al.* - this meeting). (+)Anatoxin-a and acetylcholine activated single channel currents from cultured hippocampal neurons disclosing the presence of nicotinic acetylcholine receptors which bore some functional and pharmacological homology with the neuromuscular nicotinic macromolecule. (Support: U.S. Army Med. Res. & Devel. Com. Contr. DAMD17-84-C-4219 and NIDA Grant DA 02804).

260.14 **BLOCKADE OF NICOTINIC ACETYLCHOLINE RESPONSES BY TOXIN F IN ISOLATED RAT RETINAL GANGLION CELLS AND BINDING OF RADIOLABELED TOXIN F TO RAT RETINAL HOMOGENATES.** E. Aizenman, R.H. Loring, R.E. Zigmond & S.A. Lipton. Depts. of Neurology and Pharmacology, Harvard Med. Sch., and Div. of Neurosci., The Children's Hosp., Boston, MA 02115.

Patch-clamp recordings from cultured retinal ganglion cells have revealed responses to acetylcholine that are blocked by classical nicotinic antagonists but not by alpha-bungarotoxin or atropine (Lipton et al., *Soc. Neurosci. Abstr.* 12: 635; 1986). In the present study we examined the antagonist and ligand binding properties of another fraction of the venom from *Bungarus multicinctus*, Toxin F (TXF), on retinal preparations. This toxin has been shown to block nicotinic responses in other neuronal systems (see Loring et al., *Soc. for Neurosci. Abstr.*; 1987). For the physiological studies, retinal ganglion cells were labeled *in vivo* by retrograde transport of a fluorescent dye and maintained in dissociated cultures (Leifer et al., *Science* 224: 303; 1984). Whole-cell patch-clamp recordings were performed on these cells. Currents induced by 50 micromolar acetylcholine (applied by pressure ejection from a micropipette in close apposition to the cell under study) were reversibly antagonized by 0.2 micromolar TXF. The toxin was introduced by rapid superfusion of the recording chamber; in this manner, responses were blocked within 2-4 minutes following toxin addition and partially recovered within 4-18 minutes after toxin washout. During the recovery phase from toxin block, single acetylcholine-induced channels were sometimes observed. Nicotinic channels obtained in this fashion had a slope conductance of 48 pS and a mean open time distribution with a TAU of 4.3 ms. Single nicotinic channels observed prior to toxin addition had a similar conductance.

For the binding studies, rat retinal homogenates were incubated in 3 to 100 nM radioiodinated TXF (500 Ci/mmol) in the presence and absence of cold TXF and/or alpha-bungarotoxin. We found that TXF specifically bound to two sites, one having a Kd of 14 nM and a Bmax of 18 fmol/retina, and a second having a Kd of 188 nM and a Bmax of 88 fmol/retina. We observed little or no TXF binding that was displaceable by alpha-bungarotoxin. Work by others has shown that the cholinergic pathway in the intact mammalian retina links "starburst" amacrine cells to ganglion cells and that this pathway participates in the response to slow motion of visual stimuli. Thus, the TXF binding sites may represent the TXF-sensitive cholinergic receptors on retinal ganglion cells that mediate this response.

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261.1 INTRACELLULAR RESPONSES OF HUMAN CORTICAL BIOPSIES MAINTAINED IN VITRO. B.W. Strowbridge*, G.M. Shepherd, D.D. Spencer and L.M. Masukawa. Department of Neuroscience, Yale University School of Medicine, New Haven, CT 06510.

As part of a multidisciplinary study of the neural basis of human epilepsy, we obtained neocortical biopsies from twelve patients undergoing surgical procedures to treat intractable epilepsy. We compared intracellular responses in human neocortex with those of the rat and guinea pig, and probe for evidence of the differences which might throw light on the pathophysiology of epilepsy.

Biopsies of the cingulate, temporal and parietal cortices were taken as part of the normal surgical procedure. A portion of the biopsy was immediately placed in ice-cold Krebs buffer and removed to an antechamber outside the operating room where it was blocked and vibratomed into 400 um slices. The slices were left in the vibratome bath, which was gently bubbled with 95% O_2 /5% CO_2 and transported to the recording chamber, similar to a Haas chamber.

In most areas acceptable intracellular recordings were readily obtained after a 1-3 hour recovery period. We were able to classify the neurons encountered into the 3 categories that McCormick et al. (J. Neurophysiol. 54:782, 1985) proposed for guinea pig neocortex: regular firing, bursting and fast spiking, based on the cell's response to injected current. Regular firing cells accounted for 80% of our sample and were found in all layers except layer 1. Cells which responded to current steps with a burst of action potentials were encountered in 15% of the population and were concentrated in layers 4 and 5. Fast spiking neurons constituted only 5% of the population.

We used othrodromic electrical stimulation to analyse the synaptic physiology of the neurons in the biopsies, and found considerable variation in these responses. In 5 of our 12 cases, the orthodromic responses were similar to that of the rat and guinea pig neocortex: an initial EPSP followed by a long duration IPSP. Often the IPSP could be revealed by depolarizing current injection or by superimposing the orthodromic stimulus on a train of action potentials evoked by a current step. In 4 of the cases, there was evidence for a prolonged EPSP followed by a weak inhibition. Some cells, however, exhibited strong IPSPs to orthodromic stimuli, whereas other cells in the same slice responded with little or no IPSP. Three cases had orthodromic responses consisting of bursts of action potentials at a latency of 30-50 ms. In these cases, the latency of the burst could be altered by the membrane potential; in 2 of the 3 cases, hyperpolarization decreased the latency while increasing latency in one case.

Our studies indicate a number of similarities between the physiology of the human neocortex and the rodent neocortex. We have also observed orthodromic responses which are seldom seen in healthy animal neocortex, such as the long latency burst. We postulate that such responses may represent a physiological manifestation of the epileptic tissue. (Supported by NIH NS-07609 and the Office of Naval Research)

261.2 CONTENT OF PEPTIDES & AMINO ACIDS IN THE CEREBROSPINAL FLUID OBTAINED PRE & POST SEIZURE. O Devinsky*, RJ Porter, WH Theodore, NS Nadi. NINCDS, Bethesda, MD 20892.

Investigations of human epileptic foci have shown that the content of the amino acids glutamate, glycine and aspartate, the neuropeptides somatostatin and neuropeptide Y, as well as the catecholamines are increased when compared to non-focal tissue. We therefore studied baseline and post seizure cerebrospinal fluid (CSF) levels of amino acids and neuropeptides in patients with simple and complex partial, and generalized tonic-clonic seizures to determine if similar alterations occurred. Baseline CSF was obtained in 16 patients from 10 hrs to 3 days after the last seizure, and post ictal samples between 20 min and 75 min after a seizure. Amino acids were analyzed by fluorescence, catecholamines by HPLC with electrochemical detection, and peptides were determined by radioimmunoassay. Ten sequential 1 ml aliquots of CSF were collected. The same CSF fraction number was used for each separate analysis in order to avoid changes due to gradient formation. (All patients were on carbamazepine or phenytoin, but the levels did not change between baseline and post seizure CSF). There were no significant changes in the baseline and post seizure CSF for the following amino acids: glutamate, aspartate, glycine, GABA, taurine, alanine and leucine. Furthermore, there was no difference between the CSF amino acid levels in normal volunteers (n=10) and patients (baseline or post seizure). CSF levels of the following peptides were determined: somatostatin, ACTH, neuropeptide Y, neurotensin, cholecystokinin, βendorphin, vasoactive inhibitory peptide, and met-enkephalin. Significant differences ($p<.05$ by student t-test) between baseline and post seizure were found for ACTH (mean ± SD; n=16) (4.95 ± 1.1 pg/ml vs 2.90 ± 1.0 pg/ml), βendorphin (5.95 ± 1.0 pg/ml vs 7.9 ± 0.45 pg/ml), met-enkephalin (3.49 ± 1.1 pg/ml vs 6.92 ± 1.2 pg/ml), and somatostatin (84.1 ± 25.9 pg/ml vs 174.9 ± 41.8 pg/ml), but not neuropeptide Y, neurotensin, cholecystokinin, or vasoactive inhibitory peptide. Cortisol was not significantly altered in the post seizure CSF. No time dependence of the peptide alterations were observed in patients from whom sequential post seizure CSF samples were obtained. Earlier studies from our laboratory have shown that catecholamines are also increased in the post seizure CSF. The alterations of neuropeptides and catecholamines in brain as well as CSF may provide clues for a role of these compounds in the pathophysiology of seizures.

261.3 EVIDENCE OF AUGMENTED SEROTONIN AND DOPAMINE TURNOVER IN EPILEPTOGENIC FOCI RESECTED FROM HUMAN BRAIN. G.B. Glavin*, K.M. Kiernan* and G.R. Sutherland* (SPON: F. LaBella). Dept. Pharmacol. and Ther., Univ. of Manitoba, Winnipeg, Canada, R3E 0W3.

Temporal lobe and hippocampal specimens were obtained from patients undergoing surgical resection of their epileptic foci. Samples were analyzed in triplicate by HPLC/ED and levels of noradrenaline (NA), dopamine (DA), and 5-hydroxytryptamine (5-HT) as well as their major CNS metabolites MHPG (3-methoxy-4-hydroxyphenylethyleneglycol sulfate); HVA and DOPAC (homovanillic acid and dihydroxyphenylacetic acid); and 5-HIAA (5-hydroxyindoleacetic acid), respectively, were obtained. 3 out of 4 hippocampal specimens from actively spiking electrode sites showed huge (200-400%) increases in 5-HT turnover, compared to non-epileptic brain tissue. Analysis of actively spiking temporal cortical tissue revealed little evidence of exacerbated amine turnover, however, examination of less electrically active temporal cortical specimens showed both exaggerated 5-HT and DA turnover. Since 5-HT is known to exert anti-seizure activity, we suggest that the rise in 5-HT turnover observed in these samples reflects recruitment of 5-HT for endogenous anti-epileptic activity. The significance of the enhanced DA turnover remains to be determined, however, we suggest that our method, sensitive to picomolar concentrations of monoamines and their metabolites, is a useful addition to the research armamentarium investigating the epileptogenic focus.

(Supported by the Health Sciences Centre, Winnipeg Research Foundation).

261.4 GABAERGIC SYNAPSES IN THE HUMAN EPILEPTIC HIPPOCAMPUS: LIGHT AND ELECTRON MICROSCOPY OF GAD IMMUNOSTAINING. Babb, T.L., Kupfer, W.R.* and J.E. Pretorius*. Dept. of Neurology and Brain Research Institute, University of Calif. Los Angeles 90024

Glutamate decarboxylase (GAD) immunocytochemistry of resected epileptic temporal lobes has shown that the numbers of GABA cells and terminals are not significantly and selectively decreased in epileptic hippocampus compared to the severe hippocampal sclerosis characteristic of hippocampal epilepsy. (Babb, T. Neurotransmitters and Seizures and Epilepsy III pp. 293-302, N.Y. Raven Press, 1986) This relative preservation of inhibitory interneurons and their terminals as quantified by light microscopy of GAD-stained hippocampus suggests that GABAergic inhibition is intact in human epileptic hippocampus. The present study was performed to investigate the ultrastructure of the GABAergic synapses and examine the integrity of the pre- and post-synaptic profiles in epileptic tissue. To date, 6 epileptic hippocampi have been studied extensively by combined GAD staining and electron microscopy. The hippocampi were immersion-fixed in picric acid-aldehyde, GAD-immunostained, epon-embedded and examined with transmission E.M. in fields CA_1, CA_2, CA_3 and fascia dentata (FD). Synapses were defined as GABAergic if (1) the terminals had GAD on the vesicles and around mitochondria, (2) there was a wider cleft between the pre-synaptic membrane (with denser vesicles) and post-synaptic membrane (with adjacent density). Symmetric synapses were more frequent than the asymmetric type. GABAergic synaptic profiles appeared intact in all regions of epileptic hippocampus despite adjacent glial fibrils. Cell bodies had typical organelles such as ribosomes, endoplasmic reticulum and Golgi near the post-synaptic cytoplasm. We conclude that GABAergic synaptic structure is intact in human epileptic hippocampus. NIH Grant NS02802.

261.5 VARIATIONS IN THE DELAYS BETWEEN PEAKS WITHIN POPULATION BURSTS IN CA1 RECORDED WITH A 32 ELEMENT ARRAY. J. L. Novak*[1], B. C. Wheeler[2], and F. -L. Chang[2]. [1]Department of Electrical and Computer Engineering, University of Illinois, Urbana, IL 61801 and [2]Department of Psychology, University of Illinois, Champaign, IL 61820.

Epileptiform bursts recorded in the CA1 area of the rat hippocampal slice propagate in the direction of the afferent volley evoked in the Schaffer collateral pathway. These bursts appear to be composed of two types of population spikes: the first spike which may be mediated by fast receptors involving kainic acid, and secondary spikes via NMDA. Although propagation delays for the first spikes occur as a result of the conduction velocity of the Schaffer collaterals, the mechanism for the apparent propagation of the secondary spikes remains unclear.

A planar, photoetched microelectrode array of 4 by 8 electrodes spaced 200 μm apart was used to investigate propagation of an epileptiform burst through the CA1 region of hippocampal slices from male Sprague-Dawley rats. The media contained (in mM): 124 NaCl, 26 $NaHCO_3$, 10 glucose, 5 KCl, 2.0 $CaCl_2$, 1.25 NaH_2PO_4, 0.8 $MgSO_4$. Picrotoxin (100 μM) was added to induce epileptiform responses. The slices were positioned with the Schaffer collateral pathway parallel to the major array axis. Bipolar stimulating electrodes were positioned in the s. radiatum of CA1, either near CA3 or the subiculum.

To measure the propagation of active tissue within the slice, and not the passive spread of field potentials, a 2-D current source density (CSD) analysis was performed prior to measuring peak times. This prevented artifacts which may have resulted from treating passively generated field potentials as individual events with zero interchannel delay. The use of this technique with this electrode array and preparation has been reported (IEEE Trans. BME, BME-33:1024, 1986).

The interchannel delays between active sinks corresponding to the first population spike recorded over the 200 μm interelectrode intervals were fairly regular. However, delays for the second sink appeared much more variable, often with no delay between adjacent channels. To study the variability, an index of coefficients of variation was adopted. For the first spike, the coefficient is 0.314 $\pm$ 0.183 (n = 10), and the second is 0.841 $\pm$ 0.363 (n = 10). The two coefficients of variance are significantly different, ($t = 9.76$, $p < 0.001$), suggesting that epileptiform activity in CA1 does not consist of passively conducting serially activated stereotypical responses, but rather that active localized processing may exist.

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261.6 THE PAUCITY OF BLOOD VESSELS IN THE TEMPORAL LOBE MAY ACCOUNT FOR ITS SUSCEPTIBILITY TO SEIZURE-INDUCED DAMAGE. J.P. Olson*, F.E. Samson, T.L. Pazdernik*, V.H. Gattone II* and S.R. Nelson Department of Anatomy and the R.L. Smith Center, Univ. of Kansas Medical Center, Kansas City, Kansas 66103.

Sustained seizures (kainic acid-induced) in rats result in ischemic infarction of temporal lobe (piriform-entorhinal cortex and amygdala). We hypothesize that the temporal lobe lesion may be the result of vascular insufficiency during the seizure caused by regional differences in tissue vascularity. A vascular casting technique was used to compare cortical vasculature of areas susceptible to damage (temporal cortex) with areas unaffected (parietal cortex) by seizures.

Normal rat brains were perfusion fixed with 2.5% buffered glutaraldehyde under physiological conditions and the cerebral vasculature injected with Microfil silicon casting medium. The brains were processed and cut into 4mm coronal sections for gross examination of the cerebral vasculature. Cortical regions were removed from the parietal and temporal lobes, paraffin embedded and sectioned for vascular quantitation. Quantitatively, the penetrating vessels were counted and their cross sectional areas measured. Regional vascular volume density was determined by point counting.

Gross examination of cerebral vascular casts revealed a rich supply of penetrating vessels and capillaries in parietal lobe with few penetrating vessels in the temporal lobe. This apparent difference in vascularity was confirmed by morphometric analysis of parietal and temporal cortex. Analysis revealed there were fewer and smaller penetrating vessels and less vascular volume density in temporal cortex. The temporal cortex vascular density was 0.024±0.001 compared to 0.045±0.003 mm^3/mm^3 in the parietal cortex (mean ± SEM, n=6). There were also one fourth (25.9%) the number of penetrating vessels (diameter >20um) in temporal cortex. Since blood flow is generally considered to be proportional to the vascular volume density, a 50% reduction in vascular volume would have functional significance. This paucity in vascularity may predispose the temporal lobe to ischemic damage during seizures because the blood flow cannot accommodate the increased metabolic need.

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261.7 INCREASED EXTRACELLULAR POTASSIUM UNMASKS EXCITABILITY DIFFERENCES BETWEEN THE EPILEPTIC MUTANT TOTTERING AND CONTROL MOUSE HIPPOCAMPUS IN VITRO. P.A. Rutecki and J.L. Noebels. Dept. of Neurology, Section of Neurophysiology, & Program in Neuroscience, Baylor College of Medicine, Houston, TX. 77030.

The single locus mutant mouse tottering (tg/tg) displays spontaneous generalized spike-wave epileptiform discharges and absence seizures which can be abolished by reducing their abnormal central noradrenergic (NE) hyperinnervation originating from the locus coeruleus. In this study we tested the hypothesis that a difference in excitability between tg/tg and control (+/+) mice can be demonstrated in vitro. We also evaluated whether exogenous NE can modulate convulsant-induced epileptiform activity.

Extracellular recordings were made from the CA3 subfield of 400 µM thick hippocampal slices. Neither tg/tg, nor +/+ slices displayed spontaneous epileptiform discharges in control saline containing 5 mM $[K^+]_o$.

When $[K^+]_o$ was raised to 7.5 mM, spontaneous epileptiform discharges appeared in both genotypes. The discharge frequency increased in 10 mM $[K^+]_o$. The mean frequency of occurrence of spontaneous discharges was higher in +/+ than tg/tg slices, but the difference was not significant (0.106 vs 0.062 Hz for $[K^+]_o$=7.5 mM, 0.31 vs 0.26 Hz for $[K^+]_o$=10 mM; $P > 0.05$). The mean duration of a discharge was significantly longer in tg/tg compared to +/+ slices (106.5 [N=12] vs 86.8 ms [N=18] for $[K^+]_o$=7.5 mM, 111.1 [N=9] vs 84.8 ms [N=8] for $[K^+]_o$=10 mM; $P < 0.05$). Bath application of 10 µM 4-aminopyridine had a dissociative effect similar to high $[K^+]_o$ on discharge duration but not frequency between the two genotypes. In the presence of a different class of convulsant, 10 µM picrotoxin, discharge durations in both tg/tg and +/+ slices were longer than in elevated $[K^+]_o$, but there was no significant difference in duration or rate between the two. Bath application of 10 µM NE or 1 µM isoproterenol did not initiate spontaneous discharges in low $[K^+]_o$, but did produce an increase in the frequency of discharges induced by high $[K^+]_o$ in both +/+ and tg/tg slices. Neither agonist had an effect on discharge duration.

These results demonstrate an intrinsic difference in network excitability of an isolated region of the epileptic mutant tottering brain. Although exogenous NE agonists did not prolong discharge duration, our findings confirm that NE agonists exert a neuromodulatory effect on $[K^+]_o$-induced epileptiform discharge frequency. (Supported by NIH grants NS11535, NS01049, RR-05425, and the Bluebird Circle.)

261.8 DIFFERENCES IN THRESHOLD AND PATTERN OF ELECTROSHOCK-INDUCED SEIZURES IN GENETICALLY EPILEPSY-PRONE RATS. R. A. Browning, D. L. Patrick* and P. C. Jobe. Southern Illinois Univ. Sch. of Med., Carbondale, IL 62901 and Univ. Ill. Coll. Med., Peoria, IL 61656.

Previous studies from our laboratory have suggested that convulsions characterized by facial and forelimb (F&F) clonus depend on (and presumably emanate from) a forebrain (FB) substrate, while convulsions characterized by running-bouncing (R/B) clonus and tonus depend on a brainstem (BS) substrate for expression (Browning & Nelson, Exp. Neurol. 93 546, 1986). We have also reported that minimal electroshock stimulation preferentially produces F&F clonus when corneal (C) electrodes are used and R/B clonus when ear-clip (EC) electrodes are used (Browning & Nelson, Life Sci. 37 2205, 1985). Thus, it should be possible to preferentially activate either FB or BS driven convulsions depending on whether one uses C or EC electrodes. The present investigation was designed to compare the thresholds of the FB seizure substrate in genetically epilepsy-prone rats (GEPRs) and seizure resistant (normal) rats using the minimal electroshock seizure threshold (EST) test and C electrodes. Rats used in these studies were male and female GEPRs which display either severe (tonic) seizures (GEPR-9s) or moderate (R/B clonic) seizures (GEPR-3s) in response to sound (audiogenic) stimulation and Sprague-Dawley seizure resistant (SR) rats. Female SR rats displayed F&F clonus with a threshold of 15.8±0.8 mA in response to the EST test, whereas female GEPR-9 rats failed to exhibit F&F clonus at any stimulus intensity. Instead R/B clonus was observed in GEPR-9s at very low stimulus intensities, and the threshold for this response was 6.2±0.2 mA. Higher stimulating currents (7-10mA) caused tonic seizures with full hindlimb extension in GEPR-9s. Similar results were obtained when male GEPR-9 rats were compared with male SR rats. Unlike GEPR-9s, GEPR-3s displayed F&F clonus in response to the EST test, but their threshold was lower than that of SR rats (GEPR-3s=19.5±0.1 mA; SRs=23.2±0.04 mA, $p< 0.01$). These findings suggest that in GEPR-9s the EST of the BS is less than that of the FB, whereas in SR rats and GEPR-3s, the EST of the BS is higher than the FB. Thus, in GEPR-9s one cannot trigger the FB seizure (F&F clonus) without also triggering the BS seizure. Apparently when the BS seizure occurs, it gains control of the output (spinal cord) and precludes the expression of F&F clonus.

261.9 DECREASED POTASSIUM UPTAKE IN ASTROCYTES CULTURED FROM GENETICALLY EPILEPSY PRONE RATS. J.T. Neary, L.O.B. Norenberg*, and M.D. Norenberg, Laboratory of Neuropathology, Vet. Admin. Med. Ctr. and Univ. of Miami School of Medicine, Miami, FL 33101.

A key function of astrocytes is the uptake of potassium released during neuronal activity. Pollen and Trachtenberg (Science, 167: 1252, 1970) suggested that a defect in potassium buffering by astrocytes could lead to a lower threshold for neuronal excitability, thereby contributing to epileptogenesis. The use of primary astrocyte cultures from genetically epilepsy-prone rats (GEPRs) affords a unique opportunity to study mechanisms which may be related to seizure activity in these cells. Thus, we have investigated the potassium uptake system in cultured astrocytes derived from GEPRs.

Astrocytes were obtained from neonatal cortices of normal Sprague-Dawley rats, GEPR-3 (rats which display moderate, clonic seizures), and GEPR-9 (animals with severe, tonic seizures) which were kindly provided by Dr. J. W. Dailey, Univ. Ill., Peoria. Cells were maintained in primary culture and after two weeks were treated with 0.5 mM dibutyryl cyclic AMP to induce differentiation. The age of cultures used in these studies ranged from 6 to 10 weeks. Following a 30 min. preincubation in a balanced salt solution at 37°C, potassium uptake (using 86-rubidium as a potassium analog) was measured at 3 mM KCl for 1 min.

We found that potassium uptake was reduced by about 15% in astrocytes from GEPR-9 as compared to cells from Sprague-Dawley rats (control = 75.6 $\pm$ 2.55 nmol potassium/min/mg protein, mean $\pm$ SEM; GEPR-9 = 63.8 $\pm$ 1.6; $P<0.01$). Similar results were obtained with cultures from GEPR-3 (62.3 $\pm$ 1.8 nmol potassium/min/mg protein; $P<0.01$). The ability of the cells to respond to increased potassium levels (12 mM) for a prolonged time (10 min) was also investigated. In GEPR-3 cultures, potassium uptake was decreased by about 11%. However, in GEPR-9 astrocytes (derived from rats with a more severe form of seizures than GEPR-3), potassium uptake was decreased by 31% (control = 789 $\pm$ 66 nmol potassium/10 min/mg protein; GEPR-9 = 544 $\pm$ 23; $P<0.02$).

These studies indicate that astrocytes from GEPRs have a diminished ability to take up potassium and suggest that an astroglial defect in potassium homeostasis may contribute to epileptogenesis.

261.10 SERUM GROWTH HORMONE AND BODY WEIGHT ARE DECREASED IN DEVELOPING GENETICALLY EPILEPSY-PRONE RATS. S.A. Mills, C.E. Reigel, P.C. Jobe, and D.D. Savage. Dept. Pharmacol., U. of New Mexico Sch. of Med, Albuquerque, N.M., 87131 and Dept. of Basic Sciences, U. of Illinois Col. of Med. at Peoria, Peoria, IL., 65616.

The Genetically Epilepsy-Prone Rat (GEPR) is an animal model of epilepsy in which seizures occur in response to acoustic stimuli. Recently, GEPR rats have been found to be hypothyroid between days 5 and 45 of age (Soc. Neurosci. Abs. 12:72, 1986). Neonatal hypothyroidism produces a number of deficiencies in growth and development, including central nervous system abnormalities. Hypothyroidism causes a decrease in pituitary growth hormone (GH) content, serum GH, and weight gain. Because of the effects of hypothyroidism on GH and on growth and development, body weight and serum GH levels were determined in developing GEPR rats and age matched non-epileptic Sprague-Dawley controls.

GEPR-3 and GEPR-9 rats used in these studies were derived from breeding pairs which had previously demonstrated the ability to produce progeny susceptible to audiogenic seizures. GEPR-3, GEPR-9, and control rats were weighed and sacrificed by decapitation between 1300 and 1500 hours on 5, 9, 13, 16, 22, 31, 45, and 90 days of age. Trunk blood was collected and serum stored at -70°C until assayed. Serum GH levels were determined by radioimmunoassay.

Serum GH levels in both GEPR-3 and GEPR-9 rats were decreased relative to control rats at all time points studied, and were significantly lower than control levels on Days 9, 13, and 45 of life. Weight gain in the GEPR rats was significantly less than control at all time points studied. The difference in weight gain between control and GEPR rats was most striking from 9 to 16 days of age. GEPR-3 rat weight gain was less than control until Day 16, while GEPR-9 rat weight gain was less than control through Day 31.

These results indicate that weight gain in the GEPR rat remains below control weight gain up to 90 days of age. The difference in weight gain between control and GEPR rats is greatest between Days 9 and 16 of age. This period of time coincides with the interval of the most marked decrease in serum GH in GEPR rats as compared to control. Both the decrease in weight gain and serum GH level occur at a time when serum T_4 levels are most significantly decreased in GEPR rats compared to control. These results suggest that abnormalities in GEPR rat serum GH and weight gain may be functional consequences of neonatal hypothyroidism.
(Materials for the GH radioimmunoassay were provided by the NIADDK division of the NIH)

261.11 HIPPOCAMPAL MOSSY FIBER ZINC IS DIMINISHED IN THE GENETICALLY EPILEPSY-PRONE RAT. D.D. Savage. Dept. Pharmacol., U. New Mexico School of Medicine, Albuquerque, N.M., 87131.

The axonal projection (mossy fibers) of hippocampal formation (HPF) dentate granule cells are rich in histochemically detectable zinc. The role of mossy fiber zinc in neurotransmission between dentate granule cells and CA_3 pyramidal cells is not known. Depletion of HPF zinc in rodents has been associated with a decrease in long term potentiation and decreased performance in learning paradigms. Intraventricularly administered zinc produces seizures. Elevation of whole HPF zinc has been reported in several animal models of epilepsy including the photosensitive baboon, audiogenic mice, kindling and kainate-induced seizures.

The Genetically Epilepsy-Prone (GEPR) rat has been developed by the selective inbreeding of Sprague-Dawley rats susceptible to acoustic stimulus-induced seizures. Given the evidence of an association between whole HPF zinc and epilepsy, HPF mossy fiber zinc was measured in GEPR rats by a newly developed quantitative histofluorescence technique using 8-methoxy-p-toluenesulfonamide (TSQ).

Thirty one day old GEPR-9 and non-epileptic Sprague-Dawley control rats were sacrificed and their brains frozen. Eight micron thick horizontal sections were cut through the ventral HPF, thaw mounted onto microscope slides and vacuum desiccated for 24 hours prior to analysis. Zinc:TSQ histofluorescence was examined using an image analysis system at 32.5X magnification. Eighty microliters of TSQ solution (0.003% TSQ in barbital-acetate buffer, pH 10.2) were applied to the sections, the sections coverslipped and examined one minute later. Fluorescence, expressed as grey levels, was measured and compared to a standard curve of fluorescence obtained from analysis of water soluble plastic sections embedded with known amounts of zinc. Fluorescence measurements were made in the s.lucidum of HPF CA_3 and adjacent s. radiatum. Specific mossy fiber fluorescence, expressed as femtograms zinc/100 microns^2, was defined as the difference in fluorescence between these two regions.

Six sections of ventral HPF were analyzed from six animals of each group. The results indicated a 47% reduction in mossy fiber zinc in GEPR-9 rats compared to non-epileptic controls. Whether the decrease in mossy fiber zinc is an etiological factor in the development of epilepsy in GEPR rats is unknown. However, diminished mossy fiber zinc could be one consequence of thyroid and growth hormone deficiencies during critical stages of HPF maturation, particularly mossy fiber migration and subsequent innervation of HPF CA_3 pyramidal neurons.

261.12 ONTOGENY OF MONOAMINERGIC DETERMINANTS OF SEIZURE SUSCEPTIBILITY AND SEVERITY IN THE GENETICALLY EPILEPSY-PRONE RAT. C.E. Reigel, J.J. Joliff, P.C. Jobe and P.K. Mishra. **Dept. of Basic Sciences, Univ. of Illinois Col. of Med. at Peoria, Peoria, IL 61656.**

The adult Genetically Epilepsy-Prone Rat (GEPR) has been characterized by widespread regional deficits in central norepinephrine (NE) and serotonin (5-HT) levels. Regional defects in NE or 5-HT have been implicated in the regulation of seizure susceptibility in certain areas and seizure severity in others. Severity defects are those NE or 5-HT deficits present in the severe seizure colony (GEPR-9) and not the moderate seizure colony (GEPR-3). Behaviorally, GEPR-9s exhibit full tonic extensor convulsions and GEPR-3s exhibit generalized clonic convulsions in response to sound. Pharmacological manipulations of NE or 5-HT support a role for these deficits in the regulation of seizure susceptibility and severity in the GEPR.

As a further test of the involvement of NE and 5-HT deficits in seizure processes in the GEPR, we have examined regional monoamine levels developmentally at ages prior to sound-induced seizure susceptibilty through maturation to adult patterns of convulsion. Seizure susceptibility begins in GEPR-3s at 15 days and in GEPR-9s at 16 days of age. Both colonies reach 100 percent susceptibility at 19 days, maturing rapidly to near adult patterns of characteristic seizures (clonic versus tonic) at 30 days of age. Adult seizure patterns are present at 45 days of age. Monoamine levels were measured in the telencephalon, hippocampus, cerebellum and medulla of male, seizure naive GEPR-9s, GEPR-3s and non-epileptic controls at 13, 16, 19, 21, 23, 27, 30 and 45 days of age. Monoamine levels were also measured in the telecephalon, cerebellum and remaining brain of 5, 9 and 13 day old subjects. Monoamine levels were determined by the HPLC/electrochemical detection method of Co and associates (Pharmacol Biochem Behav 16: 641-646, 1982).

Deficits in 5-HT associated with seizure severity in adult GEPRs were present only in 45 day old GEPR-9s in contrast to the appearance of tonic seizures at earlier ages. Suceptibility deficits in 5-HT were present in GEPR-3s and GEPR-9s prior to and throughout their period of seizure susceptibility. Deficits in NE associated with seizure susceptibility were present in GEPRs prior to and throughout their period of seizure susceptibility in all areas except the medulla. Adult NE severity defects were present in GEPR-9s from the age of appearance of tonic seizures to 45 days in the cerebellum and hippocampus, but not medulla or telencephalon. NE levels lower than GEPR-9 or control were present in GEPR-3s during the period that they transiently exhibited tonic convulsions. Adult susceptibility deficits in NE and 5-HT were present developmentally whereas adult severity deficits were limited to NE in two brain areas. (Supported by BRSG grant SO7 RR 05369)

261.13 **POWER-SPECTRUM OF SURFACE EEG OF GENETICALLY-EPILEPTIC CHICKENS AND EFFECTS OF INTERMITTENT PHOTIC STIMULATION.** P.K. Mishra, E.C. Crichlow and R.D. Crawford, Department of Physiological Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Sask., Canada S7N 0W0.

Intermittent photic stimulus (IPS) can induce epileptiform seizures in Genetically-Epileptic chickens. The seizures in these birds are controlled by an autosomal recessive gene 'epi'. Chickens that are homozygous for the epi gene have spontaneous seizures and can be driven into seizures by a number of external factors. The resting EEGs of the homozygous recessive birds show a slow-wave high-voltage rhythmic pattern with periodic spikes and spike-wave complexes which is characteristically different from their normal hatchmates. Upon IPS stimulus the EEG recording shows high-voltage spikes of the same frequency as the stimulus. The ictal phase is prolonged after the stimulus has stopped and in this phase a drastic reduction in amplitude is seen in the EEG.

Ten genetically epileptic (epi/epi) and ten normal chickens were implanted with stainless steel surface electrodes on their skulls. After complete recovery from surgery, EEG signals from differential as well as monopolar electrodes were recorded before during and after IPS on a FM tape recorder. These signals were later analysed by a digital computer. Fast fourier transformations of 104 sec. before IPS and 8-24 sec. after IPS were utilized to obtain the components of various physiological frequency bands in the resting and ictal phase respectively.

Slow frequency components (0-4 cps) of the interictal (resting) EEG of epileptic birds were significantly elevated (up from 37% in normal to 66% in epileptic) whereas the remaining three bands (4-8, 8-14, and 14-64 cps) were significantly reduced in the epileptic birds as compared to those of the normal birds. Although a statistically significant reduction was observed in all three high frequency components, the most significant reduction was observed in the 14-64 cps band. IPS stimulation caused a significant reduction in the 0-4 cps component and an elevation in the other three components of the power-spectrum. However, this effect was seen only in the recordings from the anteriorly placed electrodes. Recordings from the normal birds did not show any significant change in the EEG power-spectrum upon IPS stimulation. This technique may be useful in evaluating the neuropharmacological effects of various drugs, especially those, which cause no visually detectable changes in the EEG.

261.14 DEVELOPING NORMAL AND GENETIC EPILEPSY PRONE RATS HAVE A DIFFERENT SEIZURE THRESHOLD TO FLUOROTHYL. K.L. Ginter*, P.A. Schwartzkroin and J.E. Franck. (SPON: A.B. Harris) Dept. Neurological Surgery, Univ. of Washington, Seattle, WA 98195

The genetic epilepsy prone rat (GEPR) has generalized seizures in response to auditory stimuli and a lowered seizure threshold to non-auditory manipulations. We have been interested in these animals as a model of non-sensory generalized epilepsy and have examined the development of seizures in GEPRs and control rats to fluorothyl, an inhalant convulsant, which has been suggested to have a mechanism of action similar to that of pentylenetetrazol.

Sprague Dawley rats (n=46) and GEPRs (n=53; bred from progenitor stock obtained from Dr. Phillip Jobe) were individually placed in a plastic bell jar on postnatal day 10, 15, 20, 25 or 30 and fluorothyl was infused (20µl/min) onto a piece of filter paper suspended at the top of the jar. Latencies to clonic and tonic seizures were recorded. At 40 days of age all animals were tested for audiogenic seizures. Six GEPRs did not have audiogenic seizures and fluorothyl data from these animals were analyzed separately.

Control animals had clonic-tonic convulsions in response to fluorothyl with the onset of clonus showing no age dependence (all ages $\bar{X}\pm$S.D.=193±18 sec). Seizure progression to full tonic extension, however, did change with age. At 10 days, the tonic component occurred shortly following the onset of clonus (221±18 sec); by day 30, latency to tonus had increased to 291±26 sec.

The GEPRs also had generalized seizures, with a consistent, but significantly shorter, latency to clonus independent of age (all ages=162±15 sec; p=.038). Day 10 GEPRs looked much like control animals of the same age; convulsions progressed to tonus immediately following the onset of clonus. As these animals matured, however, this pattern didn't change; tonic extension consistently occurred shortly after clonus (day 30 clonic and tonic latencies 167±17 and 175±17 sec, respectively).

Six GEPRs failed to convulse to the audiogenic post-test and their fluorothyl data was examined separately to determine if GEPR seizures to different stimuli co-vary. These animals had respective clonic and tonic latencies (162 and 170 sec) almost identical to age matched audiogenic susceptible GEPRs (168 and 170 sec).

These data indicate that: 1)the GEPRs have a greater sensitivity, and different response pattern, to fluorothyl than do control animals; 2)during maturation, normal rats develop a perhaps protective mechanism which delays tonic seizures; 3)this mechanism does not mature in the GEPR, and 4) the substrates of audiogenic and non-audiogenic seizures in the GEPR can occur independently; audiogenic seizures may be a special case of a more general neural deficit which interacts with abnormalities in the auditory system. These data both support the position that the GEPR is a valuable model in the study of generalized epilepsy and raise interesting questions on the primary neural deficit involved in non-audiogenic seizures in these animals.

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261.15 THE GENETIC EPILEPSY PRONE RAT HAS ALTERED GABA RECEPTOR BINDING IN SUBSTANTIA NIGRA BUT NOT INFERIOR COLLICULUS. J.E. Franck and P.A. Schwartzkroin. Dept. Neurological Surgery, Univ. Washington, Seattle, WA 98195.

The genetic epilepsy prone rat (GEPR) has generalized convulsions to loud auditory stimuli and a lowered seizure threshold to non-auditory convulsants. A primary site of pathology in these animals appears to be the inferior colliculus which has been reported to have decreased inhibition and an altered number of GABAergic inhibitory neurons. We have examined GABA binding in the GEPR colliculus and substantia nigra, hypothesizing that this feature of inhibitory function may also be compromised in colliculus and that the nigra may be similarly involved since it modulates generalized seizures.

Conventional in vitro autoradiographic techniques were used to examine the binding of both ^{3}H-muscimol (MUS), to the high affinity GABA recognition site, and ^{3}H-flunitrazepam (FLU) to the benzodiazepine (BZ) site on the GABA receptor complex. Slide mounted tissue sections (10µ) from audiogenic susceptible GEPRs (n=13) and normal Sprague Dawley rats (n=9) were incubated in either Tris citrate buffer (0 ^{0}C; .025M; pH 7.4) containing 10nM MUS (with or without excess cold GABA), or in Tris HCl buffer (0 ^{0}C; .17M; pH 7.4) containing .5 to 12 nM FLU (to allow Scatchard analysis) (with or without excess clonazepam). Labelled slides were apposed to LKB Ultrofilm with tritium brain mash standards; the central and cortical nuclei of the inferior colliculus and the substantia nigra pars reticulata were analyzed on the resulting autoradiograms using the DUMAS/BRAIN system (Drexel University). Binding characteristics of MUS and FLU were expressed as a mean percent (±S.D.) of co-incubated control rats over five replicates.

No differences were observed in specific MUS or FLU binding to either the cortical (MUS=113±19% of control; FLU=99±3%) or central nucleus (MUS=113±30%; FLU=108±8%) of the GEPR inferior colliculus. There was, however, a dramatic reduction in MUS binding in the GEPR substantia nigra (59±12% of control; p=.0012). The binding of FLU to the nigral GABA receptor complex was examined in detail to determine if the alterations observed were specific for MUS binding sites or if other features of GABA receptor function were altered. Scatchard analysis of FLU binding demonstrated that there was no change in receptor number (B_{max}=98±4% of control values). However, the affinity of FLU binding in the GEPR nigra was decreased (K_d=125±14% of control); this difference, while suggestive, was not significant (p=.07).

These data suggest that: 1)despite reports of GABAergic abnormalities in the GEPR inferior colliculus, there are no alterations in inhibitory high affinity GABA or BZ binding sites in this structure; 2)inhibition is compromised in the substantia nigra of the GEPR; and 3)altered inhibition in the substantia nigra may be a common mechanism in the induction of both audiogenic seizures (via demonstrated nigral-collicular pathways) and in the increased propensity of the GEPR to exhibit generalized seizures in response to non-auditory treatments.

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261.16 SPECIFIC REGIONAL CHANGES IN THE CONCENTRATION OF PARVALBUMIN IMMUNOREACTIVITY IN THE EPILEPTIC (El) MOUSE. T.B. Anderson*, J.J. Miller and K.G. Baimbridge (SPON: N.W. Kasting). Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada. V6T 1W5

Parvalbumin (PV) is a soluble calcium-binding protein found associated with sub-populations of GABA containing neurons in the rat brain. We have previously shown that the concentration of a second calcium binding protein, Calbindin D28k (CaBP), is reduced in specific regions of the brains of both the kindled rat (Miller and Baimbridge, Brain Res. 278:322, 1983) and the genetically epileptic (El) strain of mouse (Mody et al, Epilepsy Res. 1:45, 1987). In the seizure sensitive gerbil, Peterson et al (Brain Res. 340:384, 1985) have demonstrated an increased number of GABAergic neurons in the dentate gyrus of the hippocampal formation. Using a specific radioimmunoassay we have now determined the regional PV concentrations of El mice in which seizures have been induced by mild vertibular stimulation and compared these values with those of a control (Swiss) strain.

With the exception of the ventral occipital cortex all other cortical regions of the El mice showed dramatically elevated levels of PV with the maximum increase being in the ventral frontal cortex (270% of Swiss strain controls). The striatum, in which PV is contained within a sparse population of GABAergic interneurons, also showed elevated levels (195%) while there were no changes in the hippocampal formationl, medulla, pons, midbrain or cerebellum. Preliminary immunohistochemical studies indicate that the number of PV-positive neurons is not significantly increased when compared to control mice. This suggests that the increased PV levels determined by RIA represents an increase in the concentration of PV within the PV-positive neurons.

The increased levels of parvalbumin in the El mice are dramatic and interestingly occur in all the brain regions where CaBP levels are decreased, with the exception of the hippocampal formation. For the most part these two proteins occur in separate populations of neurons, for example, in the cortical regions examined there are very few cells which contain both PV and CaBP. The function of these proteins is presently unknown. Both bind calcium with high affinity and neither has been shown to have any enzyme stimulatory role in either muscle of neural tissue. The changes seen in the levels of PV and CaBP in specific cortical regions of the El mice may prove to be an important factor in the predisposition of this genetic strain of mouse to seizures.

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261.17 GABA WITHDRAWAL SYNDROME IN EPILEPTIC AND NON-EPILEPTIC BABOONS AND RATS. S. Brailowsky, H. Fukuda*, C. Menini*, C. Silva-Barrat* and R. Naquet. Laboratoire de Physiologie Nerveuse, C.N.R.S., 91190 Gif-sur-Yvette, France.

We have previously shown (Brailowsky et al., Neurosci. Lett., 74:75, 1987) that local application of GABA into the fronto-rolandic cortex of photosensitive baboons produces a complete blockade of both electrographic and behavioral signs induced by light stimulation, and that these anticonvulsant effects are followed by spontaneous epileptogenic activity originating in the infused areas. In this report, we confirm the presence of this "GABA-withdrawal syndrome" (GWS) in epileptic baboons infused in other cortical territories, in amygdala-kindled rats and in non-epileptic animals from these two species.

Chronic (7 days) infusion of GABA (100 ug/ul at 10 ul/hr) into the occipital cortex of photosensitive monkeys blocked the epileptic syndrome for the whole duration of GABA infusion. This effect was not observed when the aminoacid was delivered to the pre-frontal areas. In fully kindled rats (stage 5), bilateral GABA application (100 ug/ul/hr for 7 days) into the motor cortex produced a significant decrease in the motor component of the seizures without modifying the limbic after-discharge. In both epileptic and non-epileptic rats and monkeys, the interruption of GABA treatment was followed by focal epileptic activity localized to the infused areas. In all animals, EEG activity return to normal patterns after 1-2 days in rats and 2-4 days in monkeys.

The GWS is proposed as a useful model to study the participation of GABAergic mechanisms both in genetic and acquired epileptogenesis and its possible role in the convulsive syndromes observed after chronic, anticonvulsant medication in humans.

261.18 GABAERGIC MEDIATION OF PENTYLENETETRAZOL SEIZURES IN THE ANTERIOR THALAMUS. K.D. Holland*, J.W. Miller, C.M. Hall*, and J.A. Ferrendelli, Div. of Clin. Neuropharm., Depts. of Pharmacology and Neurology, Washington Univ. Medical School, St. Louis, MO 63110.

Recent studies have indicated that the anterior thalamus may play a role in the propagation of experimental generalized seizures induced by the convulsant drug pentylenetetrazol (PTZ). In this study we have examined how manipulations of GABAergic neurotransmission in the anterior thalamus affect gross behavior and PTZ seizure threshold in the rat.

In control animals, timed, continuous, intravenous infusions of PTZ via a jugular catheter led to generalized myoclonic jerks at a dose of 25.2 $\pm$ 2.2 mg/kg, clonic seizures at 30.7 $\pm$ 2.8 mg/kg, followed by a tonic seizure at 86.8 $\pm$ 4.1 mg/kg with forelimb, but not hindlimb, extension. Muscimol (30 nmol in 0.25 µl), primarily a $GABA_A$ agonist, was injected in the midline anterior thalamus through guide cannulas. This resulted in a reduction in the myoclonic (-54%) and clonic (-52%) seizure thresholds but increased the tonic seizure threshold (+19%) compared to control animals. Preliminary results revealed that piperidine 4-sulfonic acid, a specific $GABA_A$ agonist, produced effects identical to muscimol. In contrast, (+)-baclofen (150 nmol), a $GABA_B$ agonist, injected in the same fashion, increased the threshold for all PTZ seizure components (myoclonic +60%, clonic +94%, and tonic +28%) relative to controls. In the above experiments PTZ was infused 10-20 min after intracerebral injections of saline or drug. Animals were also treated with bilateral medial anterior thalamic microinjections of gamma-vinyl-GABA (GVG) (30 µg in 0.5 µl), which increases endogenous GABA levels by inhibiting GABA-transaminase. These animals tested with PTZ infusions 24 hrs later also had increased thresholds to all seizure components (myoclonic +129%, clonic +130%, and tonic +56%). Muscimol (30 nmol) and piperidine 4-sulfonic acid caused impaired postural reflexes, decreased spontaneous activity, and in some rats impaired respiration. In contrast, baclofen produced hypokinesia without altering postural reflexes or respiration. GVG produced very little or no effect on behavior.

Bicuculline methiodide, a $GABA_A$ antagonist, produced marked hyperactivity at low doses (300 pmol), and spontaneous clonic seizures at higher doses (> 1 nmol), but 300 pmol had no significant effects on PTZ seizure thresholds.

The results of this study lead to the conclusion that GABAergic neurotransmitter systems in thalamus influence both spontaneous behavior and convulsant actions of PTZ. In addition, the data indicate that $GABA_A$ and $GABA_B$ receptors have different roles in these processes.

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261.19 INTRA-AMYGDALOID TETANUS TOXIN IN CATS: A MODEL OF LIMBIC EPILEPSY. D.S. Garant*, M.C. Mangione*, L.O. Simpson*, G.T. Golden, and R.G. Fariello (SPON: S.J. Potolicchio). VA Medical Center, Coatesville, PA 19320 and Jefferson Medical College, Philadelphia, PA 19107.

Five cats were each stereotaxically implanted with a guide cannula into one basolateral amygdala, and with epidural and depth electrodes for long-term EEG evaluation. After 1-2 weeks HPLC-purified tetanus toxin C was infused via the cannula, and EEG and behavioral observations were made daily thereafter. Infusion effects were variable. One cat given 100 pg toxin had seizures increasing in frequency and severity for 1 week, then decreasing until the cat was seizure-free by 2 weeks after infusion. Two cats given 200 pg had no seizures for 2 weeks, but within 48 hours after a second 200 pg infusion were both in limbic status epilepticus. Two cats given 200 pg were in status within 5 days. The EEG profile of each cat was, however, stereotypic. After infusion, all cats had independent multifocal spiking bilaterally in limbic nuclei: these isolated spikes were subsequently followed by recruiting afterdischarges of variable duration localized to the infused amygdala, ipsilateral hippocampus, and/or septum in 2 cats, and to leads in the vicinity of the claustrum in 3 cats. These afterdischarges propagated over time to become generalized limbic seizures in all cats, and became tonic-clonic seizures in all but the 100 pg cat. In 2 cats the initial EEG abnormality noted was bursts of fast beta activity in the infused amygdala.

These findings are similar to those obtained after limbic application of cobalt chloride (Fariello et al., Neurosci. Abstr. 11:384.17, 1985), and indicate that this technique is a potential model of limbic epilepsy. The mechanism of action of tetanus toxin remains unclear, but we plan to more fully document the electrographic and neurochemical changes in limbic function after infusion of the toxin into this and other species. The finding of seizures originating in the claustrum and this structure's early involvement in the development of the epileptic response was unexpected and deserves further study; this may be the first evidence implicating claustrum in seizure processes.

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262.1 INTRANIGRAL GAMMA-VINYL-GABA SUPPRESSION OF ELECTROSHOCK SEIZURES: ROLE OF NORADRENERGIC NEUROTRANSMISSION. J.O. McNamara and D.W. Bonhaus. V.A. Med. CTR. and Depts of Medicine (Neurology) and Pharmacology, Duke University, Durham, NC 27705.

The substantia nigra (SN) powerfully regulates seizure activity. In most seizure models the net effect of nigral output is to facilitate seizure propagation. Microinjection of the GABA mimetics muscimol and gamma-vinyl-GABA (GVG) into SN suppress tonic hindlimb extension (THE) in the electroshock (ES) seizure model. We have shown that the anticonvulsant action of intranigral muscimol, against ES seizures, is blocked by antagonism of alpha-2 adrenergic receptors; these receptors are almost certainly post-synaptic. Together with the finding that endogenous norepinephrine (NE) suppresses ES seizures, We postulated that intranigral injection of GABA mimetics produce an anticonvulsant action by increasing NE turnover.

The objectives of this work were twofold: 1) to determine whether the anticonvulsant action of GVG can be blocked by the alpha-2 receptor antagonist idazoxan (IDX); and 2) to test the hypothesis that intranigral GVG exerts its anticonvulsant action by increasing NE turnover.

Intranigral GVG produced a 37 % decrease in THE. IDX (1 mg/kg) abolished this anticonvulsant action.

	before GVG	with GVG	
vehicle	8.1 ± 0.5	5.1 ± 1.1	p<0.05
IDX	9.1 ± 1.8	10.9 ± 1.3	ns

Values are the mean ± S.E.M. of THE in seconds.

Contrary to our hypothesis, intranigral GVG did not increase NE turnover in any of the six brain or spinal cord regions tested. Using DOPA accumulation in the presence of a DOPA decarboxylase inhibiter as an index of catecholamine turnover, we found that intranigral GVG actually decreased catecholamine turnover by 28 % in the hippocampus ($p<0.05$). Intranigral GVG did not modify NE or dopamine content in any area tested.

These results suggest that, while activation of alpha-2 receptors is essential for the anticonvulsant effect of intranigral GVG on ES seizures, the mechanism of this effect does not involve increased NE turnover. Whether intranigral GVG enhances NE neurotransmission by a mechanism other than increasing NE turnover has not been tested. An alternative hypothesis is that while the basal level of interaction of NE with alpha-2 receptors is required for detection of nigral regulation of ES evoked THE, the SN and the NE systems regulate seizure activity by independent mechanisms.

262.2 ACTIVITY OF SUBSTANTIA NIGRA PARS RETICULATA (SNPR) NEURONS DURING KINDLED SEIZURES IN FREELY MOVING RATS. R.D. Russell, D.W. Bonhaus, and J.O. McNamara. Departments of Medicine (Neurology) and Pharmacology, Duke University and VA Medical Centers, Durham, North Carolina 27710.

Kindling is an animal model of epilepsy in which periodic application of an initially subconvulsive electrical stimulus results in progressively more intense seizures. The SNPR is a crucial structure in the propagation of kindled seizures activated by limbic stimulation (McNamara et al., J. Neurosci., 1984). Prior study with paralyzed-ventilated rats found that SNPR cells in kindled, but not naive (i.e., non-kindled), rats fired in bursts of action potentials which were time-locked to spike-wave complexes of afterdischarge (AD) recorded from amygdala EEG (Bonhaus et al., J. Neurosci., 1986). The objectives of the present experiment were: 1) to extend SNPR unit analysis to kindled seizures of freely moving rats; and 2) to correlate SNPR activity to behaviors which accompany the AD.

A bipolar electrode was placed in the amygdala of Sprague-Dawley rats. Single units were recorded during AD in non-kindled rats or during an AD elicited after at least three class 5 kindled seizures had been evoked. Single unit recordings were obtained in or nearby the SNPR with either a single microelectrode mounted on a microdrive or with a five-wire bundle of microwires (i.e., 25 micron diameter, teflon insulated platinum-iridium) directed at the SNPR under stereotaxic guidance.

In the non-kindled animals (n=6), neither the rate nor pattern of SNPR cell firing changed during the AD. In contrast, in kindled rats single units were recorded before and after AD but during the AD there were phasic increases in multiple unit activity which occurred time-locked to spike-wave complexes of the AD. This "population burst firing" occurred for both SNPR (4 of 5 rats) and non-SNPR recordings (10 of 12 rats) of kindled rats. The onset of clonic motor components of the seizure and the onset of burst firing varied considerably; however, once both burst firing and clonic motor activity were established, they were time-locked and terminated together.

These data extend the results of Bonhaus et al. (1986) to a more physiological preparation. The activity of cells in the SNPR, as well as nearby cells in the reticular formation, are time-locked to spike-wave complexes of the kindled seizure. Taken together, these data indicate that burst firing of SNPR is neither necessary nor sufficient for the clonic motor seizure in amygdala kindled rats.

262.3 EVIDENCE FOR ENHANCED N-METHYL-D-ASPARTATE RECEPTOR MEDIATED INHIBITION OF CARBACHOL-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS FROM KINDLED RATS. R.A. Morrisett*, J.V. Nadler & J.O. McNamara (Spon: H. S. Swartzwelder); Duke Univ./V.A. Med. Ctr., Durham, N.C., 27705

Increasing evidence implicates NMDA (N-methyl-D-aspartate) receptor mediated processes in the development of plastic changes in neuronal activity. One example is kindling, in which repeated administration of initially subconvulsive trains of electrical stimuli eventually results in the development of intense behavioral and electrographic seizures. NMDA receptor antagonists suppress kindling development. Electrophysiologic studies of hippocampal slices from kindled animals have demonstrated two findings consistent with increased NMDA receptor mediated events: 1) iontophoresis of excitatory amino acid receptor agonists into CA1 produces greater reduction of $[Ca^{++}]_o$ in kindled slices (Wadman et al., Exp. Br. Res., 57:404(1985)); 2) synaptic activation of granule cells by perforant path stimulation is inhibited by an NMDA receptor antagonist in slices from kindled but not control animals (Mody and Heinemann, Nature 326:701 (1987)). To understand the molecular basis of these electrophysiologic findings, we have initiated studies of NMDA receptors and NMDA receptor coupled second messenger systems in kindled animals.

NMDA receptor activation potently inhibits agonist-stimulated PI hydrolysis in hippocampal slices (Baudry et al., Nature, 319:329 (1986)). We hypothesized that NMDA would inhibit carbachol-stimulated PI hydrolysis to a greater extent in slices prepared from kindled compared to control animals. Sprague-Dawley rats were implanted with a stimulating electrode in the right amygdala under Nembutal anesthesia. Five animals were stimulated twice daily until each had 4-6 stage 5 kindled seizures. Kindled animals were sacrificed 24 hours after the last seizure for determination of NMDA inhibition of carbachol-stimulated PI hydrolysis. Each experimental animal was paired with a sham-operated control. PI hydrolysis was measured by determining the synthesis of radiolabelled IP_1 using anion exchange chromatography in transverse hippocampal slices (400 µm) prelabelled with ^{3}H-inositol. NMDA (10 µM) alone did not alter basal PI hydrolysis in control or kindled animals. Carbachol-stimulation of PI hydrolysis was slightly but not significantly greater in slices prepared from kindled animals. NMDA (10 µM) inhibited carbachol-stimulation of PI hydrolysis to a greater extent in slices prepared from kindled animals (17 ± 4 % NMDA inhibition in control versus 54 ± 15% NMDA inhibition in kindled slices, $p<0.03$).

These findings provide direct biochemical evidence for enhanced NMDA receptor function in hippocampal slices of kindled animals. The molecular consequences reflected in this alteration of PI hydrolysis may underlie the enhanced NMDA receptor function measured electrophysiologically.
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262.4 BRAIN SITE OF ACTION OF CLINICALLY EFFECTIVE ANTICONVULSANTS

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The brain site(s) at which clinically useful anticonvulsants exert their therapeutic action is unknown. The substantia nigra (SN) is one site which promotes seizure propagation in animal models. Iadarola and Gale have observed that the SN is a site of anticonvulsant action of some drugs which presumably act by enhancing GABA transmission. We hypothesized that the SN is one site at which clinically used anticonvulsants exert their therapeutic actions. To test this idea, we examined the effects of microinjection of phenobarbital(PB), carbamazepine (CBZ) and phenytoin (PT) into the SN bilaterally on electroshock seizures in rats. The anticonvulsant efficacy was assessed by measuring the duration of tonic hindlimb extension (THE) before and after treatment. Intranigral application of PB suppressed THE in a dose-dependent manner. Significant ($p<0.05$, student t test) reduction of THE was observed after injection of 30 (35% reduction) or 150 (40% reduction) nmol but not after 3 (3% reduction) nmol. The magnitude of the effect with 150 nmol PB was equivalent to that obtained with 440 pmol of a GABA agonist, muscimol (47% reduction). Intranigral injection of vehicle alone did not suppress THE. The anticonvulsant effect of PB was spatially specific since THE was not suppressed after injection of 30 nmol of PB 2 mm dorsal to the SN. In contrast, neither CBZ (10 or 60 nmol) nor PT (3 or 30 nmol) modified THE. Measurement of PB and CBZ content in the SN showed that differences in concentrations in the SN did not account for the lack of efficacy of CBZ. The present findings with PB extend previous evidence that intranigral application of anticonvulsants which enhance GABA transmission suppresses seizures. This suggests that at least part of the anticonvulsant action of drugs which enhance GABA mediated transmission is exerted at the SN. By contrast, CBZ and PT, drugs postulated to block seizures by suppressing sustained repetitive firing, appear not to exert significant anticonvulsant action at the SN.

262.5 DECREASED PHOSPHORYLATION OF SYNAPTIC MEMBRANE PROTEINS ASSOCIATED WITH OLFACTORY BULB KINDLING. S. E. Tan* and R. F. Berman. Dept. of Psychology and the Neuroscience Program, Wayne State University, Detroit, Michigan 48202.

Kindling refers to the observation that low intensity, initially subseizure levels of electrical stimulation delivered to one of several brain regions gradually results in the development of a seizure focus. Kindling has been considered to be a model of both epilepsy and neuronal plasticity. The neuronal mechanisms underlying the development of kindling are currently unknown. However, the phosphorylation of specific synaptic membrane proteins has been linked with other models of synaptic plasticity (e.g., long-term potentiation), and recent studies have reported altered phosphoprotein metabolism following the development of hippocampal and amygdala kindled seizures. The present experiment examined synaptic membrane phosphoproteins isolated from olfactory bulb, frontal cortex, and hippocampus two weeks following the completion of olfactory bulb kindling in rats. Briefly, eighteen adult, male Long-Evans rats were stereotaxically implanted, unilaterally with a stimulating electrode into either the right or left olfactory bulb. After recovery from surgery the animals were divided into two groups; kindled and control (n=9 each). Animals in the kindled group were electrically stimulated once daily through the implanted electrode with a level of current initially sufficient to produce a brief (3-5 sec) afterdischarge (AD threshold). Animals were stimulated daily at the AD threshold until they reached a criterion of 3 consecutive Stage 5 kindled seizures. Control animals were not stimulated. Two weeks later all animals were decapitated, the brains rapidly removed and dissected into frontal cortex, olfactory bulbs, and hippocampus. Synaptic membranes were isolated from these brain regions, homogenized, and incubated with 10 μM (^{32}P)ATP for 2.0 min at 30 deg. C. The radiolabeled samples were then further fractionated on a 10% SDS-polyacrylamide gel. The gel was stained with Coomassie Blue, dried and placed in contact with X-ray film for autoradiography. The level of ^{32}P incorporation into synaptic membrane proteins was subsequently determined by spectrophotometric scanning of the autoradiogram. The phosphorylation of a specific phosphoprotein band at a molecular weight of approximately 45,000 was found to be significantly decreased ($p<.01$) in the frontal cortex of olfactory bulb kindled rats compared to controls. Phosphorylation of this protein band accounted for approximately 16.6% of total membrane phosphorylation in the frontal cortex of control animals, and decreased to 9.5% in kindled rats. These data support the involvement of protein phosphorylation in the development of kindled seizures. (Supported by NIH grant RR08167).

262.6 ELECTRICALLY-INDUCED STATUS EPILEPTICUS IN RATS:EFFECTS OF PARTIAL AND COMPLETE AMYGDALA KINDLING. D.D. Walczak* (SPON:C.W.Gorodetzky).Dept.Clinical Neurosciences, Burroughs Wellcome Company, Research Triangle Park,NC 27709

The characteristics of status epilepticus induced by amygdala stimulation were determined in rats that were either unkindled, partially kindled, and fully kindled. All subjects were male Hooded rats implanted with bipolar depth electrodes in the left basolateral amygdala and with bilateral extradural screw electrodes over the frontal cortex. Rats were rested one month from the last kindling stimulus before status induction. Status was induced during consecutive "induction periods" consisting of 8.0 minutes of repeated stimulus trains: 2.0 second trains of 1.0 msec biphasic pulses, 90 Hz, 700-800 μA peak-peak, 2.0 seconds on, one second off. Spontaneous EEG was recorded for 2-5 minutes after each period.

Rats were subjected to a maximum of six induction periods to produce characteristic repetitive electrographic spiking that was cyclic in frequency (electrographic status). Rats exhibiting this pattern were then observed for expression of nonconvulsive status, partial convulsive status (repeated Stage 1-2), generalized convulsive status (repeated stage 3-5), and for 24-hour survival.

Partial kindling had both a facilitatory effect on status induction and a protective effect against status-related mortality. Fully kindled rats were induced more easily than partially kindled but had a higher mortality rate at equivalent levels of status induction. Nonkindled rats were difficult to induce, but those few that exhibited convulsive status had equivalent mortality to fully kindled rats. This differential responsiveness to status induction may reflect differential involvement of limbic and extralimbic structures in partial vs complete kindling.

262.7 MORPHOLOGICAL CHANGES IN THE HIPPOCAMPAL DENTATE GYRUS ACCOMPANY KINDLING OF THE ENTORHINAL CORTEX. A.J. Cronin,* T.P. Sutula and N.L Desmond (SPON: John A. Jane). Dept. Neurosurgery, Univ. Virginia Sch. Med., Charlottesville, VA 22908 and Dept. Neurology, Univ. Wisconsin, Madison, WI 53792.

Long-term potentiation (LTP) of the synapses between entorhinal cortical (EC) axons and granule cell spines in the dentate gyrus (DG) correlates with various morphological changes suggestive of enhanced pre- and postsynaptic efficacy[1]. Kindling of the EC-DG pathway also induces potentiation of the EC-DG synapses, but synaptic potentiation induced during epileptogenesis may not be equivalent to LTP. The present study seeks to identify morphological changes of the EC-DG synapses after kindling stimulation. Previous studies have not demonstrated morphological changes after kindling stimulation of a monosynaptic pathway. Chronic stimulating and recording electrodes were implanted unilaterally in the angular bundle and DG hilus, respectively, of adult rats[2]. Here we report on a kindled group (N=5) and a sham group (N=4) of animals. Each animal in the kindled group was perfused with mixed aldehydes[1] 5 days after the 3rd class 5 seizure. Sham animals were perfused with mixed aldehydes at a survival interval matched with the kindled survival interval. Blocks from the DG were processed for electron microscopy, and montages were photographed as described previously[1]. We compared the incidence of dendritic spines connected to their parent dendrite (connected spines) for the kindling group and the sham group. Across the entire proximodistal extent of the molecular layer, kindling correlates with a 36% increase in the incidence of connected spines (sham mean 7.9±0.7%). This increase in the incidence of connected spines occurs in spite of the fact that the number of spine synapses per unit area of tissue does not increase with kindling. The increased incidence of connected spines with kindling reflects a particular subpopulation of the dendritic spines, those with concave spine heads. Note that Desmond and Levy previously[1] hypothesized that the concave spines represent the population of potentiated spines following LTP-inducing conditioning stimulation. In addition, with kindling stimulation, the heads of the concave connected spines enlarge (area increases 19%), and their spine stems increase in diameter by 27%. The spine head enlargement and stem diameter increase are consistent with previously reported correlates of LTP in the EC-DG system. These data provide evidence for morphological changes in the excitatory synapses of the monosynaptic pathway which received kindling stimulation and are consistent with the enhanced synaptic efficacy associated with kindling. Supported by NIH NS15488 to W.B Levy, NSF BNS83-176795 to O. Steward, and TIDA NS00808, the Epilepsy Fdn of America & Grass Fdn to T.P.S. [1]Desmond & Levy, J. Comp. Neurol. 253:466, 476. [2]Sutula & Steward, J. Neurophys. 56:732.

262.8 AN EXPERIMENTAL TEST OF THE GABA HYPOTHESIS OF KINDLING: GENERALIZED SEIZURES IN CORTICAL- AND AMYGDALA-PREKINDLED RATS FOLLOWING ACUTE ADMINISTRATION OF GABA-COMPLEX AND ADENOSINE ANTAGONISTS. N.S. Mingo* and W.M. Burnham. Dept. of Pharmacology, Univ. of Toronto, Canada, M5S 1A8.

The GABA hypothesis of kindling - as recently outlined by Burnham et al. - suggests that the development of secondary generalization in the kindling model is associated with a loss of GABA-mediated inhibition. If this is true, it follows that blockade of GABAergic function should produce secondary generalization even in subjects that have not been kindled.

The present study was designed to test this prediction of the hypothesis. Drugs were applied at appropriate intervals before the triggering of a focal seizure in a non-kindled animal, and electrographic and behavioural measures of secondary generalization were taken. Three different GABA complex antagonists were tested in a dose response paradigm: bicuculline, a specific antagonist for the GABA recognition site, picrotoxin, a drug that antagonizes GABA by binding to the associated chloride ionophore, and norharmane, a putative inverse agonist for the benzodiazepine receptor. In each case, the drugs were tested on both cortical and amygdala- implanted subjects, in order to assess possible differences related to the site of stimulation. For comparison, the adenosine agonist, aminophylline - already known to facilitate generalization in partially kindled subjects - was tested in the same paradigm. In line with the hypothesis, it was predicted that all GABA-complex antagonists would promote secondary generalization in prekindled rats.

Contrary to expectations, it was found that bicuculline and norharmane were ineffective at either site even at "near convulsant" doses. Picrotoxin was effective in facilitating generalization only in subjects implanted in the cortex. On the other hand, aminophylline was effective in promoting generalization in both the amygdala and cortically implanted prekindled subjects, although it was much more effective in animals with amygdala implants.

The failure of bicuculline and norharmane to produce "early trial" generalization raises serious questions about the GABA hypothesis of kindling. The contrasting actions of the different GABA complex antagonists raises corresponding questions concerning the current model of the GABA macromolecular complex. (Supported by MRC (Canada) grant #MA 5611) .

262.9 THE TEMPORAL RELATIONSHIP OF AMYGDALOID AND HIPPOCAMPAL AFTER-DISCHARGE ACTIVITY DURING AMYGDALOID KINDLING. L. J. Burdette. Department of Neurology, Graduate Hospital, Philadelphia, PA 19146

Kindling is a process that involves repeated electrical stimulation of temporal lobe structures until a generalized motor seizure is elicited. For the kindling process to occur, current intensity must trigger an electroencephalographic spike train, called the primary afterdischarge (PAD). During amygdaloid kindling, PAD duration increases and a secondary afterdischarge (SAD) frequently occurs during middle or late kindling stages. The present objective was to assess the temporal relationship of the PAD and SAD recorded from the amygdala and hippocampus during amygdaloid kindling.

Male Long Evans rats (N=23) were implanted with chronic bipolar electrodes in basolateral amygdala and dorsal hippocampus. Following recovery, amygdala leads were stimulated daily with current (2 s train, .3 ms bipolar 50 Hz pulses) that was 400% of amygdaloid PAD threshold. Behavioral seizure development was scored according to Racine (1972). The kindled state was defined by the presence of three consecutive stage 5 seizures. PAD and SAD duration were measured as the time that spike amplitude was greater than half that of the prestimulation EEG. The duration of the intervening depression in spike activity was bounded in time by the termination and initiation of the PAD and SAD, respectively. Afterdischarge variables were averaged for each seizure stage prior to analysis.

The total duration of the PAD, spike depression and SAD was significantly shorter in the hippocampus than in the amygdala only during the first kindling stage. This effect is explained by the failure to observe a PAD in 25% of the hippocampal records early in the kindling process. When the analysis was restricted to only those trials in which an amygdaloid SAD was present, the pattern of afterdischarge activity varied by recording site and kindling stage. The incidence of amygdaloid SAD peaked during the middle kindling stages and remained constant until the kindled state was reached. In contrast, the hippocampal SAD rarely accompanied the amygdaloid SAD until late in the kindling process, and then was of shorter duration. The duration of the intervening spike depression decreased and increased in the amygdala and hippocampus, respectively, with each kindling stage. PAD duration did not differ between recording sites at any time. Together, these data suggest that the appearance of the amygdaloid SAD is not due to propagation of hippocampal afterdischarge activity.

262.10 **EFFECT OF CORTICAL KINDLING ON THE FUNCTIONAL ANATOMY OF CORTICAL CIRCUITS.** E.M. Santori and R.C. Collins Neurology and Neurological Surgery Dept., Washington U.,St. Louis, MO 63110

Electrical stimulation of the anterior neocortex in rat can trigger a focal clonic motor seizure. Repeated induction of such seizures results in a permanent increase in the strength and duration of the evoked seizure response. The present study was undertaken to determine whether the kindling of forelimb cortex alters its functional anatomy.

Rats were kindled within the forelimb sensorimotor cortex with 2 second trains of 1 msec pulses delivered at 50 HZ, at currents above the afterdischarge (AD) threshold applied once every other day. Animals were sacrificed after a mean of 32 stimulations. Mean AD duration increased from 5 seconds to 27 seconds and seizure responses changed from clonic forelimb movements and upper body bobbing to include rearing, tonic muscle contractions, and loss of balance.

In control and kindled subjects the pattern of functional metabolic changes induced by stimulation of the forelimb zone was studied. [14C]-Deoxyglucose (DG) was given during repetitive train stimulation: 0.1 sec. trains applied once/ two sec. consisting of 500 HZ bipolar pulses (0.1 msec and 1mAmp). Controls responded to these trains with discrete left arm jerks. During the later half of the 45 minute DG trace, stimuli would occasionally induce brief post-stimuli forelimb tremor or rarely a generalize seizure would occur. By contrast, kindled subjects responded to this stimulation with an almost immediate generalized seizure. This was followed by a period of simple or complex forelimb jerks eventually leading to another seizure. Several such cycles occurred during the course of the trace.

There were no consistent differences between control and kindled animals in either the size or magnitude of DG labeling within the focus. In three of the five kindled subjects, [14C] concentrations within the mirror focus and bilateral substantia nigra were more than two standard deviations greater that mean concentrations of controls (n=6). In addition, temporally averaged rates of glucose utilization within the nucleus of the lateral olfactory tract, ventral pallidum, entopeduncular nucleus, posterior thalamus were more than two standard deviations greater than the mean rates for control subjects. Finally, in two kindled subjects glucose utilization within the amygdala and hippocampus was markedly enhanced.

These studies indicate that cortical kindling results in new circuits participating in the functional response to cortical stimulation. Whether these pathways play any role in the mediation of kindling remains to be explored.

262.11 **POST-KINDLING REFRACTORINESS TO THE DEVELOPMENT OF STATUS EPILEPTICUS IN JUVENILE AND ADULT RATS.** H.B. Michelson and G.G. Buterbaugh. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, Maryland 21201.

Amygdala-kindled rats pretreated with pilocarpine nitrate (20 mg/kg i.p.) will, following stimulation, show prolonged seizures lasting at least four hours. We investigated the role of kindling in the development of status epilepticus (SE) in this model.

Adult rats were kindled with daily stimulations through two consecutive Stage 5 seizures and tested 24 hours, and at weekly intervals thereafter for the development of SE following pretreatment with 20 mg/kg pilocarpine. Rats were refractory to the development of pilocarpine-facilitated SE (pfSE) during the first week after kindling. Two weeks after kindling, 50% of rats tested developed pfSE; three weeks after kindling 80% of rats tested developed SE when electrically stimulated after pilocarpine administration. Stimulation of rats pretreated with higher doses of pilocarpine will elicit SE at shorter intervals after kindling in adult rats; 100 mg/kg pilocarpine will induce SE within 24-48 hours after kindling; 50 mg/kg will elicit SE within 1-2 weeks following kindling.

Twenty-eight day old rats were kindled with hourly stimulations and similarly tested for the emergence of SE. Juvenile rats are refractory to the development of SE during the first 5-6 weeks after kindling (until approx. 65 days of age). Nearly all juvenile-kindled rats developed SE when tested 6-7 weeks after kindling. Hourly-kindled adult rats will develop pfSE within 3-4 weeks after kindling, indicating that this difference in post-kindling refractoriness to pfSE is not a result of the use of a different kindling protocol. Juvenile rats will also develop SE after administration of high doses of pilocarpine (400 mg/kg) or lithium/pilocarpine coadministration, indicating that this maturational difference in the development of pfSE is not due to an inability of young rats to support prolonged seizure activity.

These findings suggest a state of relative enhanced post-kindling inhibition which steadily decays over time. This enhanced inhibition may be comparatively greater or may take longer to dissipate in juvenile-kindled rats. (Supported by USPHS MH09301.)

262.12 **PATHOLOGY AND 2-DEOXY-D-GLUCOSE UPTAKE ASSOCIATED WITH EARLY AND LATE STATUS EPILEPTICUS IN KINDLED RATS.** G.G. Buterbaugh, H.B. Michelson, D.O. Keyser and B.R. Jones*. Depart. of Pharmacol. and Toxicol., Univ. of Maryland School of Pharmacy, Baltimore, MD 21201.

Pilocarpine-facilitated status epilepticus (pfSE) is a model of status in which the seizures evoked by amygdala stimulation of amygdala kindled rats pretreated with pilocarpine (20 mg/kg, i.p.) continue for at least 4-5 hours. Stable, bilateral, 10-12/sec, spiking EEG discharge is characteristic of the first 2-3 hours of pfSE, while increasingly more severe convulsive behavior typically begins after 2-3 hours. This study correlated 2-deoxy-D-glucose (2-DG) uptake and neuropathology with this time-course of pfSE.

Rats received 2-DG (100 uCi/kg; i.v.), were killed 45 min later and brains removed and frozen sectioned for contact autoradiography. Rats injected after 10 min of seizures (minimal partial motor convulsions) showed extensive, bilaterally symetric 2-DG uptake throughout the brain, in particular the amygdala, hippocampus, lateral septum, most thalamic nuclei, substantia nigra, medial geniculate and all cortical regions. Rats injected after the appearance of more severe convulsions (2-2.5 hours) showed comparatively less bilateral 2-DG uptake in the hippocampus, medial geniculate and cortical regions. Uptake was notably absent in the thalamus and lateral septum and limited to the medial portion of the substantia nigra.

Seizures were pharmacologically terminated in separate rats which were killed 3-4 days later for histology. One hour of seizures was associated with cell loss in the ipsilateral hippocampus (CA1) and bilateral amygdala-pyriform regions and with bilateral necrosis of the substantia nigra. In some rats, damage was limited to the amygdala-pyriform region. Two or more hours of seizures, accompanied by increasingly severe convulsive behavior, were associated with bilateral damage in CA1/CA3 (ipsilateral CA4) hippocampal and amygdala-pyriform regions and in the paraventricular thalamic nuclei and substantia nigra.

The results indicate that the emergence of more severe motor convulsions during prolonged pfSE is associated with (1) diminished 2-DG uptake and (2) more extensive cell damage. (Supported by USPHS MH09301 and NS20670.)

262.13 ALPHA-2 ADRENERGIC RECEPTORS IN THE AMYGDALA ARE TRANSIENTLY ELEVATED DURING THE EARLY STAGES OF KINDLING. M.J. Chen, A. Vigil*, C. Laubert*, D.D. Savage and G.K. Weiss*. Departments of Pharmacology and Physiology, University of New Mexico School of Medicine, Albuquerque, New Mexico, 87131.

Our laboratory has recently demonstrated that the auto-inhibitory alpha-2 adrenergic receptors on the locus coeruleus (LC) neurons increase in number in the early stages of kindling. This enhanced inhibitory effect on the noradrenergic system may be important in allowing the progressive intensification and spread of amygdala kindling seizures. The purpose of this study was to determine if autoinhibitory alpha-2 adrenergic receptors are altered in other portions of the brain during the development of kindling.

Bipolar electrodes were stereotactically implanted in the right basolateral nucleus of the amygdala. Kindling stimulations (80-300 microampere biphasic square wave pulses, for 1 msec at 60 Hz for a total train duration of 1 sec) were administered three times per day at intervals of 90 minutes. Rats were kindled to either two Class 1 or one Class 5 kindled motor seizures. Ninety minutes after the last kindling stimulation, the rats were sacrificed and the brains frozen. Eight micron coronal sections were thaw mounted onto microscope slides and processed for in vitro autoradiography. Sections were incubated with 20 nM 3H-RX781094 in the absence or presence of 1 uM phentolamine. The sections were apposed to Ultrofilm and the film exposed for 15 days. Optical density measurements of 3H-RX781094 binding were made in the lateral septum, stria terminalis, substantia inominata, central nucleus of the amygdala, dorsal hippocampal s. moleculare and para-ventricular grey using computer-assisted microdensitometry.

Specific 3H-RX781094 binding was elevated significantly in the amygdala of Class 1 kindled rats compared to control. With the exception of the stria terminalis, binding was elevated, but not significantly, in the other brain regions analyzed. By contrast, there was a slight, but insignificant, decrease in binding in the brains of rats kindled to Class 5 motor seizures. Saturation of binding studies indicated that the elevation of 3H-RX781094 binding in the amygdala after two Class 1 kindled seizures was due to an increase in the number of binding sites with no change in affinity constant. This result is similar to the elevation in 3H-RX781094 binding in the LC reported previously. We speculate that a transient increase in the number of amygdaloid inhibitory alpha-2 receptors during the early stages of kindling would promote a progressive reduction in noradrenergic inhibition of kindled seizure spread. (Supported by the Minority Biomedical Research Support Program and NIH-NS23262.)

262.14 KINDLING OF SEIZURES WITH PROCAINE. G.A. Cottrell, B.K. Doane* and H.A. Robertson. Departments of Pharmacology and Psychiatry Dalhousie University, Halifax, N.S., CANADA B3H 4H7.

Several lines of evidence suggest that systemically administered procaine HCL has a selective limbic activating effect, i.e., it preferentially activates subcortical epileptic foci in animals and man. However, it is not known whether procaine can induce an epileptic focus. The following set of experiments addresses this issue by demonstrating that seizures can be pharmacologically kindled with procaine and by characterizing the kindling.

Male Sprague-Dawley rats were injected once daily with 150 mg/kg, i.p. procaine HCL (Sigma). Initially this dose produced no overt seizure activity. After an average of 13.4 injections, the rats exhibited mild front-paw clonus with hind-paw tonus. When only one of these seizures was induced and the rats were tested 2 weeks later, poor retention was observed. Only 3 of 8 rats responded to the procaine injection with a seizure. Further injections will induce a more severe seizure response characterized by front-paw clonus alternating with myoclonus almost continually for 30 min. These seizures are quite different from electrically kindled seizures. They also appear to differ from lidocaine-kindled seizures as neither omniphagia/coprophagia nor aggression was observed. Procaine kindling to this more severe stage took an average of 19.7 injections. Following 2-3 consecutive seizures, good retention was observed. On a 2 week retest, 7/7 rats exhibited seizures. Further, seizures have now been observed after 88 drug-free days. This dose of procaine does not induce seizures in age-matched controls. A group of rats with a single recording electrode in the amygdaloid complex, were kindled with procaine. During the kindling process, the amygdala EEG showed no seizure activity. The first after-discharge was observed at the time of the first behavioral seizure.

To summarize: Repeated daily administration of procaine HCL (150 mg/kg, i.p.) results in convulsive seizure activity of increasing severity. Thus, we have shown that procaine is a pharmacological kindling agent. Further, the phenomenon appears to be 'true' kindling as it is not observed on the first injection, the response gradually becomes more severe but once developed it can be reliably elicited and it is permanent or at least long-lasting. However, it does not appear to be caused by development of an epileptic focus in the amygdala.

This work was supported by the Canadian MRC and Department of Psychiatry, Dalhousie University.

262.15 FORMATION OF ABERRANT MOSSY FIBER PROJECTION BY LIMBIC KINDLING: EVIDENCE FOR SPROUTING INDUCED DURING DEVELOPMENT OF EPILEPTIC SEIZURES T. Sutula, He X.*, C. Hurtenbach*, Depts. of Neurology and Anatomy, and Neurosciences Training Program, University of Wisconsin, Madison, WI 53792

Kindling has been proposed as a mechanism for development of epileptogenesis after focal brain lesions. Lesions of CA3 are common in epilepsy (Ammon's horn sclerosis), and result in denervation of the dentate gyrus (DG), followed by sprouting of mossy fiber axons that form synaptic connections in the inner molecular layer (Laurberg, J. Comp. Neur. 200:433, 1981; Tauck, Nadler, J. Neurosci. 5(4):1016, 1985). The sprouted mossy fibers can be identified by supragranular staining in the DG with the Timm method.

To identify any relationships between CA3 lesions, sprouting, and rate of kindling, lesions of CA3 were prepared in rats by intraventricular kainic acid and electrolytic methods. Kindling was induced by daily 60Hz perforant path stimulation in lesioned groups and a matched unlesioned control group. After three Class 5 seizures, animals were perfused and adjacent horizontal sections were stained with the Timm method, and with cresyl violet.

Sprouting and reinnervation in the DG was observed in both lesion groups, as indicated by supragranular Timm staining with neuronal loss and gliosis in CA3-4. Unexpectedly, supragranular Timm staining was also observed in 7 of 8 kindled unlesioned control animals, and in four animals kindled by stimulation of the olfactory bulb. Phase contrast microscopy revealed clusters of Timm positive granules around cell bodies and radially oriented dendritic shafts in the inner molecular layer. There was no evidence of neuronal loss in CA3-4, and the hippocampal pyramidal layer appeared intact except for mild gliosis.

The results are evidence for a previously unrecognized anatomic plasticity associated with epileptic phenomena. The anomalous supragranular Timm staining in kindled animals is most likely an aberrant mossy fiber projection, and suggests that axonal sprouting and alterations in neuronal connectivity occur during development of kindling. These anatomical alterations may contribute to the development of kindling, and could have implications for emergence of neuronal dysfunction in response to repeated seizures. The findings also raise the intriguing possibility that abnormal patterns of activity can exert a potent influence on neural connectivity. (Supported by TIDA K07-NS00808, and the Epilepsy Foundation of America).

262.16 EFFECTS OF LESIONS OF THE CEREBELLAR CORTEX ON KINDLED SEIZURE DEVELOPMENT IN THE RAT. C.D. Applegate, M. Krieger* and J.L. Burchfiel. Departments of Neurology and Neuroscience, The Children's Hospital, Boston, MA 02115.

Previous work has suggested a role for the cerebellum (CB) in the behavioral and EEG expression of seizures. In general, results indicate an involvement of the CB in seizure suppression. Thus, CB stimulation has been shown to be capable of attenuating evoked cortical afterdischarge (AD) activity. Conversely, lesions of the CB have been reported to increase seizure durations following cortical application of epileptogenic agents and to increase AD durations following amygdala kindling. Preliminary data from our laboratory suggest that large radiofrequency lesions of the pontine brainstem which extensively damage the major output pathway of CB, the superior cerebellar penduncle, eliminate the development of seizure suppression typically observed at one focus following concurrent, alternate kindling stimulation of the septal nucleus and entorhinal cortex (kindling antagonism). Our data suggest that the CB may be involved in the development of patterns of site suppression in the kindling antagonism model and support earlier data indicating a role for the CB in the suppression of brain seizure activity. In the present study we have begun to examine the effects of lesions of the CB cortex on the development and subsequent expression of kindled seizures elicited from the septal nucleus.

The lateral CB cortex was removed bilaterally through aspiration in male, rats (N=6) under deep pentobarbital anesthesia. At the time of lesioning, animals were implanted with chronic stimulating-recording electrodes into the left septal nucleus. Control animals (N=7) were implanted unilaterally with electrodes into the septal nucleus as above. Following recovery from surgery, AD thresholds were established and daily kindling stimulations were administered until fully generalized stage 5 seizures were elicited.

Lesions resulted in a >50% loss of CB cortex bilaterally. The vermis and paraflocculi were largely spared. Lesions resulted in significant increases in focal AD durations. Increases in AD durations were present at initial trials (X=29.2 vs 18.1s;$p<.05$) and continued to be significantly longer when fully generalized seizures were elicited (X=80.5 vs 55.1s;$p<.01$). The latency to motor seizure as defined by the onset of bilateral forelimb clonus was also significantly increased (X=42.4 vs 12.0s;$p<.05$) following lesions of the CB cortex. Neither the rate of kindled seizure development nor thresholds for the elicitation of AD were significantly altered by CB lesions, although there was a trend toward lower thresholds in lesioned animals ($p=0.14$). These data suggest a complex role for the cerebellum in motor seizure development and expression.

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262.17 STUDIES OF BRAIN SPECTRIN (FODRIN) IN THE HIPPOCAMPUS AND CORTEX OF RATS WITH KINDLED SEIZURES. M Pintor*, KP Wayns-Lee*, NS Nadi. (Spon: PH Sheridan). NINCDS, Bethesda, MD. 20892.

Brain spectrin (fodrin) is a 220,000 - 240,000 MW dimer which lines the cell membrane and is suggested to play an important role in the maintenance of the cytoplasmic structure. Changes in the levels of the spectrin molecule have been associated with a variety of disorders ranging from muscular dystrophy (increased spectrin) to elliptocytosis (decreased spectrin). In the excitable tissues such as chromaffin cells release of catecholamines has been associated with the breakdown of fodrin (Perrin and Aunis, Nature, 315:589, 1987). When the breakdown of fodrin was prevented by pretreating the cells with fodrin antibody, release was blocked (Perrin et al., Nature, 326:498, 1987). Because the proteases involved in the breakdown of fodrin are calcium-dependent and there is a calcium flux into the cell during the state of seizure, we hypothesized that a breakdown of fodrin may occur during convulsions thus facilitating the release of transmitters and altering membrane structures at the seizure focus. To test this hypothesis rats were kindled to stage 5 using a bipolar electrode implanted unilaterally into the amygdala. Control rats were identically implanted with an electrode but were not stimulated. The rats were killed in mid seizure after having achieved at least 5 consecutive stage 5 seizures, and the brain was rapidly frozen and dissected into several parts including the hippocampus and cortex. The brains were then homogenized and run in SDS-containing buffer and analyzed in a 7.5% acrylamide-containing gel electrophoresis system. The gels which included purified fodrin were stained with coomassie-blue and read on a Biomedical Instruments densitometer. In the kindled rat hippocampus fodrin found in the 220,000 -240,000 MW range was decreased when compared with the sham operated rats. The cortex, on the other hand, showed an increase in the quantity of fodrin molecules in the kindled rat when compared with the sham operated rats. The decrease in the number of fodrin molecules in the hippocampus may imply an involvement of fodrin in the seizure process. The localization of this change and its involvement in transmitter interactions will be discussed.

262.18 RESPONSE PROPERTIES OF RAPIDLY RECURRING HIPPOCAMPAL SEIZURES. E.W. Lothman, J.B. Perlin, R. Salerno, Dept. Neurology, Univ. Virginia, Charlottesville, VA 22908.

Repetition of certain stimuli leads to a progressive enhancement of seizure responses (kindling) until a stereotyped convulsion occurs with each stimulus. The final kindled state, a model of chronic focal epilepsy, is typically achieved and studied with stimuli given many hours apart. With this standard approach, it takes days to weeks to reach a kindled state. Previously we have shown that rapidly recurring hippocampal seizures (RRHS) cause a kindling response over hours (Br. Res. 360:83, '85). We report here the epileptic responses to RRHS after completion of rapid kindling.

Adult albino rats stereotactically implanted with bipolar electrodes in the hippocampus were rapidly kindled with supramaximal tetani every 5 min for 6 hr. On alternate days thereafter, 14 afterdischarges were elicited at 30 min intervals. The first and last were produced by a series of tetani (50 Hz, 1 msec biphasic pulses for 10 sec) of increasing current until afterdischarge (ICTAD stimuli); the others were provoked with the same tetanus at current intensities suprathreshold for after-discharge production (STC stimuli). Behavioral seizure scores (BSS) and afterdischarge durations (ADD) were assessed according to convention and 4 STC responses were blocked together for analysis. For the first week kindled responses were seen, but BSS and ADD showed variability. Thereafter, responses stabilized so that kindled BSS and ADD responses were constant within and among separate test days. Afterdischarge thresholds (ADT) were stable throughout the experiment. This established the basic test protocol. Interposing blocks of 4 ICTAD stimuli in the test period increased the variability of BSS and ADD, but ADT remained unchanged. When the number of STC stimuli given on a test day was increased, mean BSS and ADD fell after 18 hours and ADT rose. Upon resumption of the basic test protocol, BSS, ADD, and ADT returned to stable, baseline kindled values over the next several days. Using the basic test protocol, but on consecutive days, BSS and ADD were lowered after 4-5 days. Returning to alternate test days normalized responses within a week.

These experiments show that with appropriate stimuli a kindled state can be established quickly with RRHS and that, after this, multiple stable responses can be elicited at a rate of every 30 minutes. With the basic test protocol the effect of various experimental manipulations can readily be studied, including their time course. However, even with the supramaximal stimuli used, repetitive seizures can eventually result in postictal "inhibitory" processes that can persist for several days.

262.19 RAPID KINDLING IN THE IMMATURE BRAIN. James L. Thompson and Gregory L. Holmes, Dept. of Neurology, Medical College of Georgia, Veterans Medical Center, Augusta, GA 30912-2366.

Kindling, the phenomenon whereby repeated administration of an initially subsconvulsive electrical stimulus results in progressive intensification of seizure activity culminating in a distinctive convulsion, is a widely used animal model of partial seizures. In immature rats the typical kindling paradigm used has been 1 sec., 60 Hz stimulations with a biphasic waveform of intensity sufficient to induce an electrical afterdischarge. Inter-stimulus intervals of 15 minutes to 24 hours are used. Lothman et al. (Brain Res. 360:83, 1985) reported that in mature animals, 10 sec. trains of low-frequency suprathreshold stimulations resulted in kindling in 2-3 hours. The purpose of this study was to determine whether rapid kindling could be achieved in immature animals using low frequency, long duration stimulations with a short inter-stimulus interval.

Bipolar electrodes, stereotaxically implanted in the hippocampus (CA3 region) or the amygdala of immature rats, were used to deliver 1 or 10 sec trains of suprathreshold (1000 μA) stimulations at frequencies of 10 or 60 Hz. The stimuli were administered every 5 minutes. Rapid kindling was achieved with 10 second but not 1 second stimulation with both 10 and 60 Hz frequencies. Ten second stimulations with a 60 Hz frequency resulted in more rapid kindling (mean number of stimulations to stage 5 seizures = 8.88±1.17) than 10 second stimulations with a 10 Hz frequency (mean = 14.93±1.54) ($t = 3.06$, $p<0.01$). When 10 sec., 10 Hz stimulations were administered hourly kindling also occurred although the animals required statistically more stimulations to reach their first stage 5 seizure (mean = 22.17±1.94) than in the animals stimulated every 5 minutes (mean = 14.93±1.54) ($t = 2.92$, $p<0.01$). The rate of kindling was similar in animals with hippocampal and amygdala electrodes. This study demonstrates that rapid kindling can be achieved with long duration stimulations and short interstimulus intervals in the immature animal.

262.20 CHRONIC CARBAMAZEPINE INHIBITS THE DEVELOPMENT OF COCAINE-KINDLED SEIZURES. S.R.B. Weiss*, M. Costello*, R. Woodward*, D. J. Nutt* and R.M. Post. BPB, NIMH, LCS, NIAAA, Bethesda, MD 20892.

Carbamazepine has been used to treat seizure disorders, paroxysmal pain syndromes and, more recently, manic-depressive illness. Its potency appears greatest for limbic system seizures but depends on the stage of development and the etiology of the seizure. Cocaine possesses both psychomotor stimulant and local anesthetic properties. In high doses, repeated administration produces increased motor responses (behavioral sensitization) as well as a convulsive response (pharmacologic kindling). Since the development of lidocaine-kindled seizures is blocked by carbamazepine, we evaluated the effects of carbamazepine on the development of cocaine-kindled seizures and stereotypic behavior, and on acute, high-dose cocaine seizures.

Rats were placed on a control or carbamazepine-containing diet for four days prior to the studies and were kept on these diets throughout the period of cocaine injections. In separate studies cocaine was injected once-daily at doses of 65 or 40 mg/kg and the animals were observed for one-half hour following treatment for stereotypy (40 mg/kg only), seizures, and death. By the third day of the experiment cocaine (65 mg/kg) produced seizures and deaths in all rats (N=15) on the control diet. In contrast, 12/15 rats treated with carbamazepine survived for three days and 7 survived nine days of cocaine injections.

At the 40 mg/kg dose of cocaine, carbamazepine markedly inhibited the development of cocaine-kindled seizures and lethality. While 80% (16/20) of control rats ultimately had seizures and 50% died, only 25% (5/20) of the carbamazepine-treated rats had seizures and only one animal died (5%) over the course of sixteen days of cocaine injections. Carbamazepine reduced the intensity of the stereotypy, but did not attenuate the overall pattern of sensitization of the response.

Carbamazepine (15, 25 or 50 mg/kg, i.p.), administered acutely, did not suppress seizures induced by high-dose cocaine (65 mg/kg), and lethality may have been potentiated. However, chronic pretreatment with carbamazepine (4 days) did decrease acute cocaine-induced seizures and associated lethality.

In summary, chronic dietary carbamazepine inhibits the development of lidocaine- and cocaine-kindled seizures and lethality. Chronic, but not acute pretreatment with carbamazepine inhibits high-dose cocaine seizures. The cocaine and the lidocaine findings suggest important interactions of carbamazepine with local anesthetic mechanisms mediating the progressive development of seizures. Effects at the type II sodium channels (batrachotoxin-sensitive) should be further explored since both carbamazepine and the local anesthetics potently interact at this site.

263.1 IMMUNOHISTOCHEMICAL LOCALIZATION OF BENZODIAZEPINE/$GABA_A$ RECEPTORS IN THE HUMAN AMYGDALOID COMPLEX. C.R. Houser, R.W. Olsen, J.G. Richards and H. Möhler*. VA Medical Center, West Los Angeles; Brain Research Institute, UCLA, Los Angeles, CA 90024; and Research Department, F. Hoffmann La-Roche, CH-4002 Basel, Switzerland.

Monoclonal antibodies to a purified $GABA_A$/benzodiazepine receptor complex from bovine cerebral cortex (Schoch, P. et al., Nature 314:168, 1985) have been used to determine the normal localization of this receptor in immunohistochemical preparations of the human amygdaloid complex. Within the basolateral group of amygdaloid nuclei, the densities of immunolabeling ranged from high to low. High levels of labeling were observed in the lateral amygdaloid nucleus, and this was the most heavily labeled nucleus within the entire amygdaloid complex. Moderate densities of labeling were present within the accessory basal nucleus. In contrast, the basolateral nucleus contained relatively low densities of receptor labeling. Within the corticomedial group of amygdaloid nuclei, the levels of staining were moderately high in the plexiform layer of the cortical nucleus but were moderate to low in other nuclei of this complex including the medial and central nuclei. The lowest levels of labeling were observed in the cortico-amygdaloid transition area that intervenes between the pyriform cortex and the amygdaloid nuclei.

The cellular localization of the benzodiazepine/$GABA_A$ receptor also varied among the different nuclei. In regions with high densities of receptor labeling, such as the lateral nucleus, immunoreaction product was distributed diffusely throughout the neuropil and could not be localized to specific cellular elements. However, in more lightly stained regions, labeled neuronal elements could be clearly distinguished. For example, in the basolateral nucleus, large multipolar neurons with long dendritic processes were outlined by reaction product. Similarly in the cortico-amygdaloid transition zone, neurons with elongated cell bodies and dendrites extending from both poles of the neuron were prominently labeled. In the anterior and central nuclei, many dendrities were enveloped with reaction product and merged with similarly labeled processes within the substriatal gray.

These findings indicate that different nuclei of the human amygdaloid complex have distinctive patterns of benzodiazepine/$GABA_A$ receptor localization. Knowledge of these patterns can now be utilized in studies of receptor localization in patients with neurological disorders that may involve the amygdala such as temporal lobe epilepsy. Supported by VA Medical Research Funds and NIH Grants NS21908 and NS22071.

263.2 AUTORADIOGRAPHIC LOCALIZATION OF GABA-A AND M1 MUSCARINIC RECEPTORS IN DEAFFERENTED PREPIRIFORM CORTEX OF RAT. A. P. Thomas*[1] and L. E. Westrum[2]. (SPON: P. D. Swanson). Depts. of Neurological Surgery[1,2] and Biological Structure[2], Univ. of Washington, Seattle, WA 98195.

The distribution of benzodiazepine (BZD) binding sites (which are complexed with the GABA-A receptor) and M1 muscarinic cholinergic receptors in the prepiriform cortex (PC) of deafferented rats were studied by in vitro autoradiography, using 3H flunitrazepam (FNZP), and 3H quinuclidinyl benzilate (QNB) respectively. The PC of Sprague-Dawley rats was deafferented by unilateral ablation of the olfactory bulb (OB) on the day of birth (PNO) or in adulthood. Deafferented rats were examined at 22 or 16 weeks survival respectively. Cryostat sections (10 μm) were incubated in 3H FNZP (3nM) with or without unlabeled diazepam, and adjacent sections in 3H QNB (3nM) with or without unlabeled atropine. This was followed by preparation for autoradiography using LKB Ultrofilm. The films were exposed for 3 or 4 weeks, and the resultant autoradiograms were referenced to co-exposed brain mash standards, and optical densities (OD) were measured using a computer-assisted Drexel-DUMAS densitometer. Adult deafferented animals showed significant increases in OD of BZD receptor binding in the superficial layer of PC ipsilateral to the lesion as compared to the control side. In PNO deafferented subjects the difference was not as pronounced as in adults. The adult deafferented subjects demonstrated very highly significant increases in M1 muscarinic receptor binding, while changes in PNO-operated ones appeared to be very subtle. This increase in muscarinic receptors may indicate an enhanced activity of subcortical cholinergic afferents to PC, to compensate for the reduced sensory input with OB removal, or increased concentrations of receptor proteins resulting from heightened activity of intracortical association systems that generate excitatory synaptic potentials. In neonates the deprivation of primary sensory input during the critical period of muscarinic and GABAergic synaptogenesis in layer 1 of PC may have influenced the observed change in these receptor densities. The increase in BZD receptor binding in adult deafferented subjects may be indicative of: a) compensatory proliferation of GABA contacts onto pyramidal cells from GABA cells; b) effects of shrinkage resulting from the OB removal; c) possibly some of the glial fraction of GABA showing some binding in layer 1. Thus GABAergic and muscarinic receptor bindings not only are affected by deafferentation, but are age-dependent.

(Supported by NIH Grants NS07144, NS20482, and NS09678. LEW is an affiliate of the CDMRC, Univ. of Washington.)

263.3 ORGANIZATION OF THE GABAergic SYSTEM IN THE RAT HIPPOCAMPUS. W. Woodson*, L. Nitecka* and Y. Ben-Ari (SPON: N. Lugo-Garcia). INSERM U-029, Hopital de Port-Royal, 123 Boulevard de Port-Royal, 75014 Paris, France.

Specific antibodies against GABA were used to study the GABAergic system in the rat hippocampus. Both the number of GABA-Like (Li) immunoreactive somata and neuropil density was assessed in semi-thin sections. Cell counts revealed that 9% of the hippocampal neuronal population show GABA-Li immunoreactivity.

Each laminar region had a characteristic organization of GABA-Li elememts. In the Ammon's horn 80 to 95% of the neuronal somata within the apical and basal dendritic regions were GABA-Li postive. Within the pyramidal cell layer 5 to 8% of the cells were GABA-Li in the CA1 to CA3 sectors of Ammon's horn and only 3% were postive within that portion of the pyramidal cell layer which inserts into the hilus. Only slight differences were observed in the density of the GABA-Li neuropil within the CA1 to CA3 dendritic regions. A dense band of GABA-Li label was found in the stratum lucidum associated with many postive cells. Counts of immunoreactive grains localized on the perimeter of pyramidal (CA1 to CA3) and granule somata revealed more terminal boutons on the CA3 cells as compared to CA1 and granule neuronal somata.

A clear topographical distribution of GABA-Li somata and neuropil was found in the fascia dentata: There the label particularly concentrated in its suprapyramidal and rostro-lateral portions. Approximately 40% of neurons in the molecular layer, 60% in the polymorph layer, and 18% within the hilar region proper showed GABA-Li immunoreactivity. Within the granule cell layer only 2% of the neurons were GABA-Li postive. Distinct differences in the density of the GABA-Li neuropil were present in the molecular, polymorph and hilar regions of the fascia dentata.

While the morphology of GABA-Li neuronal somata varied according to their laminar region the most heterogeneous cell types were found in the inferior region of the hippocampus.

These data demonstrate a clear topography in the distribution of GABA-Li in the hippocampus. While little differences in both the percentage of somata and neuropil density were found in the Ammon's horn, distinct variations were observed in the fascia dentata. Moreover, these results suggest the presence of a unique GABAergic plexus aligned along the mossy fibers.

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263.4 **DUAL ULTRASTRUCTURAL LOCALIZATION OF TYROSINE HYDROXYLASE AND GABA IN RAT NUCLEUS ACCUMBENS: PRE- AND POSTSYNAPTIC INTERACTIONS BETWEEN DOPAMINE AND AMINO ACID TRANSMITTERS.** V.M. Pickel, A.C. Towle, T.H. Joh and J. Chan, Divs. of Neurobiology and Molecular Biology, Cornell Univ. Med. Coll., New York, NY 10021.

Cellular substrates for known physiological interactions between dopaminergic afferents and neurons containing gamma-aminobutyric acid (GABA) were examined in the rat nucleus accumbens. Peroxidase-antiperoxidase labeling for GABA and immunoautoradiographic labeling for the catecholamine synthesizing enzyme, tyrosine hydroxylase (TH) were differentially identified within single sections examined by light and electron microscopy. The GABA-like immunoreactivity (LI) was seen in two distinct types of medium-sized (10-20 μm) perikarya. The first, characteristically spiny type, showed low intensity GABA-LI; whereas, the second, morphologically characterized as aspiny, showed more intense immunoreactivity. The GABA-LI was also seen in proximal dendrites, axons, axon terminals, and glia.

In dual labeled sections, terminals showing GABA-LI and immunoreactivity for TH exclusively formed symmetric synapses or appositions characterized by equally spaced, non-electron dense membranes. The two types of terminals differed in that the TH-labeled terminals were smaller (mean diameter 0.3 versus 0.6 μm) and less frequently detected (138 versus 300 terminals in an area of 2,400 um^2). The TH-labeled terminals also more frequently formed junctions with the soma (4% versus 1%) and proximal dendrites (14% versus 6%) of GABAergic neurons, and less frequently (8% versus 24%) formed junctions with unlabeled proximal dendrites. However, in some cases, both types of labeled terminals formed synapses on common proximal dendrites that were either with or without GABA-LI. They also formed junctions with common or separate unlabeled dendritic spines. Presynaptic interactions were seen between the TH- and GABA-labeled terminals and unlabeled terminals exhibiting asymmetric, typically excitatory, synapses on the common spine. We conclude that GABAergic and TH-labeled (principally dopaminergic) terminals have certain common post-synaptic sites of termination on soma and proximal dendrites, with GABA being the principal inhibitor of non-GABAergic neurons and dopamine interacting primarily with the GABAergic neurons. Furthermore, GABA and dopamine may selectively modify the output from the nucleus accumbens through convergence on common dendritic spines of known projection neurons or through axonic interactions with each other or with excitatory afferents.

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263.5 IN VIVO IMAGING OF RAT C6 GLIOMAS BY [125-I] IODINATED BENZODIAZEPINES AND ISOQUINOLINES. B.J. Ciliax, S. Starosta-Rubinstein, D.M. Wieland, M.E. Van Dort, D.L. Gildersleeve, J.B. Penney and A.B. Young. Depts. of Pharmacology, Neurology, and Internal Medicine. University of Michigan, Ann Arbor, MI 48104.

The C6 rat glioma cell line has very high concentrations of the peripheral benzodiazepine binding site (PBS). The function of this site is unknown but it is distinct from the well known central/neuronal benzodiazepine receptor (CNR), which is coupled to the GABA-A receptor/Cl^- channel complex. The two benzodiazepine (BDZ) binding sites can be distinguished pharmacologically: diazepam and flunitrazepam bind to both sites, clonazepam binds only to the CNR, while Ro 5-4864 and the isoquinoline compound, PK 11195, bind only to the PBS. Intracranial C6 gliomas can be imaged in vitro and in vivo by PBS radioligand binding (Starosta-Rubinstein et al. PNAS 84:891, 1987). Several human tumors (two high grade glioma, one breast, one kidney, and one meningeal tumor) grown in athymic rodents also had high concentrations of the PBS. Thus, these tumor types might be imaged via positron emission tomography (PET) or single photon emission computed tomography (SPECT) if the appropriate ligands were available. To this end, we have synthesized and tested several iodinated [125-I]-labelled BDZ analogues ultimately for use in SPECT imaging in our rat C6 glioma model.

We examined [125-I]-7-iodo-diazepam, [125-I]-7-iodo-Ro 5-4864, [125-I]-4'-iodo-Ro 5-4864, and [125-I]-2'-iodo-PK 11195 for tumor imaging. Wistar rats were injected intracerebrally with 5 µl of a C6 glioma cell suspension. After two weeks, the animals were catheterized for i.v. injections of [125-I]-labelled compounds (400 µCi/250g body wt). (Animals receiving the diazepam analog were predosed with 5 mg/kg clonazepam to block binding to CNR). All compounds imaged tumor tissue, selectively binding to the PBS, with little background binding to normal brain. However, the time course for optimal signal-to-noise ratios differed substantially for each drug. For example, [125-I]-7-iodo-diazepam had its best in vivo binding ratio (tumor to brain) early (10 min post injection) while [125-I]-4'-iodo-Ro 5-4864 required approximately two hours to achieve optimal binding. The differences in the binding kinetics could be very important when selecting a ligand for human use in SPECT.

Supported by USPHS grant NS 15655.

263.6 AUTORADIOGRAPHIC EVIDENCE FOR POSTSYNAPTIC GABA-B RECEPTORS IN RAT STRIATUM AND HIPPOCAMPUS. D.C.M. Chu, J.B. Penney and A.B. Young. Neuroscience Program and Dept. of Neurology, University of Michigan, Ann Arbor, MI 48104.

Lesion techniques were combined with [3H]GABA quantitative autoradiography to examine the hypothesis that GABA-B receptors exist on presynaptic terminals of certain pathways where baclofen has been shown to block synaptic release of glutamate. We performed lesions in anesthetized rats of both pre- and postsynaptic elements of two glutamate pathways which are affected by baclofen: the corticostriatal pathway and the perforant pathway. The presynaptic corticostriatal pathway was disrupted by aspiration of frontoparietal cortex; intrinsic striatal neurons were destroyed by intrastriatal injection of ibotenic acid (126 nmol in 1.0 µl). Presynaptic input from the entorhinal cortex was disrupted by knife-cut lesions of the angular bundle; destruction of postsynaptic dentate granule cells was achieved with the selective toxin colchicine (2 injections, each 8 nmol in 0.7 µl). GABA-B and GABA-A binding in the striatum were evaluated one month after lesioning by quantitative autoradiography using [3H]GABA. All side-to-side comparisons were made by two-tailed paired Student's t-test.

There were no changes in striatal GABA-B nor GABA-A receptor numbers or affinities following unilateral decortication (n=7). However, destruction of striatal neurons with ibotenic acid (n=9) led to dramatic decline in the number of both GABA receptor types with no significant change in affinity. Four and 30 days after unilateral entorhinal knife-cuts (each n=6), GABA-B receptor numbers were significantly increased by 20-40% in dentate gyrus molecular layer ipsilateral to the lesion. This suggested that GABA-B receptors upregulate following removal of excitatory afferents. Conversely, unilateral colchicine lesions (n=12) of dentate granule cells led to total loss of both types of GABA receptors in the molecular layer of dentate gyrus two weeks later. It appears that GABA-B receptors are not on presynaptic nerve terminals of these glutamate pathways, but may have a postsynaptic neuronal localization. Upregulation of GABA-B receptors following removal of entorhinal inputs suggests that these excitatory inputs synapse directly on GABAergic neurons in the dentate gyrus and may mediate feed-forward inhibition of granule cells.

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263.7 MULTIPLICITY OF BRAIN L-GLUTAMATE DECARBOXYLASE AND CHOLINE ACETYLTRANSFERASE. J.-Y. Wu, J.Y. Liu*, D.M. Evans*, H.S. Lin* and C.T. Lin. Dept. of Physiol., Penn State Univ. Col. of Med., Hershey, PA 17033.

It is generally accepted that the level of neurotransmitters such as GABA and acetylcholine (Ach) is normally governed by their synthetic enzymes e.g. L-glutamate decarboxylase (GAD) and choline acetyltransferase (CAT), respectively. Multiple forms of GAD differing in molecular weight [WU, SU, Lam, Brandon and Denner, Brain Res. Bull. 5 (Supp. 2) 63-70 (1980)], in affinity for pyridoxal phosphate [Denner and Wu, J. Neurochem. 44, 957-965 (1985)], in subunit structure [Denner, Wei, Lin, Lin and Wu, Proc. Natl. Acad. Sci. 84, 668-672 (1987)] and in hydrophobic properties [Spink, Wu and Martin, J. Neurochem. 40, 1113-1119 (1983)] have been reported. With the use of monoclonal antibody against GAD, we were able to obtain two distinct forms of GAD from rat brain. One appeared to have two identical subunits with a molecular weight of about 67,000-dalton and the other one appeared to have two subunits with a molecular weight of 40,000- and 80,000-dalton. The immunoaffinity purified GAD still retained GAD enzyme activity and GAD immunoreactivity as shown in the immunodot and immunoblot tests. In addition to GAD, we have also identified multiple forms of CAT in the rat brain. Two distinct forms of CAT were obtained by CM-cellulose column. One was eluted at relatively low ionic strength, about 30 mM potassium phosphate buffer, pH 7.2 containing 1 mM β-mercaptoethanol and 1 mM EDTA. The other one was eluted at much higher ionic strength, about 150 mM potassium phosphate containing the same amount of protectors. These two forms of CAT could be further distinguished by immunoblot test using monoclonal antibody against CAT. The former showed a single protein band corresponding to a molecular weight of 68,000-dalton, whereas, the latter showed two protein bands corresponding to lower molecular weight, 55,000- and 60,000-dalton in addition to the 68,000-dalton protein band. The 68,000-dalton form has been purified by immunoaffinity and the purified preparation still retained CAT enzyme activity and CAT immunoreactivity. We are currently using the same approach to purify the second form of CAT. Eventually, we plan to localize various forms of GAD and CAT at cellular and subcellular levels so that their functions, particularly those related to the regulation of GABA and Ach levels can be analyzed. (Supported in part by grants from NIH, #NS20978, #EY05385 and #NS20922.)

263.8 IMMUNOCYTOCHEMICAL LOCALIZATION OF L-GLUTAMATE DECARBOXYLASE AND CATECHOLAMINE-SYNTHESIZING ENZYMES IN THE RETROPERITONEAL SYMPATHETIC TISSUE OF NEWBORN RAT. L.Eränkö 1, M.Ahonen1, O.Häppölä 1, T.H. Joh 2 and J.-Y.Wu 3. 1Dep. of Anatomy, Univ. of Helsinki, Siltavuorenpenger 20, 00170 Helsinki, Finland, 2Lab. of Neurobiology, Dep. of Neurology, Cornell Univ. Med. College, New York, NY 10021, U.S.A., 3Dep. of Physiology, Pennsylvania State Univ., Milton S. Hershey Med. Center, Hershey, PA 170033, U.S.A.

All sympathetic cells, i.e., sympathetic ganglion cells, adrenal medullary cells and paraganglionic cells use catecolamine as neurotransmitter and/or hormone. The main retroperitoneal paraganglion of newborn rat consists of several kinds of catecholamine containing cells: 1) small, intensely tyrosine hydroxylase (TH) immunoreactive (paraganglion-type) cells, 2) larger, moderately TH-reactive (neuron-like) cells, 3) some small intensely TH- and phenylethanolamine N-methyltransferase (PNMT)-reactive cells and 4) some TH-negative cells. In addition to catecholamines, other neuroactive substances have been localized in sympathetic cells. γ-aminobutyric acid (GABA), which is known to function as inhibitory neurotransmitter in the mammalian central nervous system, has also been identified in mammalian peripheral tissues. L-glutamate decarboxylase (GAD) is the enzyme synthesizing GABA from glutamate. GAD has been shown to be present in chromaffin cells of the adrenal medulla, which also contain catecholamines. In this study, indirect immunofluorescence method and a specific GAD antiserum were used to localize GAD in newborn rat retroperitoneal sympathetic tissue.

The retroperitoneal paraganglia and the adrenal medullas of newborn rats were immersion fixed in 4% paraformaldehyde, pH 7.3, for two hours and indirect immunofluorescence method was applied using antisera to TH, PNMT and GAD. To study coexistence of GAD with catechoamines, consecutive sections and the method of Tramu et al. (1978) were used.

In the main retroperitoneal paraganglion all small intensely TH-reactive cells were also intensely reactive to GAD. Larger, neuron-like cells were moderately reactive to TH but non-reactive to GAD. PNMT immunoreactivity was localized in a small number of cranially situated small intensely TH- and GAD-positive cells. In the adrenal medulla a large number of chromaffin cells showed intense immunoreactivity to TH and they all were PNMT- and GAD-reactive, too. A subpopulation of chromaffin cells were only moderately TH-reactive and non-reactive to PNMT and GAD.

The results suggest that in the retroperitoneal paraganglion of newborn rat the small noradrenaline containing cells are also GAD-immunoreactive. Adrenaline containing cells in the paraganglion and in the adrenal medulla are GAD-positive. In addition to adrenal medulla, rat retroperitoneal paraganglionic tissue contains cells in which catecholamine-synthesizing enzymes and GAD are immunocytochemically colocalized.

263.9 EXPRESSION OF GLUTAMATE DECARBOXYLASE mRNA BY NEURONS OF THE CEREBRAL CORTEX. D.L. Benson*, P. Isackson, S.H.C. Hendry, E.G. Jones and A.J. Tobin. (SPON: R.S. Morrison). Departments of Anatomy and Neurobiology, and Biological Chemistry, University of California, Irvine and Department of Biology, University of California, Los Angeles.

A plasmid was constructed for the preparation of glutamic acid decarboxylase (GAD) RNA probes by inserting the 2.4 Kb EcoRI fragment of cat GAD cDNA (Kaufman et al., Science 232: 1138, 1986) into the EcoRI site of pBS (Stratagene). The resultant plasmids, pBS GAD-1 and pBS GAD-2, contain the GAD cDNA in opposite orientations flanked by the T_3 and T_7 RNA polymerase promoters. A 300 base RNA antisense probe has been prepared by transcription of Hind III linearized pBS GAD-1 with T_7 RNA polymerase. This probe, labeled with α^{35}S-thio-UTP, has been used for in situ hybridization experiments. Northern blot analysis shows that this probe, as well as a 2.4 Kbase antisense probe, reacts specifically and equally well with GAD mRNA from mouse, cat and monkey brain poly A^+ RNA preparations under the same hybridization conditions (65°C with 50% formamide) being used for in situ localization.

Autoradiographs from sections of fresh frozen cat and monkey cerebral cortex show hybridization of the GAD RNA probe to neurons in all layers of the sensory-motor and visual cortices. The same proportions of cortical neurons show hybridization as those stained immunocytochemically for GAD or gamma aminobutyric acid (GABA) and similar populations of large and small non-pyramidal neurons show hybridization as those stained immunocytochemically.

In situ hybridization of the GAD RNA probe is now being applied to the visual cortex of monocularly deprived animals in order to determine if activity dependent changes in GAD and GABA levels result from changes at the transcriptional level.

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263.10 AUTOANTIBODIES TO GLUTAMIC ACID DECARBOXYLASE IN A PATIENT WITH STIFF-MAN SYNDROME AND TYPE I DIABETES MELLITUS. F. Folli*, M. Solimena+,**, S. Denis-Donini+, G.C. Comi#, G. Pozza*,#, A. Vicari* and P. De Camilli+. *Clinica Medica VII and #Clinica Neurologica of the "Istitute S. Raffaele"; +Dept. of Medical Pharmacology, Univ. of Milano, 20129, Milano, Italy.

Stiff-man syndrome (SMS) is a rare CNS disorder of unknown etiology characterized by spontaneous muscle rigidity and painful spasms due to a simultaneous activation of agonist and antagonist muscles. A case of stiff-man syndrome associated with epilepsy and diabetes mellitus is described.

The patient is a 45 year-old woman whose HLA phenotype is: A 1/28, B 8/44, Cw 5/x and DR 3/4. DR 3 and DR 4 antigens are known to be associated with an increased incidence of autoimmune diseases. The diabetes is a type 1B diabetes (autoimmune diabetes) (Bottazzo, G.F. et al., unpublished observations). IgGs oligoclonal bands are present in the cerebrospinal fluid (CSF). These findings prompted us to search for signs of autoimmunity against the CNS. To do so we tested the serum and the CSF of the patient for the presence of antibodies directed against nervous system antigens both by light microscopy immunocytochemistry and by Western blotting. Both the serum and the CSF produced an identical prominent immunostaining of gray matter regions in sections of rat and human brains, and of a subpopulation of nerve cells in primary neuronal cultures from mouse brain. They also produced an identical staining of rat pancreatic islets. Double-immunofluorescence experiments revealed that in all cases the staining was identical to that produced by antibodies to glutamic-acid decarboxylase (GAD) (gift of Oertel and co-workers). High concentrations of both GAD and GABA in pancreatic β-cells had been previously reported (some evidence indicates that GABA may play a local regulatory function in the endocrine pancreas). Western blots of proteins from total homogenates of rat and human brain indicated that the major antigen recognized by both the serum and the CSF antibodies is a protein comigrating with GAD.

On the basis of pharmacological and clinical studies, it has been suggested that SMS is due to a disorder of GABA-ergic neurons. An abnormal function of GABA-ergic systems has also been implicated in at least some types of epilepsy. Although autoantibodies directed against GAD are probably not pathogenic since GAD is an intracellular antigen, our findings raise the possibility of a common primary autoimmune pathogenesis in a syndrome which appears to involve GABA-ergic neurons and GABA-ergic neuroendocrine cells. (Supported in part by an MDA grant and by grants from the Italian Ministry of Education to PDC).

263.11 DISTRIBUTION OF GAMMA-AMINOBUTYRIC ACID-POSITIVE NEURONS AND ACETYLCHOLINESTERASE IN THE MONKEY THALAMIC INTRALAMINAR NUCLEI. C.A. Hunt and E.G. Jones. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

To gain more knowledge about the organization of the primate intralaminar complex, we studied the precise distribution of gamma-aminobutyric acid (GABA)-immunoreactive neurons and acetylcholinesterase (AChE) in the monkey. Three adult Macaca fascicularis were perfused transcardially with either 10% formalin and 0.05% glutaraldehyde (one monkey) or with 2.5% paraformaldehyde and 0.25% glutaraldehyde (two monkeys). Frozen sections were stained immunocytochemically for GABA using a rabbit antiserum and the avidin-biotin-peroxidase technique. Adjacent sections were stained histochemically for AChE. Monkeys were not pretreated with colchicine. The distribution of GABAergic neurons and of AChE staining were plotted onto camera lucida drawings of frontal sections through the thalamus.

GABAergic neurons were present in all nuclei of the intralaminar complex. They were uniformly distributed in the central medial, rhomboid, centre médian and parafascicular nuclei. The latter two nuclei, particularly the centre médian nucleus, contained considerably fewer GABAergic neurons than neighboring thalamic nuclei. GABA-immunoreactive neurons in the paracentral and central lateral nuclei tended to be distributed in small clusters interspersed among the fibers of the internal medullary lamina.

The intralaminar nuclei could be clearly distinguished from adjacent nuclei by the presence of intense AChE staining. No AChE-positive somata were seen. The pattern of AChE staining varied somewhat among individual nuclei, in a manner approximately parallel to the distribution of GABAergic neurons. The centre médian nucleus contained the least intense AChE reactivity, and AChE staining in the paracentral and central lateral nuclei had a patchy appearance. Areas of darker staining could sometimes be superimposed upon clusters of GABAergic neurons.

These data show that the intralaminar nuclei of the monkey, like other thalamic nuclei, contain a discrete population of GABAergic neurons, and that the intralaminar nuclear complex can be characterized by a high level of acetylcholinesterase staining, which is distributed in a pattern similar to that of the GABAergic neurons.

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263.12 DEVELOPMENT OF CORTICAL INHIBITION: AN IN VITRO HRP AND GABA-IMMUNOCYTOCHEMICAL STUDY IN TURTLE. M. Blanton and A. Kriegstein. Dept. of Neurology, Stanford U. Sch. of Med., Stanford, CA 94305.

Pyramidal cells in developing turtle cortex are generated in a brief time period (Goffinet, JCN 243:106, '86) and occupy a single layer. When mature, their dendrites receive GABAergic, inhibitory inputs from nonpyramidal cells and excitatory inputs segregated in distinct sublaminae of the molecular layer. These temporal and spatial features aid resolution of developmental events and encouraged us to study the anatomy and physiology of synaptic systems in developing turtle cortex. We used focal applications of horseradish peroxidase (HRP) and GABA-immunocytochemistry to follow cellular and synaptic differentiation. We found pyramidal and non-pyramidal neurons to be distinguishable early in corticogenesis and to later form distinct synaptic patterns.

Pseudemys scripta embryos were staged (Yntema, J. Morph. 125:219, '68), perfused transcardially with saline, and the brains vibratomed at 300-500 µm. HRP-coated electrodes were placed in desired locations in the brain slices, which were maintained in vitro for six hours, then fixed and processed with standard techniques.

Pyramidal cells develop from vertically or obliquely oriented bipolar cells that appear at stage 15. They differentiate in relative synchrony and acquire multiple ascending dendrites to become pyramidal-like at the time of cortical plate appearance (stage 20). In subsequent stages, pyramidal cells give rise to multiply branching axons, which issue recurrents containing varicosities and form an axonal network extending mediolaterally and rostrocaudally. Apical dendrites acquire spines over a similar time course. At hatching, these cells appear mature, except for the presence of spines more proximally than in adult neurons.

Horizontally oriented nonpyramidal cells, the first relatively differentiated cells to appear, are intensely immunoreactive for GABA from the earliest stages studied (stage 16), well before cortical plate formation (GABA antisera and protocol kindly provided by O. Ottersen and J. Storm-Mathisen). This population of cells is split by intercalation of the cortical plate, composed predominantly of GABA-negative somata (presumably pyramidal cells). HRP-filled and GABA-positive fusiform cells acquire multiple ascending secondary dendrites with development, and more heterogeneous nonpyramidal cell types, covered with numerous fine branches and growth cones, occupy all layers. Punctate structures (putative terminals), present at stage 20, increase in density to a nearly adult-like distribution by hatching.

GABAergic neurons are thus present and distinct from pyramidal cells at early stages of corticogenesis, prior to cortical plate formation and synaptogenesis. Analysis of postsynaptic effects of GABAergic neuronal circuits may be facilitated in this system because of sequential, synchronous pyramidal cell differentiation.

263.13 DEVELOPMENT OF CORTICAL INHIBITION: FUNCTIONAL $GABA_A$ RECEPTORS AT EARLIEST STAGES OF CORTICOGENESIS. J.M. Shen, J.R. Huguenard, and A.R. Kriegstein. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The balance between excitation and inhibition is critical for normal cortical function and may be disordered in pathological states such as epilepsy. To understand how this balance may be attained during corticogenesis, one must first examine the time course of development of excitatory and inhibitory systems. Since GABA is the principal inhibitory neurotransmitter in cortex, we have begun by studying the development of GABA responsiveness in embryonic cortical neurons.

The whole-cell patch clamp technique was used to record from acutely dissociated cortical neurons from embryonic turtles (Pseudemys scripta). Animals between embryonic stages 16 (early corticogenesis) and 26 (hatching) were studied (staged according to Yntema, C.L., J. Morph. 125:219, 1968). This preparation holds several advantages: 1) turtles and mammals are phylogenetically related and their cortical structures share certain anatomical and physiological features; 2) the relatively simple organization and developmental pattern of turtle cortex facilitate the analysis and interpretation of results, and 3) patch clamp recordings are easily obtainable from embryonic turtle cortical neurons.

Neurons from the earliest stage tested (stage 16) were responsive to applications of 0.5 mM GABA; 96% of all neurons tested across all developmental stages responded (n=50). The response was accompanied by an increase in membrane conductance (mean increase across all ages = 7.4-fold). This GABA-mediated conductance became larger with developmental age, increasing 3-fold from stage 16 to 26. In cells from all stages, the GABA-mediated response inverted polarity at E_{Cl^-} and was reversibly blocked or greatly reduced by 10 μM bicuculline methiodide, suggesting that the receptors mediating the response were predominantly of the $GABA_A$ subtype. Analysis of current-voltage relationships revealed that a fast, transient, voltage-dependent inward current did not appear until stage 19/20.

These results indicate that $GABA_A$ receptors are present and functional in neuronal membranes at the earliest stages of corticogenesis (stage 16) before the formation of the cortical plate (stage 19/20), identification of synaptic profiles (stage 20), or appearance of a voltage-activated inward current (stage 19/20). Cells immunoreactive for GABA are also found in the cortex at stage 16 (see preceding abstract). The role of these precocious GABAergic elements and the time at which this inhibitory system becomes functional have yet to be determined.

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263.14 DEVELOPMENT OF CORTICAL INHIBITION: APPEARANCE OF SYNCHRONIZED NEURONAL DISCHARGES. A.R. Kriegstein and J.M. Shen. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Agents that antagonize inhibitory function in cortex can produce spontaneous epileptiform activity. However, for drug-induced synchronized neuronal discharges to occur, functional excitatory circuits and inhibitory mechanisms that normally keep check on excitation must both be present. To determine the earliest stage at which both systems are functional, cortices of embryonic turtles (Pseudemys scripta) were exposed to bicuculline, a $GABA_A$ receptor antagonist, and the timetable for appearance of synchronized neuronal discharge was established.

The entire cortex was dissected out from brains of animals between embryonic stage 17 (early corticogenesis) and hatchlings 1 week of age. The cortical slab was placed in a recording chamber at room temperature perfused with 10 μM bicuculline methiodide in turtle ringer. Field potentials were recorded from the ependymal surface and stimulation was provided by tungsten electrodes placed in the molecular layer.

Cortex from embryonic stages prior to stage 21 (n=5) failed to develop spontaneous or evoked epileptiform activity even after 4 hours of bicuculline exposure. Epileptiform discharges were first apparent in stage 21 embryos and were observed in cortices obtained from all later stages (n=12). The mean amplitude of spontaneous population events increased 3-fold from stage 21 to hatchling animals, and the mean frequency of discharge decreased from 0.17 Hz to 0.12 Hz and became more regular. Data from adult cortex indicate that epileptiform discharges originate in the dorsomedial subfield (DMC) and spread to other cortical areas. Simultaneous recordings from the dorsal cortex (DC) and DMC in embryonic tissue revealed that discharges in DMC led those in DC, and the velocity at which discharges propagated nearly doubled from 14.8 mm/sec in stage 21 animals to 28.3 mm/sec in hatchlings. Furthermore, at younger stages, discharges recorded in DMC did not always propagate to DC, suggesting that the ability to generate repetitive synchronized neuronal discharge matures in advance of the mechanism underlying propagation of epileptiform activity.

Our data indicate that the mechanisms for synchronizing excitatory neuronal activity become functional at stage 21. This timetable parallels other events in corticogenesis: cortical plate formation, synaptogenesis (Goffinet, A.M., J. Comp. Neurol. 125: 219, 1983), and development of a voltage-dependent inward current. Our results do not exclude the possibility that functional GABA-mediated inhibition develops even earlier. The previous abstracts show that GABA-immunoreactive neurons and functional $GABA_A$ receptors are present by stage 16. This raises the possibility that GABA may play a non-transmitter role in early corticogenesis.

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263.15 FURTHER EVIDENCE FOR A GABAERGIC PROJECTION FROM THE LATERAL HABENULA TO THE DORSAL RAPHE NUCLEUS: NEUROTRANSMITTER LABELING OF FASCICULUS RETROFLEXUS AXONS AND KAINIC ACID LESIONS. M.R. Park and H.T. Chang, Department of Anatomy and Neurobiology, University of Tennessee, Memphis, College of Medicine, Memphis, TN 38163.

Progress in understanding neuronal organization has been the greatest in the layered structures of the brain. In them, the segregation of individual neuronal elements provides additional criteria for characterizing components that is not available in more homogenous structures. While the habenular nuclei are not themselves layered, their afferents and efferents traveling in the fasciculus retroflexus are segregated in a way that allows conclusions to be drawn about the organization of habenular circuits from their patterns of labeling.

The fasciculus retroflexus is divided into a central core containing medial habenular axons and a surrounding mantle containing efferents from the lateral habenula (Herkenheim & Nauta, J. comp. Neurol., 187: 19, 1979). Afferents to the habenular nuclei also travel in the fasciculus retroflexus, as do some fibers from the stria terminalis. Little is know of the division habenular afferents between core and mantle.

We now report that immunohistochemical labeling for γ-amino butyric acid (GABA) labels axons in the mantle of the fasciculus retroflexus where efferents from the lateral habenula are confined. Substance P immunoreactivity is confined to axons lying in the core of the fasciculus retroflexus; the mantle is not labeled. GABA immunoreactivity is also found among axons of the stria terminalis. Since some stria terminalis axons course uninterupted through the habenular complex and join the fasciculus retroflexus, we performed kainic acid lesions in the lateral habenula to test the source of labeled axons. Lesions confined to the lateral habenula diminish the number of GABA positive fibers in the mantle of the fasciculus retroflexus. Substance P labeling is unaffected. Serotonergic immunoreactivity is present in axons of both the core and mantle. However, phaseolus vulgaris leucoagglutinin (PHAL) injections in the dorsal raphe nucleus label only mantle fasciculus retroflexus axons. The source of the serotonergic fibers found in the core could then well be the median raphe nucleus.

These results bear on our continuing efforts to clarify the nature of the lateral habenular-dorsal raphe projection. We have previously reported the presence of axonal terminal fields in the dorsal raphe nucleus from the labeling of lateral habenula efferents with the anterograde PHAL technique. Physiological evidence of a monosynaptic lateral habenula-dorsal raphe connection comes from our intracellular recording experiments of dorsal raphe neurons. The present findings indicate that at least some lateral habenular efferent axons are GABAergic. If these same axons reach the dorsal raphe nucleus, then they could account for a monosynaptic GABA mediated inhibition in neurons of that nucleus. On the other hand, the pattern of substance P labeling does not seem to permit the hypothesis of a substance P projection from lateral habenula.

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263.16 GABA DISTRIBUTION IN A PAIN MODULATING ZONE OF TRIGEMINAL SUBNUCLEUS INTERPOLARIS. M. A. Matthews and T. V. Hernandez*. Dept. Anat., LSU Medical Center, New Orleans, LA 70119

Physiological and behavorial studies have shown that painful stimuli from the central face and oral cavity are retained following a trigeminal tractotomy at the obex and that many WDR and NS units with receptive fields in these same areas are located in subnucleus interpolaris (Vi). We have defined a subdivision of Vi rich in Substance-P, enkephalin and serotonin, together with those categories of cellular elements involved in pain modulation (Matthews et al., 1987).

A recent model for control of spinal and medullary nociceptive neurons (Basbaum and Fields, 1984) incorporates a GABA-ergic cell into this circuitry and indicates that such elements could act as one substrate for presynaptic inhibition of primary afferents. We therefore examined the distribution of GABA-ergic activity in Vi by focusing on the types of cells, together with dendritic and synaptic profiles, which are immunocytochemically labeled with an antiserum against glutamic acid decarboxylase (GAD).

GAD occurred throughout Vi but was most concentrated in the ventrolateral quadrant and interstitial nucleus. It was localized to groups of small neurons with two to three primary dendrites, and within numerous punctate profiles suggestive of synaptic elements. Electron microscopy revealed labeled dendrites, some of which were post-synaptic to scalloped terminals of presumptive primary afferents. Other labeled dendritic elements, which were quite variable in size, engaged both GAD-labeled and unlabeled synapses. Most GAD synapses displayed clear round vesicles and formed contacts with unlabeled perikarya and a variety of dendritic processes. Numerous GAD-positive synapses were also incorporated into axo-axonic clusters, in which the GAD element was presynaptic to scalloped terminals. Others engaged in serial arrays with other unlabeled terminals which, in turn, were presynaptic to dendrites. Occasionally, GAD synapses formed contacts with GAD-positive dendrites.

These data show that GABA-ergic neuronal elements occur in spatial arrangements providing an anatomical substrate for post-synaptic modulation of activity in this area. GABA terminals also appear to be involved in a pre-synaptic inhibitory mechanism which may, in some instances, affect transmission in primary afferents. Double labeling immunocytochemical studies are currently in progress to define this circuitry.

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264.1 CHANGES IN EXTRACELLULAR POTASSIUM EVOKED BY GABA AGONISTS IN THE HIPPOCAMPAL SLICE. A.W. Barclet and M.E. Morris. Departments of Pharmacology and Anaesthesia and the Playfair Neuroscience Unit, University of Toronto, Toronto, Canada, M5S 1A1.

The effects of GABA (gamma-aminobutyric acid) and related compounds on extracellular potassium ion concentration ([K^+]o) were examined using isolated slices of guinea pig hippocampus. Double-barrelled K^+-selective microelectrodes were used to record from the CA1 region in 400 um slices mounted in a Haas tissue bath, perfused with physiological saline containing 4.25 mM K^+ at 33.5 °. Bath applications of GABA (0.5 - 10 mM) resulted in reversible dose-dependent depressions of field potentials evoked by stimulation (0.5 Hz, 100 uA, 100 us) of the Schaffer collaterals. These were accompanied by increases in [K^+]o which were followed by graded undershoots during wash-out. Changes in [K^+]o were greatest in the pyramidal cell layer at depths of 100 - 200 um and characterized by an apparent ED_{50} of 3 mM and mean maximal increase of 1.8 (SE ±0.36, n = 7) mM. The $GABA_A$ agonist THIP (0.1 - 1 mM) evoked changes of similar magnitude but shorter duration than those with GABA. Applications of baclofen (± racemic mixture, 0.01 - 1 mM), caused small (≤ 0.5 mM) but definite and prolonged accumulations of K^+. Potassium levels were also raised by beta-alanine and glycine (1 - 10 mM) but not with taurine (10 mM). Bicuculline methiodide (100 uM) or picrotoxin (100 uM) reduced the increases in [K^+]o evoked by 10 mM GABA by approximately 25 %. Pentobarbital (100 - 1000 uM) and ethanol (20 - 100 mM) also caused dose-dependent increases in [K^+]o (≤1.5 mM), and both GABA- and THIP-evoked changes in [K^+]o were strongly potentiated by pentobarbital (200 uM). The apparently greater potency of THIP (which is taken up less avidly than GABA) as well as the demonstrated antagonist sensitivy of the GABA-evoked [K^+]o increases suggests that there is a receptor-mediated increase in potassium conductance. However, the K^+ accumulations evoked by GABA agonists/analogs may also receive contributions from electrogenic Na/K pumping of GABA or K/Cl co-transport.

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264.2 DESENSITIZATION AND CHANNEL OPENING OF γ-AMINOBUTYRATE (GABA) RECEPTORS FROM RAT BRAIN. D.J. Cash and K. Subbarao*. Neurochemistry Unit, Missouri Institute of Psychiatry and Department of Biochemistry, University of Missouri-Columbia, School of Medicine, St. Louis, MO 63139

GABA Mediated chloride ion exchange and receptor desensitization were measured using $^{36}Cl^-$ isotope tracer and quench flow technique. The responses of different receptors could be resolved from the kinetic measurements. Two major receptors differed in their rates of desensitization, their modulation by pentobarbital and their activity (concentration).

Cerebral cortex from male Sprague-Dawley rats were homogenized and centrifuged at 270 g, 4 min. The supernatant was pelleted, resuspended and centrifuged in a 4-12% Ficoll gradient at 110,000 g, 1 hr. The middle band was diluted, pelleted and resuspended to give a membrane suspension containing sealed vesicles. The measurement of GABA mediated chloride exchange was initiated by mixing the membrane with $^{36}Cl^-$ in the presence of GABA and terminated by mixing with bicuculline methiodide. Two major phases of chloride exchange limited by two phases of receptor desensitization were detected in two types of experiment. (a) The time course of the $^{36}Cl^-$ influx progressed in two phases. (b) The $^{36}Cl^-$ influx was decreased by preincubation with GABA in two phases.

The measurements supported the existence of two distinguishable receptors on the same membrane, which were desensitized with time constants τ (=1/k) = 47 ms and 740 ms and gave chloride exchange into the vesicles with τ = 105 ms and 444 ms respectively at saturation with GABA. The 1/2 response concentrations were similar for both receptors, 150 μM and 114 μM GABA for desensitization and 105 μM and 82 μM GABA for chloride exchange for the faster and slower desensitizing receptors respectively. The two receptors are present in a ratio circa 1:4, similar to the ratio of low affinity to high affinity sites in binding experiments. Desensitization rates and channel opening equilibria have different dependencies on GABA concentration. Minimal molecular models are presented which explain the results over a wide GABA concentration range.

264.3 INWARD Ca CURRENT REQUIRED TO OPEN GABA RECEPTOR Cl CHANNELS. M. Gyenes*, T.T. Gibbs*, and D.H. Farb Department of Anatomy and Cell Biology, SUNY Health Science Center, Brooklyn, New York 11203

Whole cell current recordings under voltage-clamp conditions were used on spinal cord neurons from 6 d chick embryos maintained in cell culture. GABA, drugs and extracellular solutions of varying ionic composition were applied by microperfusion from 7-barrel pipets. All experiments were carried out at 22°C.

Adding 10 mM Ca or 10 mM Ba augments the GABA-activated current at -60 mV by 23.8 ± 2.6% (N=7) and 143 ± 27% (N=11), respectively, whereas 100 uM Cd, 10 mM Mn or 10 mM EGTA inhibit the GABA response respectively by 71.4 ± 6.3% (N=8), 67 ± 7.8% (N=4) and 48 ± 6.1% (N=4). Similarly, the somatic currents induced at -60 mV by externally applied GABA are enhanced by elevated internal Ca or Ba and inhibited by internal Cd. While the expected reversal potential for the GABA response in our internal and external solutions is around 0 mV, the observed values were +3.7 ± 0.75 mV (N= 9), +7.4 ± 0.06 mV (N= 6) and +11.9 ± 0.9 mV (N= 13) with internal solutions that contained respectively, 1 mM Ca and 11 mM EGTA, 10 mM Ba and 11 mM EGTA and 11 mM EGTA. External picrotoxin (100 uM) reduces by 80% the Ba-enhanced GABA response both at -60 mV and +40 mV. Near the apparent reversal with 2 mM Ba added to the external solution, the GABA-activated current could be separated into two sequential components: a rapid inward current followed by a slow outward current.

The results indicate that GABA cannot activate the Cl channel when the inward Ca current is blocked. Two possible explanations seem the most likely: GABA activates a separate Ca channel close to the Cl channel or GABA activates a single channel that allows both Ca and Cl to pass through sequentially. Ca could act catalytically as cofactor changing the channel's selectivity filter, and thereby coordinating Cl permeation.

264.4 DESENSITIZATION OF THE GABA RECEPTOR-GATED CHLORIDE CHANNEL IN BRAIN: EFFECTS OF NON-COMPETITIVE BLOCKERS OF NICOTINIC RECEPTOR-GATED CATION CHANNELS. Rochelle D. Schwartz and Meredethe R.Cone*. Department of Pharmacology, Duke University Medical Center, Durham, NC 27710.

The functional activity of the GABA receptor complex was studied by measuring muscimol stimulation of ^{36}chloride ($^{36}Cl^-$) uptake in rat cerebral cortical synaptoneurosomes. The effects of muscimol were blocked uncompetitively by picrotoxin (IC_{50}=44uM), a blocker of the GABA receptor-gated Cl^- channel. The muscimol response was blocked non-competitively by several non-competitive inhibitors (NCI) of nicotinic receptor (nAChR)-gated cation channels such as quinacrine, triphenylphosphonium, phencyclidine and chlorpromazine (IC_{50}s=20, 50, 250 and 300uM, respectively). Other antagonists at the nAChR complex such as cocaine, propranolol, amantadine, mecamylamine and hexamethonium were ineffective up to concentrations of 1mM. Since many of these NCI inhibit nicotinic responses via enhancement of desensitization of the nAChR, their role in desensitization of the GABA receptor was investigated. We previously demonstrated that "desensitization" of the GABA receptor complex occurs following preincubation of synaptoneurosomes with GABA agonists, barbiturates or ethanol (Schwartz et al., Mol. Pharmacol. 30, 419-426, 1986). In the present study initial rates of $^{36}Cl^-$ uptake were measured so that desensitization of the GABA receptor could be studied in more detail. Analysis of the time course for muscimol-stimulated uptake (10uM, 1-10 sec, 30°C) indicated a decrease in the apparent dissociation rate constant (k_d) at the initial time points, consistent with a desensitization process, with a transition $t_{1/2}$ of 0.58±0.23 sec. In order to slow the rate of muscimol-induced desensitization, $^{36}Cl^-$ uptake was measured at 22°C, resulting in a relatively constant k_d over the first 5 sec. However, in the presence of triphenylphosphonium (50uM), the rate of desensitization was enhanced (transition $t_{1/2}$=1.04±0.15 sec). Similar results were obtained with phencyclidine. When Ca^{2+} was omitted from the medium, the inhibition of muscimol-induced $^{36}Cl^-$ uptake by each NCI except picrotoxin was significantly attenuated. Ca^{2+} also decreased muscimol-stimulated $^{36}Cl^-$ uptake in a concentration-dependent manner (IC_{50}=30uM) primarily via decreasing muscimol potency. These data suggest that the desensitization of the GABA receptor promoted by NCI could be mediated by Ca^{2+} mobilization. Under identical assay conditions, the NCI inhibited [^{3}H]t-butylbicycloorthobenzoate binding to synaptoneurosomes with the same IC_{50}s for inhibition of muscimol responses, indicating that these NCI of nAChR-gated cation channels also interact with convulsant sites on the GABA receptor-gated Cl^- ion channel.

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264.5 DIFFERENTIAL ACTIONS OF PENTOBARBITAL AND PHENOBARBITAL ON GABA-ACTIVATED SINGLE CHANNEL CHLORIDE CURRENTS. R.E. TWYMAN, C.J. ROGERS AND R.L. MACDONALD. (Spon: S.Gilman) Department of Neurology, University of Michigan, Ann Arbor, MI 48104.

Barbiturates enhance GABAergic inhibition. To determine the mechanisms of pentobarbital (PB) and phenobarbital (PhB) enhancement of GABA-activated chloride (Cl^-) currents, we recorded single channel Cl^- currents from excised outside-out patches from mouse spinal cord neurons grown in dissociated cell culture.

Bathing and intrapipette recording medium consisted of a HEPES-buffered, equal Cl^- concentration solution. Strychnine (200 nM) was used in all solutions to block glycine-coupled Cl^- currents. Patches were held at the potassium equilibrium potential (-75 mV). Pressure ejection pipettes were used to apply GABA (2μM) alone or with PB (50 μM) or PhB (500 μM). Due to desensitization of GABA Cl^- currents, GABA applications were limited to 5 seconds. Responses were digitized at 8 kHz with 1 kHz Bessel filtering and analyzed by computer using the analysis program IPROC (Sachs et al., Pflugers Arch 395:331, 1982).

Two conductance states were recorded, but the larger, dominant state (~26 ps) was used for these analyses. Excised patches which were GABA sensitive had infrequent spontaneous, non-bursting Cl^- currents. GABA increased the probability of channel opening and evoked bursting currents. In the presence of PB or PhB, GABA-evoked currents had the same conductance but openings were more frequent, with an increase in burst number and a longer mean open time. In addition, PB, but not PhB, increased the mean number of openings per burst.

Both PhB and PB increased burst duration. However, PhB prolongation of burst duration was due to the prolongation of individual openings within the burst while PB prolongation of bursts was due to an increased duration of individual openings within the burst and an increased number of openings. Enhancement of GABA-activated Cl^- currents by PB and PhB may be due to 1) an increased number of channel openings, 2) an increased duration of individual channel openings and 3) an increase of burst duration. The effect of PB on burst duration was more pronounced than that of PhB and may account for the ability of PB to prolong GABA currents more effectively than PhB.

264.6 GABA-ACTIVATED CHLORIDE CURRENTS HAVE MULTI-STATE KINETICS IN CULTURED MURINE SPINAL CORD NEURONS. C.J.Rogers, R.E.Twyman and R.L.Macdonald. Dept. of Neurology, University of Michigan, Ann Arbor, Michigan, 48104.

Gamma aminobutyric acid (GABA) produces inhibition by increasing membrane chloride conductance. GABA-activated chloride currents have been shown to have three conductance states and to open in bursts (Bormann, Hamill and Sakmann, J. Physiol. (Lond) 385:243, 1987). We have studied the steady state gating properties of the largest (~26 pS) GABA-activated chloride conductance state. By analyzing frequency plots of open and shut durations we have determined that the GABA-activated chloride channel has multiple open and shut states.

GABA-activated Cl^- currents were studied using excised outside-out patches of mouse spinal cord neurons. Dissociated fetal neurons were cultured for 3-6 weeks prior to use. The medium used in the recording pipette contained in mM: KCl 153.33; $MgCl_2$ 1; Hepes 10; EGTA (EGTA 5, Ca^{++} .5, K^+ 12); NaOH 1; KOH 2; pH = 7.38. The bathing medium used for recording contained in mM: NaCl 142; $CaCl_2$ 1; KCl 8.09; $MgCl_2$ 6; glucose 10; Hepes 10; pH = 7.4. Strychnine (100 nM) was added to the bathing medium and to the GABA-containing solutions to block glycine-activated Cl^- currents. Patches were clamped at -75 mV. Micropipettes (tips broken to 20-30 μM were used to apply 2 μM GABA for periods of 5-10 seconds. Responses were digitized at 8 KHz with a 1 KHz 8 pole bessel filter. Data were analyzed using a modified version of the IPROC analysis program (Sachs et al., Pflugers Arch 395:331, 1982).

Two conductance states were recorded but the larger, dominant state (~26 pS) was used for these analyses. Excised patches which were GABA sensitive had infrequent spontaneous single opening, non-bursting currents. GABA (2 μM) increased the frequency of non-bursting currents and evoked bursting currents. Bursting was defined by periods of rapid channel opening and closings separated by designated longer shut periods. Frequency plots of open and shut durations were fit using the least squares method. The frequency plot of open durations could be fit by four exponentials and the frequency plot of shut durations could be fit by six exponentials. These data suggest that the GABA-activated chloride channel has at least ten states, four open states and six closed states. There may be also longer shut and open states which were not detected in our experiments due to the relatively short periods of GABA application. A method of burst analysis was developed using the shut state time constants. Four "burst terminators" were defined and burst properties were characterized using these burst terminators. These analyses have shown that the largest GABA-gated chloride channel has multi-state kinetics.

264.7 ACTIONS OF AVERMECTIN B_{1a} ON CHLORIDE CHANNELS IN RAT BRAIN SYNAPTONEUROSOMES. K. Matsumoto, M.E. Eldefrawi and A.T. Eldefrawi. Dept. Pharmacol. & Exp. Therap. Univ. Maryland Sch. of Med., Baltimore, MD 21201.

The macrocyclic lactone anthelmintics, avermectin B_{1a} (AVM) and ivermectin (dihydroavermectin), have been shown to interact with γ-aminobutyric acid (GABA) receptor-chloride channel complex in the central nervous system. These drugs not only stimulate GABA release from brain synaptosomes but also increase chloride channel conductance in cultured chick spinal neurons (Matsumoto et al., Neurosci. Lett. (1986) 69, 279). Preliminary data from our laboratory showed that AVM inhibited ^{35}S-*t*-butylbicyclophosphorothionate (^{35}S-TBPS) and ^{3}H-muscimol binding to rat brain synaptic membranes and also stimulated $^{36}Cl^-$ flux in rat brain microsac preparations (Abalis et al., (1986) 1,69). In the present study, we investigated the effects of AVM on $^{36}Cl^-$ efflux and ^{35}S-TBPS binding assays. Rat brain synaptoneurosomes were obtained by slightly modifying the method of Schwartz et al.(FEBS Lett., (1984) 175, 193). Whole brains were minced into small pieces and homogenized in 20 vol. of ice-cold Krebs-HEPES buffer. The homogenate was passed through 3 layers of nylon mesh (130 μm), then through a Millipore filter (10 μm). The filtrate was centrifuged at 1000 *x* g for 15 min. The pellet was washed by resuspension and recentrifugation to remove endogenous GABA, then used for efflux and binding assays. The time course of $^{36}Cl^-$ efflux from synaptoneurosomes was multiphasic. AVM (3 μM) significantly increased $^{36}Cl^-$ efflux compared to basal (control) efflux. The stimulatory effect of AVM on $^{36}Cl^-$ efflux was partly temperature-dependent (21° > 30° > 0°C). Muscimol (10^{-5}M) had no effect on $^{36}Cl^-$ efflux at various temperatures tested. Bicuculline methiodide, picrotoxinin and 4,4'-diisothiocyano-2,2'-stilbene disulfonic acid (DIDS) inhibited AVM-induced $^{36}Cl^-$ efflux. These agents also decreased basal $^{36}Cl^-$ efflux in the absence of AVM. AVM-induced $^{36}Cl^-$ efflux was not changed by other Cl^- transport blockers like anthracene 9-carboxylic acid and 2,4-dichlorocarboxylic acid (10^{-4} M). AVM-induced $^{36}Cl^-$ efflux was not decreased by pretreatment with 20 μM muscimol for 5 min. On the other hand, effects of AVM on ^{35}S-TBPS binding was biphasic: stimulating binding to a maximum at $3x10^{-8}$ M, with less stimulation up to 10^{-7} M and inhibition above 10^{-6} M. These results suggest that AVM directly activates chloride channels that are regulated by GABA and those that are not, through sites that are different from the GABA receptor.

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264.8 **MEMBRANE CURRENTS INDUCED BY GLYCINE IN NEURONS ISOLATED FROM *SPASTIC* AND CONTROL MICE.** D.L. Tauck, W.F. White and S.A. Lipton. Depts. of Neuroscience, Neuropathology & Neurology, Children's Hospital and Harvard Medical School, Boston, MA, 02115.

Behavioral and electromyographic abnormalities in the mutant mouse *spastic* suggest a decrease in inhibitory neurotransmitter function. Previous studies using receptor binding techniques have demonstrated an 80-90% decrease in the specific binding of ^{3}H-strychnine, a ligand for the post-synaptic glycine receptor, to the spinal cord, brainstem and midbrain of *spastic* compared with littermate control mice. Preliminary autoradiographic data indicate that the density of glycine receptors is decreased in the retina. Since mammalian retinal ganglion cells are known to be directly sensitive to glycine [Tauck et al., (1986) *Soc. Neurosci. Abstr.* 12(1):635], and since the cells can be labelled for unequivocal identification in culture, these are the neurons that were chosen for this study. Using the whole-cell mode of the patch-clamp technique, the present study compares the membrane currents induced by glycine in isolated central neurons from *spastic* and control mice.

The electrophysiological responses to glycine of cells isolated from *spastic* animals were indistinguishable from those of controls. Glycine, 100 μM, produced large increases in membrane conductance in all ganglion cells studied. The reversal potential of the current induced by glycine was dependent on the distribution of chloride ions across the membrane, consistent with chloride being the charge carrier of the glycine-induced current. The responses to 100 μM glycine were reversibly antagonized by 1 μM strychnine. Finally, the currents elicited by glycine decayed exponentially with a time constant of approximately 5 seconds.

These results demonstrate that the function of those glycine receptors which mediate inhibition in retinal ganglion cells is not compromised in the mutant mouse *spastic*. This discrepancy suggests that the receptors studied with autoradiographic techniques may be a different population than the ones that mediate fast synaptic transmission; alternatively, the discrepancy may indicate that only a small percentage of the glycine receptors studied with autoradiographic techniques are necessary to mediate the whole-cell currents measured with these techniques.

264.9 EFFECT OF AMMONIUM ON E_{GABA} IN CULTURED RAT HIPPOCAMPAL NEURONS. M.K. Walton*, W. Raabe and J.L. Barker.(SPON: J.M.H. ffrench-Mullen) Lab of Neurophysiology, NIH, Bethesda MD 20892 and Dept. of Neurology, VA Med. Ctr., Minneapolis, MN 55417

Ammonium levels are elevated in several clinical disorders and are believed to have a role in the pathophysiology of these disorders by effects on both inhibitory and excitatory pathways. In cerebral cortex, thalamus and spinal cord ammonium has been shown to readily shift the equilibrium potential of the IPSP by inactivation of extrusion of chloride from these neurons. However, there have been conflicting reports of the effect of ammonium on the IPSP equilibrium potential in mammalian hippocampus in vivo or in hippocampal slice. To re-examine the effects of NH_4^+ on hippocampal cells this study investigated the effect of NH_4^+ on the equilibrium potential for the GABA activated current (E_{GABA}) in cultured rat hippocampal neurons.

Hippocampal neurons from E19 rat embryos were grown in culture for 2 to 4 weeks. Cells were placed in a physiologic medium with 1uM TTX added to suppress spontaneous activity. Intracellular recording was carried out in control medium and in medium with NH_4Cl added (1-5 mM) using conventional sharp microelectrode pipettes filled with potassium acetate. GABA (20uM) was applied by brief pressure pulses from a pipette in close proximity to the recorded neuron. Intracellular current was used to briefly shift membrane potential to determine E_{GABA}, and to maintain a constant membrane potential between test pulses.

Ammonium shifted E_{GABA} in a depolarizing direction. NH_4^+, 1 mM, shifted E_{GABA} 2 mV, 2 mM NH_4^+ shifted E_{GABA} by 4 mV, and 5 mM NH_4^+ shifted E_{GABA} by 7 mV. NH_4^+ had no consistent effect on passive membrane properties.

In rat hippocampal neurons E_{GABA} is dependent on the chloride gradient across the neuron's membrane. The effect of NH_4^+ on E_{GABA} found in this study implies an increase in intracellular chloride. These results suggest that rat hippocampal neurons in tissue culture do have a mechanism for chloride extrusion that is sensitive to inactivation by ammonium.

264.10 **ALPHA ETHYL, METHYL THIOBUTYROLACTONE POTENTIATES GABA CURRENTS IN CHICK SPINAL CORD NEURONS.** C.F. Zorumski, J. Yang, K. Baker, D.F. Covey, D.B. Clifford; Departments of Psychiatry, Anatomy and Neurobiology, Pharmacology, and Neurology; Washington University Medical School, St. Louis, Missouri.

Alpha alkyl substituted thiobutyrolactones (TBLs) are heterocyclic molecules with anticonvulsant activity. Previously it was shown that these compounds are effective against pentylenetetrazole and maximal electroshock seizures. Binding, chloride flux and molecular modelling studies suggest that the anticonvulsant effects occur throughan action at the GABA chloride ionophore complex and specifically through an action at the picrotoxinin (PTXN) binding site. The present study was undertaken to investigate the electrophysiological effects of alpha ethyl, methyl thiobutyrolactone (aEMTBL),one of the most potent of the TBLs yet synthesized, on GABA currents in voltage clamped neurons.

Embryonic chick spinal cord neurons (SCN) were dissociated and grown in primary culture. Between 3-7 days after plating SCN were studied using gigaseal recording techniques. Pressure applications of aEMTBL produced a reversible dose dependent potentiation of GABA currents. The half maximal effective concentration was 7uM with saturation at 500uM. aEMTBL potentiated the response to all concentrations of GABA, increasing the response to a saturating GABA dose by 56%. The potentiation was not due to a GABA-mimetic effect as concentrations of aEMTBL up to 10mM failed to induce current.

Based on binding studies it is likely that TBLs interact with the PTXN site. The physiological consequences of an interaction of aEMTBL with this site was examined by attempting to reverse the block of GABA currents produced by 1mM PTXN. 500um aEMTBL produced a partial relief of PTXN block leading to a small inward current with a large increase in membrane noise during application of GABA. Neither phenobarbital (PB) nor diazepam (DZP) had any effect on PTXN block suggesting a difference between aEMTBL and other GABA potentiators. aEMTBL also enhanced GABA responses in the presence of saturating concentrations of either PB or DZP.

At a single channel level the potentiation of GABA currents by aEMTBl was manifest as an increase in the frequency of channel opening without a change in the single channel conductance (28-30ps for GABA alone and GABA+aEMTBL) or mean channel open time (8.3+/-1.7msec for GABA and 9.3+/-1.5msec for GABA+aEMTBL). In the presence of aEMTBL the GABA channel showed a near doubling of the probability that the channel is in an open state (17% vs. 9%).

In summary aEMTBL potentiates GABA currents in chick SCN. We propose that this potentiation occurs through an action at the PTXN binding site and that aEMTBL has features which differ from either barbiturates or benzodiazepines.

264.11 HETEROLOGOUS DESENSITIZATION OF THE GABA RECEPTOR COUPLED CHLORIDE ION CHANNEL BY ETHANOL: REVERSAL BY THE IMIDAZOBENZODIAZEPINE Ro15-4513. P.D. Suzdak, R.D. Schwartz and S.M. Paul*. Section on Molecular Pharmacology, Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892.

The ability of ethanol, to "desensitize" the -aminobutyric acid (GABA) receptor-coupled chloride (Cl^-) ion channel was studied using a cell-free subcellular brain preparation (synaptoneurosomes) for measuring $^{36}Cl^-$ flux. Ethanol (20-100 mM) stimulates $^{36}Cl^-$ uptake into rat cerebral cortical synaptoneurosomes in a biphasic manner, with a maximal response at 50-70 mM (EC_{50} 30mM) [Suzdak et al., PNAS 83, 4071 (1986)] and a maximal stimulation of $^{36}Cl^-$ uptake of 11.5 nmole/mg protein/5 sec. Higher concentrations of ethanol (160 mM) resulted in a progressively smaller stimulation of $^{36}Cl^-$ uptake and blocked both muscimol (5 μM) and pentobarbital (500μM)-stimulated $^{36}Cl^-$ uptake. Following continuous exposure of synaptoneurosomes ($\leq$ 20 minutes) to ethanol (50mM), $^{36}Cl^-$ uptake diminished to a new steady state level with a $t_{1/2}$ of 1 minute. The decrement in response to ethanol was dependent upon both concentration and length of exposure, and was reversible. No decrement was observed in the ability of subthreshold concentrates of ethanol (10 mM) to enhance muscimol stimulated $^{36}Cl^-$ uptake following a 20 minute incubation. "Heterologous desensitization" to both muscimol and pentobarbital was observed in experiments where these agents were added following a 20 minute preincubation with ethanol (50 mM). The ability of ethanol to produce "heterologous desensitization" of the $GABA_A$ receptor was prevented by coincubation with Ro15-4513 (0.1 μM) but not with β-carboline inverse agonist DMCM (1 μM). By contrast Ro15-4513 did not block pentobarbital or muscimol-induced desensitization of the GABA receptor-coupled Cl^- ion channel confirming previous studies on its specificity in blocking ethanol stimulated $^{36}Cl^-$ uptake in vitro (Suzdak et al., Science 234, 1243, 1986). These data further suggest that ethanol, at pharmacologically relevant concentrations, interacts with brain GABA receptors and that Ro15-4513 is an antagonist of the action of ethanol at this receptor complex.

264.12 **IN VITRO SLICE PREPARATION AS A MODEL FOR INVESTIGATION OF GABA/BENZODIAZEPINE INTERACTIONS.** **S. Hussain,* J. Bagust,* C.R. Gardner*[1] and R.J. Walker. Dept. Neurophysiol., Southampton Univ. Southampton SO9 3TU, and [1]Roussel Labs., Swindon SN3 5BZ, England.**

In vitro brain slice preparations are a popular tool for neuropharmacological studies. Many areas of the mammalian brain have been used including the hippocampus, olfactory cortex, lateral geniculate nucleus and spinal cord (Kerkut, G.A. & Wheal, H.V., Electrophysiol. Isolated Mammalian CNS Preps., Academic Press, Lond., 1981). The cerebellum has been studied by a number of workers (Llinas, R. & Sugimori, M., J. Physiol. 305, 171, 1980) and its cellular organization is known. The pharmacological actions of Benzodiazepines (BDZs) are at least partly mediated by enhancing GABA transmission (Costa, E. et al., Life Sci., 17, 167, 1975) and certain clinically effective anticonvulsants interact with sites in the GABA-BDZ-chloride ionophore complex.

In the present study extracellular recordings were made from the Purkinje cell layer in the rat cerebellar slice (500 μm thick). Slices were continuously perfused with oxygenated Krebs at 25°C and a flow rate of 15 ml min^{-1}. The firing rate of the cells was in the range 5-80 Hz. Drugs were applied either by bath addition or ionophoretically using a multibarrel electrode placed in close proximity to the recording electrode.

GABA (0.1M in electrode) inhibited spontaneous activity in a current dose related manner (range, 5-100 nA, 15 sec duration). This inhibition was blocked by bath application of bicuculline salts, 0.5 μM. Pitrazepin and picrotoxin, both 5 μM, antagonised bath applied GABA inhibition of cell activity. Bath applied BDZs, eg. Flurazepam and RU 32007 also inhibited activity at mM concentration. At lower concentrations the BDZs produced a significant potentiation of submaximal GABA responses. For example, GABA, 50 nA, and Flurazepam, 50 nA, separately applied only caused slight inhibition of cell activity. When applied together they produced almost complete inhibition. This potentiation of GABA inhibition was blocked by the BDZ antagonist RO 15-1788 (0.1 M in electrode, 50 nA) All effects were readily reversed. Similar effects to flurazepam were obtained with RU 32007. The beta-carboline DMCM, a potent inverse agonist, did not appear to have any obvious effects. Bath application of up to 50 μM or ionophoretic application did not potentiate or inhibit GABA responses.

This work clearly demonstrates the modulatory effects of BDZs on GABA transmission. The use of cortical slice preparation and isolated spinal cord preparations for the analysis of GABA/BDZ interactions is also under investigation. In preliminary studies using isolated cord BDZs depress dorsal root afferent activity in a dose dependent manner but this depression is irreversible.

264.13 PHARMACOKINETICS OF FLURAZEPAM AND ITS METABOLITES IN RAT SERUM AND BRAIN. S. Dolan*, C.E. Lau*, M. Tang* and J. L. Falk. Dept. of Psychology, Rutgers Univ., New Brunswick, NJ 08903.

Flurazepam is a hypnotic agent used for the treatment of insomnia. Its pharmacokinetics and metabolic fate is known mainly from research with dogs, cats and humans but remains largely unknown in rodents. Since diazepam and its major metabolite desmethyldiazepam are eliminated differently in rats, compared to humans and other animals, flurazepam was studied to evaluate a similar possibility.

Groups of adult, male, albino rats were injected with flurazepam (16 mg/kg, i.p.). Brain and serum levels of flurazepam and its major metabolites were determined at various times between 15 min and 24 hr. Serum samples (50 ul) were buffered with borate buffer (pH 9) and extracted with diethyl ether. Following evaporation of the ether layer the residue was resuspended in mobile phase. Whole brain was homogenized in distilled water (1:4) and similarly extracted. Samples (20 ul) were analyzed by HPLC with UV detection at 230 nm on a 5 um Ultrasphere C18 2mm I.D. column. Mobile phase consisted of 0.03 M sodium acetate buffer (pH 2.9), acetonitrile and methanol (60:25:15 v/v). The flow rate was set at 0.3 ml/min.

This method identifies simulaneously flurazepam and the following metabolites: didesethyl flurazepam, monodesethyl flurazepam, N-1-hydroxyethyl flurazepam and desalkyl flurazepam, all of which were present in serum and brain with Tmax at 30 min. The elimination rates appear to be parallel for flurazepam and desalkyl flurazepam in both compartments with relatively short half-lives. In serum the t1/2 was 1.76 hr for flurazepam which is quite similar to the value for other species. In contrast, we found that the serum t1/2 for desalkyl flurazepam was 1.44 hr in the rat compared to values of 40 to 200 hours in dogs and humans. Hence, it appears that the pharmacokinetics of the major metabolites of both diazepam and flurazepam are quite different in rats and humans.

264.14 ALTERATIONS IN BRAIN CELLULAR METABOLISM OF RATS FOLLOWING IN UTERO EXPOSURE TO DIAZEPAM. M.C.K. KELLOGG AND R. MIRANDA. Dept. of Psychology, Univ. of Rochester, Rochester, N.Y. 14627.

Previous work has shown that exposure to diazepam (DZ) during the last week of gestation can induce alterations in the adult rat offspring that resemble the actions of DZ given acutely to naive adult rats. Additionally, concomitant exposure to DZ and the central benzodiazepine (BZ) antagonist RO15-1788 prevented the effects of DZ (Brain Res., 293:73 and 307:38,1984). While the central BZ receptor is considered to be associated with neural membranes, BZs also bind to a peripheral type receptor in the CNS that may be associated with subcellular membranes such as the mitochondrial outer membrane (Anholt, J.Biol. Chem. 261:576; 1986). The objective of the present study was to determine whether late gestational exposure to DZ altered cellular metabolism in the offspring, a possible consequence of a mitochondrial receptor location. To examine this possibility malondialdehyde(MDA)-like material was measured as an index of metabolic activity. MDA is an endproduct of a series of autocatalytic peroxidation reactions and of the synthesis of prostaglandins and thromboxines. Pregnant dams (Long Evans) were divided into groups and all injections were given subcutaneously once daily over gestational days 14-20. MDA was measured in 6 brain regions of exposed offspring at 3-4,16-18, and 24-26 months of age.MDA levels increased in most regions of uninjected and vehicle injected rats from 3 to 24 months; in the diencephalon and midbrain, however, the levels increased from 3 to 18 mo. and then decreased from 18 to 24 mo. Prenatal exposure to DZ elevated MDA levels at 3 months with the lower exposure dose (1.0 mg/kg) producing a larger increase than the higher dose (2.5 mg/kg). The pattern of aging-related changes in MDA levels was also altered by early DZ exposure in a dose-related manner. Exposure to 2.5 mg/kg induced an aging pattern in all regions similar to that seen only in control diencephalon and midbrain, whereas exposure to 1.0 mg/kg induced a profile similar to telencephalic regions and cerebellum of controls. Concurrent exposure to DZ (2.5 mg/kg) and RO15-1788 (10 mg/kg) did not prevent the effect of DZ and exposure to the antagonist alone produced an effect in young adults that was comparable to the effect of the low dose of DZ. The effect of the peripheral type antagonist PK-11195 is being evaluated. The results suggest an environmentally modifiable, dynamic, age-related involvement of BZ binding sites with intracellular metabolism. The central and peripheral sites may both be involved. Supported by grant MH-31850.

264.15 ALTERATION IN RAT BRAIN ^{31}P-NMR SPECTRA FOLLOWING IN UTERO EXPOSURE TO DIAZEPAM. R.MIRANDA, T.CECKLER*, R.GUILLET*, and C.K.KELLOGG. Depts of Psychology and Biophysics, Univ. of Rochester, Rochester, N.Y., 14627.

Exposure to diazepam (DZ) between gestational days 14-20 alters malondialdehyde (MDA) levels in young adult and aged offspring in a dose-dependent manner. The central antagonist RO15-1788 (RO) did not reverse this effect. Administered alone, RO induced an effect comparable to DZ. These findings suggest a dynamic age related involvement of benzodiazepine (BDZ) receptor subtypes with intracellular metabolism.

We analysed the effects of prenatal exposure to BDZs on intracellular metabolism using in vivo ^{31}P-NMR spectroscopy. Following prenatal exposure of Long Evans rats to vehicle, DZ(2.5mg/kg/day), RO(10mg/kg/day) or DZ+RO, ^{31}P spectra were acquired at 3-4, 16-18 or 24-26 months of age. A 2cm surface coil was placed over the head of a rat anesthetised with Ketamine and Xylazine. Spectra were acquired using a GE 2.0 Tesla CSI system (^{31}P-frequency 34.635 MHz). Parameters were: repetition rate, 3 sec; sweep width, 3 KHz; pulse width, 20 µsec. Convolution difference techniques were used to eliminate the broad lipid/bone resonance.

Intracellular pH (pHi) decreased with age in uninjected and vehicle controls, as did the ratio of phosphocreatine (PCr) to inorganic phosphate (Pi). The ratio PCr/ßATP did not change with age. At 3-4 mo. and at 24-26 mo. of age, DZ exposed animals showed a significant decrease in pHi from control levels. At 16 -18 months of age however, the differences in pHi were not significant. The 16-18 month period may be a critical period in the life span of the animal, representing a transition between late adulthood and old age that might involve changes in cellular energy metabolism.

Prenatal exposure to DZ did not alter PCr/Pi ratios from control values. The PCR/ßATP ratios differed between exposed and control groups only at 24-26 mo. If ßATP is relatively constant throughout the animal's lifespan, this could imply that there was an increase in PCr and Pi levels with treatment and age. One explanation of these results is that the cellular composition of brain tissue may change with age and be modified by prenatal drug exposure. At 3-4 months of age, pHi was also significantly reduced in animals exposed to RO alone, but not in animals exposed to DZ+RO. Also, RO administered alone or along with DZ, decreased PCr/Pi ratios in 3-4 mo. old animals without changing PCr/ßATP ratios. These results indicate that prenatal exposure to BDZs may alter relationships between PCr utiliztion and pHi. Alternatively, observed changes in ratios may be due to changes in relaxation rates of the different phosphate groups, indicating a change in their physical environment. Present research centers on the role of peripheral BDZ receptor ligands (PK11195), the effects of acute exposure to BDZs, and the relationship between PCr utilization and pHi. Supported by grant MH 31850.

264.16 MODULATION OF GABAERGIC FUNCTION IN THE SUBSTANTIA INNOMINATA BY THE MESOLIMBIC DOPAMINE SYSTEM. M.C. Austin and P.W. Kalivas, Dept. of VCAPP, Washington State University, Pullman, WA 99164.

It has been suggested that the behavioral hyperactivity produced by increased dopaminergic neurotransmission in the nucleus accumbens (NA) is mediated by inhibition of the GABAergic efferents from the NA to the substantia innominata (SI). Studies have demonstrated that pretreating the SI with the GABA agonist muscimol attenuates the locomotor activity elicited by dopamine injection into the NA. We evaluated whether the locomotor activity produced by cholinergic stimulation of the NA could be inhibited by pretreating the SI with muscimol. We also investigated the effect of 6-OHDA lesions of the VTA on GABA levels in the SI.

Male S.D. rats were implanted with chronic bilateral injection cannulae into the NA and SI. One week after surgery, rats were adapted to the photocell cages for one hour, pretreated with 2 or 5 µg/side muscimol intra-SI followed by an intra-NA injection of 0.33 µg/side carbachol and placed back into the photocell cages for 120 min. The carbachol-induced motor stimulant effect was significantly reduced by pretreating the SI with 2 or 5 µg/side muscimol.

To determine the effect of 6-OHDA lesions of the VTA on GABA levels in the SI, rats were unilaterally injected with 6-OHDA into the A10 region. Ten days following surgery, rats were sacrificed by head-focused microwave and the NA and SI dissected from the brain. Measurement of GABA was obtained by pre-column derivatization of the supernatant with t-butylthiol o-phthaldialdehyde for 6 min. and injection of the sample on HPLC. Lesion of the VTA significantly increased GABA levels in the SI compared to the nonlesioned side, whereas GABA levels were decreased in the NA. This may be explained as a disinhibition of GABA turnover in the SI by reducing DA inhibitory tone in the accumbens. These findings provide further support for the postulate that DA in the NA modulates GABAergic function in the SI. Studies are also planned to investigate the effect of direct stimulation of the NA on GABA levels in the SI.

264.17 COCAINE DECREASES GABA TURNOVER IN THE VENTRAL PALLIDUM AND NUCLEUS ACCUMBENS. A.J. Bordelais and P.W. Kalivas* (SPON: J.W. Wright), Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA.

It is known that GABAergic neurons in the nucleus accumbens (NA) project to the ventral pallidum (VP) (Br. Res. 177, 325, 1979). Furthermore, the increase in spontaneous motor activity produced by injection of dopamine (DA) agonists into the NA is prevented by pretreatment with a GABA agonist injection into the VP, which has led to the postulate that DA may be acting in the NA to inhibit tonic GABA modulation of neurons in the VP (Prog. Neurobiol. 14, 69, 1980). To more directly evaluate this hypothesis, GABA metabolism by GABA-transferase was inhibited by injection of aminooxyacetic acid (AOAA; 40 mg/kg, ip) which results in a linear increase in GABA levels over time that can be used as a measure of GABA turnover.

Male S.D. rats were injected with AOAA, and 15 min later injected with saline, ip, or cocaine (5, 10 or 20 mg/kg, ip). 45 min later, the rats were again injected with saline or the same dose of cocaine, and killed by head-focused microwave one hr later. The VP, NA, pedunculopontine region and A10 DA region were punched from the brain, and GABA levels measured with HPLC-EC using a t-butylthiol, o-pthaldehyde derivatization of GABA and 5-aminovaleric acid (internal standard). The derivatized GABA was separated on a C-18 reversed-phase column, and oxidized at +0.7 V. It was found that cocaine produced a decrease in the level of GABA in all brain areas examined. The decrease was dose-related in the VP, NA and A10 where only the highest dose of cocaine decreased GABA accumulation. This decrease was up to a 40% decline over control levels. In the pedunculopontine region, the lowest dose of cocaine was most effective at decreasing GABA accumulation. Since cocaine increases DA neurotransmission in the NA by preventing DA reuptake into the presynaptic terminal, these data support the hypothesis that DA release into the NA inhibits tonic GABAergic influence in the VP.

264.18 EFFECT OF GABA AGONISTS AND ANTAGONISTS ON EVOKED GABA RELEASE FROM PARS COMPACTA AND RETICULATA OF THE RAT SUBSTANTIA NIGRA. B. Floran *, I. Silva *, C. Nava *, and J. Aceves *. (SPON. H. Brust Carmona). Department of Physiology. Centro de Investigación del IPN. Apartado Postal 14-740. 07000 México, D.F.

In previous comunications we have presented data suggesting the presence of GABA "A" presynaptic autoreceptors on the GABAergic terminals of the pars compacta of the substantia nigra, and of GABA "B" receptors (autoreceptors?) probably on the GABAergic terminals of the pars reticulata. Continuing our characterization of these presynaptic GABA receptors, we are now reporting the results of a comparative study on the effects of muscimol, benzodiazepines (diazepam), baclofen, bicuculline and picrotoxin on the release of GABA from gabaergic terminals in nigra compacta and reticulata. Experiments were done in slices of either pars compacta or reticulata of the substantia nigra. To prevent the uptake by glial cells, the labelling with tritiated GABA was done in the presence of β-alanine (10 μM). To prevent part of the reuptake of the released GABA, the superfusion medium contained nipecotic acid (10 μM). The release was induced by continuous superfusion with high (15 mM) potassium. Muscimol inhibited in a dose-dependent manner the release from compacta, but had no effect on the release from reticulata. Diazepam (100 μM) potentiated the inhibitory effect of muscimol, without having any effect by itself on the release from compacta. Baclofen (100 μM) inhibited the release from reticulata without affecting the release from compacta. Both picrotoxin (100 μM) and bicuculline (10-100 μM) facilitated the release from compacta, but, in reticulata, picrotoxin had no effect and bicuculline inhibited the release. Confirming previous results the present ones strongly suggest the presence of two types of GABA receptors modulating the release of GABA from the gabaergic terminals of substantia nigra: the classical type "A" receptor (activated by muscimol, blocked by bicuculline, affinity increased by benzodiazepines) in pars compacta, and the type "B" receptors (activated by baclofen), where picrotoxin has no effect and bicuculline (surprisingly) behaves as an agonist. (Supported by CONACYT- México).

264.19 COMPARISON OF THE ACTIONS OF THE GABA-A AGONIST, MUSCIMOL AND THE BENZODIAZEPINE RECEPTOR AGONISTS, CGS 9896 AND DIAZEPAM ON GABA AND GLUTAMATE TURNOVER IN THE RAT CORTEX AND CEREBELLUM. G.A. Rowan, C.F. Flaherty, H.S. Kim, C. Cosi*, D.L. Cheney and P.L. Wood. Dept. of Psychology, Rutgers Col, New Brunswick, N.J., 08903 and Neuroscience Research, Research Dept., Pharmaceutical Division, CIBA-GEIGY Corp., Summit, N.J. 07901.

Studies of benzodiazepine receptor ligands in vitro have clearly indicated differences in both the affinity and efficacy of coupling between benzodiazepine and GABA receptors. There are, however, few detailed studies of the in vivo consequences of these differences in efficacy of coupling between these receptors at the synaptic level. We have, therefore compared the effects of the GABA-A receptor agonist, muscimol; the benzodiazepine receptor agonist, diazepam; and the agonist/antagonist benzodiazepine receptor ligand, CGS 9896, on GABA and glutamate turnover in the rat cerebellum and prefrontal cortex.

For these studies, rats were injected intravenously with infusions of [^{13}C]glucose and the incorporation of this label into CNS glucose, glutamate and GABA was monitored by gas chromatography-mass fragmentography. Using this approach, muscimol reduced both GABA and glutamate turnover in the cerebellum and prefrontal cortex. GABA turnover was also decreased in both brain regions by CGS 9896 and only in the prefrontal cortex by diazepam. The decrease in GABA turnover elicited both by muscimol and the benzodiazepine receptor agonists was greatest in the prefrontal cortex. In marked contrast to the direct GABA agonist, the benzodiazepine receptor ligands did not alter glutamate turnover in either brain region.

These data indicate regional differences in the efficacy of coupling of the benzodiazepine/GABA receptor complex regulating GABAergic tone. Also, with this method, no benzodiazepine modulation of basal glutamatergic tone was detected. However, the role of benzodiazepine/GABA receptor coupling in synaptically- or pharmacologically-activated glutamatergic neurons remains to be determined.

265.1 IN VIVO LABELLING OF CENTRAL BENZODIAZEPINE RECEPTORS WITH ^{3}H-RO 15-4513. Bernard Sadzot*, J. James Frost, and Henry N. Wagner, Jr.* (SPON: H. Mayberg). Division of Nuclear Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Ro15-4513 is a high affinity triazine derivative of the benzodiazepine (BZ) antagonist Ro 15-1788 and is classified as a partial inverse agonist. Ro 15-4513 has the unique property of antagonizing ethanol-stimulated GABA receptor-mediated uptake of $^{36}Cl^{-}$ and blocking the anticonflict and intoxicating actions of ethanol in vivo (P.D. Suzdak, et al., Science, 1243-1247, 1986). This unique property suggests that Ro15-4513 could be an interesting ligand to image benzodiazepine receptors in man using positron emission tomography. Current ligands for imaging BZ receptors in man include the nonbenzodiazepine agonist ^{11}C-suriclone and the agonist ^{11}C-Ro 15-1788.

Parallel in vivo binding experiments were carried out in mice using ^{3}H-Ro 15-4513 and ^{3}H-Ro 15-1788 (0.2-0.5 ug/kg). Some animals were pretreated with Ro 15-1788 (15 mg/kg, i.p.) or flunitrazepam (3 mg/kg, i.v.) 15 min. prior to the injection of each tritiated drug to define nonspecific binding. Mice were killed at 2-120 min. after ^{3}H- drug administration. The percent of the injected dose in the brain was high for both tritiated drugs (ca. 10%/g). The percent displacable binding for ^{3}H-Ro 15-4513 was greatest in the cerebral cortex and hippocampus (>90%) and lowest in the cerebellum (60-70%). For ^{3}H-Ro 15-4513, the relative regional distribution revealed particularly high specific binding in the hippocampus relative to the other regions. The ratio of specific binding for ^{3}H-Ro 15-4513 to ^{3}H-Ro 15-1788 at 15 min. post injection was computed for 11 brain regions. This ratio varied from 1.85 in the hippocampus to 0.27 in the cerebellum demonstrating that the regional binding is quantitatively different for the two ligands.

These results demonstrate that Ro 15-4513 can be used to determine the distribution of central BZD binding sites in vivo. The distribution of Ro 15-4513 binding is different from that of Ro 15-1788 which may relate to the unique ability of Ro 15-4513 in reversing the effects of ethanol. Ro 15-4513 is a ligand which could be used in PET studies to understand the receptor-mediated effects of ethanol.

265.2 MUSCIMOL BINDING DIFFERS IN KREBS-RINGER'S BICARBONATE AND TRIS-CITRATE BUFFERS: DISPLACEMENT WITH GABA. Geoffrey Fuller and Paul Madtes Jr. (SPON: V. Wahby). Departments of Biology and Chemistry, Point Loma Nazarene College, San Diego, CA 92106.

Characterization of the GABA receptor frequently involves the use of a TRIS-citrate (TRIS) buffer. This has allowed extensive analysis of the receptor. However, recent evidence suggests that this receptor is sensitive to the presence of various ions, such as sodium, bicarbonate, and chloride ions. Consequently, it has been suggested that a physiological buffer should be used to determine GABA binding. One ligand which often is used to measure GABA binding is muscimol, a potent agonist which binds to the GABA receptor. We previously reported the effect of different buffering conditions on ^{3}H-GABA binding, with displacement by GABA, and on ^{3}H-muscimol binding, with displacement by muscimol. We found that high-affinity GABA binding is maximal if measured under physiological conditions and is not dependent on the buffer used to prepare the tissue. In contrast, high-affinity muscimol binding is maximal if the tissue is prepared using TRIS and is not dependent on the assay buffer. Since apparently there are more muscimol receptors than GABA receptors, we studied whether the displacement of ^{3}H-muscimol by GABA differed from that by muscimol, depending on the buffering conditions. Fresh bovine retinas were isolated, homogenized in sucrose, and stored frozen until assayed for ^{3}H-muscimol, displacing with unlabelled GABA. Membranes were washed in either TRIS or Krebs-Ringer's bicarbonate (KRB) buffer and assayed in either the same or the opposite buffer. Both high- and low-affinity binding were higher when assayed in TRIS and lower when assayed in KRB. GABA-displaceable, high-affinity binding was low when membranes were washed and assayed in KRB. These data suggest that the conditions for muscimol binding which is displaceable by muscimol differ from those for muscimol binding which is displaceable by GABA. Therefore, the buffering conditions may reveal different subclasses of GABA receptors. Thus, the characteristics of GABA binding may depend upon the ionic environment during both tissue preparation and assay. This work was funded by a grant from Research Corporation.

265.3 BRAIN GABA-A RECEPTORS ASSOCIATED WITH CHLORIDE CHANNELS AND INNATE ALCOHOL SENSITIVITY. K. Nieminen, O. Malminen* and E.R. Korpi. Research Laboratories of the Finnish State Alcohol Company, Alko Ltd., POB 350, SF-00101 Helsinki, Finland.

Alcohol may enhance the actions of GABA, the main inhibitory neurotransmitter in the brain. In a series of studies on the role of GABAergic mechanisms in ethanol-induced motor impairment, we have now compared the brain GABA/benzodiazepine receptor-chloride ionophore complexes in two rat lines selectively bred for low (AT) and high (ANT) acute ethanol sensitivity.

In the cerebral cortex of naive ANT and AT rats the maximal stimulation of [^{3}H]flunitrazepam by GABA was slightly greater in the AT's than the ANT's, suggesting a larger number of GABA-A receptors associated with benzodiazepine receptors in the AT's. Scatchard analyses of [^{3}H]flunitrazepam binding in repeatedly washed cortical membranes, however, did not reveal any difference in the binding parameters between the lines. In solubilized (CHAPS) receptors from the cerebral cortices, GABA was found to enhance the binding of [^{3}H]flunitrazepam slightly more in the ANT's. In several AT samples, a clear GABA stimulation was observed, however, in the presence of ethanol.

We did not find any significant difference between the lines in cerebrocortical or cerebellar membranes in the binding parameters of a chloride channel ligand, t-[^{3}H]butylbicycloorthobenzoate ([^{3}H]TBOB). Also the GABA inhibition of [^{3}H]TBOB binding and the enhancement of this GABA effect by lorazepam was similar in both rat lines. Preliminary experiments using muscimol-stimulated $^{36}Cl^{-}$ flux assay in a preparation of cerebrocortical and cerebellar microsacs have not yet shown any meaningful differences between naive AT and ANT animals.

In conclusion, our present data do not suggest that any major modification in the rat brain GABA/benzodiazepine receptor-chloride ionophore complexes has occurred as a result of selective breeding for high and low sensitivity to the motor impairment produced by moderate doses of ethanol.

265.4 **METHYLXANTHINES DIMINISH GABA/BENZODIAZEPINE RECEPTOR FUNCTION THROUGH AN ADENOSINE RECEPTOR-LIKE MECHANISM.** D.J. Roca* and D.H. Farb (SPON: M. Halpern) Dept of Anatomy and Cell Biology, SUNY Health Science Center, Brooklyn, New York 11203

Benzodiazepines (BZDs) are thought to exert their behavioral effects by enhancing the activity of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Biochemically, GABA-BZD interactions can be observed in binding studies, where it has been shown that BZDs enhance GABA binding and GABA enhances BZD binding. Chronic BZD exposure can diminish the ability of GABA to potentiate BZD binding. We refer to this reduction in the interaction between GABA and BZD binding sites as functional "uncoupling." Interestingly, methylxanthines (MXs), such as caffeine and theophylline, also bind to the BZD binding site and enhance GABA activity. Thus, we thought it would be of interest to examine the effects of prolonged methylxanthine treatment on BZD binding and its modulation by GABA. Seven day old primary chick brain cell cultures were treated for 36h with either theophylline, caffeine, or isobutylmethylxanthine (IBMX). Following treatment cells were harvested, homogenized and washed exhaustively. Reversible binding of 1 nM [^{3}H]flunitrazepam (FNZM) was determined by filtration in the presence and absence of GABA. Potentiation is expressed as the percentage increase of [^{3}H]FNZM binding over binding without GABA. Maximum enhancement of [^{3}H]FNZM binding by GABA was 70+3% (n = 31) in control cultures. In contrast, maximum GABA potentiation of [^{3}H]FNZM binding was reduced to 49+9% in theophylline treated cultures (EC_{50} = 0.89 nM, 25 pooled experiments), 45+9% in caffeine treated cultures (EC_{50} = 10 nM, 15 pooled experiments) and 55+3% in IBMX treated cultures (100 uM, n = 9). Chronic theophylline treatment did not significantly affect the B_{MAX} or K_D of [^{3}H]FNZM binding or the potency of GABA for potentiating [^{3}H]FNZM binding. Protein synthesis and degradation were unaltered after 36h exposure to theophylline, indicating that chronic treatment did not adversely affect cell viability. Chronic treatment of membranes did not result in uncoupling, suggesting that uncoupling requires active cellular function and is not due to a passive conformational change. Chloroadenosine (a stable adenosine receptor agonist) blocked the ability of chronic theophylline treatment to produce uncoupling, but did not alter the uncoupling induced by chronic treatment with flurazepam. This suggests that the MXs induce uncoupling through an adenosine-like receptor. In contrast the BZDs are thought to induce uncoupling through the BZD receptor. These results may help explain some of the side effects observed with chronic methylxanthine exposure.

265.5 THE CAGE CONVULSANT SITE OF THE LOCUST GABA RECEPTOR COMPLEX DIFFERS FROM ITS COUNTERPART IN MAMMALIAN BRAIN
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We have described a GABA receptor complex in locust brain that has a basic organization of interacting binding sites similar to the well characterized receptor of mammalian brain (Robinson, T. N. et al J. Neurochem. 47, 1955, 1986). Nevertheless there are significant differences in the detailed pharmacology of the GABA/muscimol and benzodiazepine binding sites. We have now looked at the cage convulsant site of the locust receptor and some important differences have emerged.

Binding of [^{35}S]TBPS to locust brain membranes at equilibrium over the range 2 to 150nM gives a K_d of 10nM but such data produce Scatchard plots indicative of positive cooperativity. Such effects are not seen in parallel binding studies on rat brain membranes. Further examination of the kinetics of binding in the locust show that both the on and off rate constants vary with [^{35}S]TBPS concentration in a manner consistent with positive cooperativity.

The interaction of the TBPS binding site with other sites on the locust receptor complex was also examined. We observed enhancement of [^{35}S]TBPS binding by GABA, (+30-80%) diazepam (+20-40%) and pentobarbital (+25-50%) that is, the converse of what is generally seen in studies on mammalian brain GABA receptor. Additionally [^{35}S]TBPS binding in the locust was insensitive to picrotoxinin whereas in mammalian brain strong antagonism is seen. It has been reported that in housefly thorax preparations picrotoxinin is a weak inhibitor of TBPS binding and GABA enhancement of the binding is seen (Cohen, E. and Cassida, J. Pestic. Biochem. Physiol., 25, 63, 1986). In fly head membrane preparations [^{35}S]TBPS binding shows complex kinetics, is insensitive to muscimol but is inhibited by picrotoxinin (Szamraj, O. I., Miller, T. and Olsen, R. W. Abstr. Neurosci. 12, 656, 1986). Thus it seems that the cage convulsant site of the insect GABA receptor complex not only differs from the corresponding site in mammalian brain but differences between insect species may occur.

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265.6 ALTERATIONS IN [^{35}S]TBPS BINDING BY HALIDE IONS.
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An integral component of the GABA-A receptor complex (GAR) is the chloride ionophore (CI). Other components of the GAR (i.e. binding sites for GABA, benzodiazepine and barbiturates) are known to allosterically interact with the CI as well as with each other. The "cage convulsant", [^{35}S]-t-butylbicyclophosphorothionate ([^{35}S]-TBPS), binds to a site associated with the CI. Previous studies have shown that [^{35}S]-TBPS binding is dependent on anions (Squires et al. 1983; Havoundjian et al. 1986). We have conducted experiments to further examine the mechanism by which halide ions stimulate [^{35}S]-TBPS binding in rat cortical membranes. Fluoride, Cl, Br and I produce a concentration dependent increase in [^{35}S]-TBPS binding. Binding at sub-saturating concentrations of [^{35}S]TBPS (2 nM) is greatest in Br followed by I > Cl > F. Iodide produces a biphasic response; increases at $\leq$ 100 mM and decreases > 200 mM. The EC_{50} values for F, Cl and Br are comparable (150-200 mM), however, that for I is much lower (16 mM). Scatchard analyses of [^{35}S]TBPS were conducted at various concentrations of the four ions. As the ion concentration increases the number of binding sites (Bmax) increases, whereas the KD for [^{35}S]TBPS is not altered. For example, the Bmax (pmol/mg prot) ranges from 2.3 in 25 mM to 5.6 in 100 mM NaCl. The KD is 138 nM over this range. For KI, the Bmax ranges from 4.5 to 6.6 in 25 mM and 100 mM, respectively. The KD under these conditions is 88 nM. It appears that the halide ions can affect [^{35}S]TBPS binding in two ways: 1) the affinity of the receptor is dependent on the specific halide ion and 2) the number of receptors is dependent on the concentration of the ion. Data will be presented on [^{35}S]TBPS binding for a number of other ionic conditions. The question as to whether all halide ions induce the appearance of the same maximal number of [^{35}S]TBPS binding sites will be discussed.

265.7 DIFFERENTIAL EFFECTS OF HALIDE IONS ON ALLOSTERIC INTERACTIONS OF THE GABA-A RECEPTOR COMPLEX.
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[^{35}S]t-butylbicyclophosphorothionate ([^{35}S]-TBPS) binds to a site on the GABA-A receptor complex (GAR) that is associated with the chloride ionophore. The GAR contains sites for other pharmacophores (i.e. benzodiazepines, barbiturates and GABAergic compounds) that are known to allosterically interact with each other. We have investigated the modulation of [^{35}S]TBPS binding by the above pharmacophores in the presence of various anions. Benzodiazepine (BDZ) agonists stimulate binding in F, Cl, and low Br (< 100 mM), but have no effect in I or high Br ($\geq$ 100 mM). Chloride allows the greatest stimulation (60%) followed by F and Br. The BDZ antagonist RO 15-1788 minimally stimulates binding in F and Cl (12% at 200 mM) and has no effect in Br or I. BDZ inverse agonists inhibit binding in F and Cl and have no effect in Br. Unlike BDZ agonists and antagonists, the inverse agonists stimulate binding in I (40% at 200 mM). GABA agonists and barbiturates produce biphasic effects in F, Cl and Br (< 50 mM), however, only the inhibitory phase is observed in I and high Br. Bicuculline, a GABA antagonist, stimulates binding in I, yet has no effect in F, Cl and Br. The stimulatory effects of bicuculline are similar to those seen for the BDZ inverse agonists and suggests an inverse agonist activity for bicuculline. RO 15-1788 specifically antagonizes the effects of BDZ agonists and inverse agonists and does not alter GABA and barbiturate effects. In addition, at concentrations with no intrinsic activity, bicuculline blocks GABA effects but not those of BDZs and barbiturates. These data suggest the following: 1) the effects of ligands for the GAR on [^{35}S]TBPS binding are qualitatively dependent on the specific halide ion and quantitatively dependent upon the concentration of the ion, 2) the effects of the various drugs are specific for the site of the GAR at which they interact, and 3) there is a continuum (inhibition → stimulation) of drug effects on [^{35}S]TBPS binding which correlates to the range (convulsion → sedation) of pharmacological responses for these drugs.

265.8 CHARACTERIZATION OF PERIPHERAL BENZODIAZEPINE BINDING SITES IN Nb2 NODE LYMPHOMA CELLS: EFFECTS ON PROLACTIN-STIMULATED ORNITHINE DECARBOXYLASE ACTIVITY AND PROLIFERATION.
H.E. Laird II, K.C. Duerson*, A.R. Buckley*, D.W. Montgomery*, and D.H. Russell*. Depts. of Pharmacol. & Toxicol. and Pharmacol., Colls. Pharmacy & Medicine, Univ. of Ariz.,Tucson, AZ, 85721.

^{3}H-Ro 5-4864, a specific ligand for the peripheral-type Benzodiazepine (P-BZ) receptor, binds to Nb2 Node Lymphoma cells in a specific saturable and reversible fashion. Scatchard analysis of the specific binding data reveals a single, homogeneous class of binding sites with an equilibrium dissociation constant of 3.941 ± 0.217 nM and a receptor density of 155 ± 11 fmol / 2 x 10^6 cells. Evaluation of the binding potencies of selected benzodiazepine (BZ) recognition site ligands showed a pattern characteristic of the P-BZ receptor (Ro 5-4864= PK11195>Diazepam>Flunitrazepam >>>>Clonazepam).

The range of high affinity ^{3}H-Ro 5-4864 binding paralleled the concentrations which enhance prolactin (PRL)-stimulated ornithine decarboxylase activity and ^{3}H-thymidine incorporation in Nb2 cells. Binding of Ro 5-4864 to high affinity P-BZ receptor sites (10^{-10},10^{-9} M) enhanced PRL-stimulated mitogenesis to values as high as 200% of PRL alone, whereas binding to low affinity binding sites (10^{-6} M) inhibited PRL-stimulated mitogenesis to values less than 80% of PRL alone, similar to the extent of inhibition reported in mouse thymoma cells in response to 10^{-6} M Ro 5-4864 (Wang et al., PNAS 81:753,1984). PRL and BZs may interact in the regulation of proliferation and/or differentiation in certain cell types. PRL and P-BZ receptors have similar tissue distribution patterns in rats. Interestingly, both have been reported to have tumor promotion properties in chemically-initiated rat liver, and both are implicated in nuclear oncogenic expression.

These data coupled with the known requirements for de novo RNA and protein synthesis for cell cycle pregression in response to a mitogenic stimulus, suggest a nuclear site of action for mitogenic or co-mitogenic properties. In support of this hypothesis, preliminary evidence has revealed high affinity binding sites for ^{125}I-PRL and ^{3}H-Ro 5-4864 in intact and functional nuclei from Nb2 Node Lymphoma cells. Studies are in progress to characterize these binding sites and to determine their function in regulating cell proliferation.

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265.9 CHARACTERIZATION OF PERIPHERAL BENZODIAZEPINE (P-BZ) BINDING SITES IN THE RAT LIVER. K. C. Duerson*, A.R. Buckley*, D.W. Montgomery*, D.H. Russell*, and H. E. Laird II (Spon: P. Consroe). Depts. of Pharmacol. & Toxicol. and Pharmacol., Colls. of Pharmacy & Medicine, Univ. of Ariz., Tucson, AZ, 85721.

Evidence suggests that the P-BZ binding site plays a role in the regulation of cellular proliferation (Wang, et al., PNAS 81:753,1984). Benzodiazepines (BZ) and Prolactin (PRL) may interact in the regulation of proliferation and/or differentiation in certain cell types. P-BZ and PRL receptors have similar tissue distribution patterns in rats. Interestingly, both have been reported to have tumor promotion properties in chemically-initiated rat liver, and both are implicated in nuclear oncogenic expression. The association between the P-BZ and PRL receptors in cellular proliferation has not been examined in this tissue.

The high affinity binding of ^{3}H-Ro 5-4864, the specific ligand for the P-BZ binding site, has been characterized in a crude membrane preparation from rat liver. ^{3}H-Ro 5-4864 binds in a saturable and reversible fashion to a single, homogenous class of binding sites with an equilibrium dissociation constant (Kd) of 4.9 + 0.3 nM and a receptor density (Bmax) of 658 ± 67 fmoles/mg protein. Evaluation of the binding potencies of selected benzodiazepine recognition site ligands showed a pattern characteristic of the P-BZ receptor: Ro 5-4864 = PK11195 > Flunitrazepam > Diazepam >>>> Clonazepam.

Examination of the subcellular distribution of P-BZ sites in the liver showed an increased density of sites in the nuclear fraction compared with the mitochondrial and microsomal fractions (Bmax = 1260, 938, and 936 fmoles/mg protein respectively). In order to characterize the P-BZ binding site, intact nuclei were isolated (Haddox and Russell, J. Cell Physiol. 109:447, 1981) from rat liver. Preliminary data using this preparation revealed a high affinity P-BZ binding site with Kd = 3.7 nM and Bmax = 1641 fmoles/mg protein. The binding in this preparation showed a two fold enrichment of P-BZ binding sites over the crude membrane preparation. In addition, there was an improvement in the degree of specific binding from 72% in the crude membrane preparation to 96% in the nuclear preparation.

Studies are in progress to characterize the nuclear P-BZ binding site and to explore its relationship to the PRL receptors in regulating cellular proliferation in the liver.

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265.10 RESPONSE TO VARIOUS BENZODIAZEPINE RECEPTOR LIGANDS FOLLOWING CHRONIC DIAZEPAM EXPOSURE IN RATS: SN RETICULATA SENSITIVITY AND GABA MODIFICATION OF RECEPTOR BINDING. M.A.Wilson, C.Heninger and D.W. Gallager, Dept. Psychiatry, Yale U.Sch.Med., Conn. Mental Hlth. Ctr., New Haven, CT 06508.

We previously reported that substantia nigra pars reticulata neurons (SNr) demonstrate reduced responses to systemically or iontophoretically applied benzodiazepines (BZ) following chronic diazepam (DZ) exposure(Wilson and Gallager,Eur.J.Pharm,1987). Such treatment also decreases the ability of GABA to enhance agonist binding. Following chronic agonist treatment, a shift in the spectrum of responsiveness of BZ ligands toward inverse agonists has been postulated (Little et al.,Br.J.Pharm.83, 1984; Peterson and Jensen, Eur.J.Pharm.133,1987). We have examined SNr responses to the antagonist Ro 15-1788 and the inverse agonist DMCM following chronic DZ exposure in rats. We have also examined the ability of chronic DZ treatment to alter GABA's effects on agonist, antagonist and inverse agonist binding.

Male rats were continuously exposed to constant levels of DZ (250 ng/g) for 3 weeks by the s.c. implantation of silastic capsules filled with crystalline DZ. Control animals received empty capsules. The administration of 1 mg/kg of the BZ antagonist Ro 15-1788 (i.v.) did not significantly alter the firing rate or iontophoretic sensitivity to GABA in control SNr. After chronic DZ treatment, Ro 15-1788 caused a significant increase in SNr firing rate to 42 ± 13 % above the pre-injection rate. GABA senstivity, however, was not altered by Ro 15-1788 administration (11 ± 8 % increase) and no GABA shift in Ro 15-1788 binding was observed in cortical membranes following chronic DZ exposure.

The systemic administration of the inverse agonist DMCM (0.05 up to 1.6 mg/kg,i.v.) induced a mean increase of 62% over basal firing rate in controls, which was reversed by Ro 15-1788. SNr responses to DMCM were apparently attenuated after chronic DZ treatment, at least in this dose range (6 ± 6 % vs 31 ± 9% in controls at 0.3 mg/kg). Ro 15-1788 administration following DMCM did not alter firing rate further. Although DMCM's effect on the GABA-induced shift in agonist binding was attenuated in cortical membranes from chronic DZ treated rats, this effect is complicated by a reduction in the GABA response after DZ treatment. We are currently analyzing GABA's effects on inverse agonist binding following chronic DZ exposure.

Thus, following chronic agonist administration, SNr responses to agonists and inverse agonists appear reduced. Reductions in both agonist and inverse agonist responses following chronic agonist treatment would suggest that such treatment modifies some common element coupling various BZ ligands with the GABA effector system.

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265.11 CHARACTERIZATION OF THE BENZODIAZEPINE/ALCOHOL ANTAGONIST RO 15-4513 BINDING SITES IN CEREBELLUM. D.M. Turner* and R.W. Olsen (SPON: A.K. Cho). Dept. of Pharmacology, Mental Retardation Research Center and Brain Research Institute, Univ. of California, Los Angeles,CA 90024.

The pharmacological properties of binding sites for the benzodiazepine and alcohol antagonist Ro 15-4513 were investigated. Several antagonist and inverse agonist ligands of the benzodiazepine receptor were found to inhibit binding to both those sites which were sensitive to diazepam and those unique sites which were insensitive to diazepam. The binding of [^{3}H]Ro 15-4513, described by Möhler, et al., Eur. J. Pharmacol. 102, 191-192 (1984) as a photoaffinity label for the benzodiazepine (BZ) receptor,was found to label a 51 kD band in various brain regions corresponding to the central BZ receptor labeled with [^{3}H]flunitrazepam, as well as a 57 kD band in cerebellar granular layer not labeled with [^{3}H]flunitrazepam and not blocked by 10 µM diazepam (Sieghart, et al.,J. Neurochem. 48, 46-52 (1987)). We measured reversible [^{3}H]Ro 15-4513 binding to cerebellar membranes and found two populations, diazepam sensitive (DZ-S) and diazepam insensitive (DZ-IS) sites. The DZ-S site had a K_d of 7 nM for Ro 15-4513 while the DZ-IS site had a K_d of 4 nM in frozen-thawed bovine cerebellum and in fresh rat cerebellum, with a four to five-fold greater abundance (B_{max}) of DZ-S sites to DZ-IS sites. Like cold Ro 15-4513 itself, several other benzodiazepine receptor ligands at 10 µM inhibited the [^{3}H]Ro 15-4513 binding to a greater extent than did 10 µM diazepam, demonstrating the binding of these compounds to the DZ-IS site. These compounds included Ro 15-1788, CGS-8216, DMCM and ZK93426, indicating that the DZ-IS site is inhibited by some antagonist and inverse agonist ligands including β-carbolines and a pyrazoloquinoline as well as the imidazobenzodiazepinones. Ro 15-4513 was reported (Suzdak, et al., Science 234, 1243-1247 (1986)) to antagonize the actions of ethanol in animal intoxication and in in vitro neurochemical activity involving potentiation of GABA-regulated ^{36}Cl- flux in synaptoneurosomes. However, in our studies at 0°C ethanol up to 100 mM did not affect the binding of [^{3}H]Ro 15-4513 to either the DZ-S site or the DZ-IS site; 200-500 mM ethanol inhibited only 5-15% of the control binding. Numerous drugs that modulate DZ-S (central BZ) receptors were tested on [^{3}H]Ro 15-4513 binding. Binding to DZ-S sites at 0°C was allosterically inhibited by etazolate, pentobarbital, muscimol and GABA, indicating a partial inverse agonist efficacy for Ro 15-4513. These ligands, however, did not appear to affect binding to DZ-IS sites, providing no direct evidence for association with GABA receptors. Thus the relevance of the DZ-IS binding sites to pharmacological actions of this unique imidazobenzodiazepinone drug remains to be explored.

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265.12 GENETIC DIFFERENCES IN BENZODIAZEPINE RECEPTOR SENSITIVITY IN MICE. D. J. Nutt*, R. G. Lister*, M. Costello* (SPON: M. Eckardt) LCS/DICBR, NIAAA, Bldg. 10/3B19, 9000 Rockville Pike Bethesda, MD 20892.

The present studies investigate differences in benzodiazepine receptor function in inbred strains of mice using the partial inverse agonist FG 7142. In outbred mice FG 7142 is proconvulsant, producing a maximal effect at 40 mg/kg but does not cause seizures at any dose (1). When given at a dose of 40 mg/kg to inbred strains weighing approximately 30 gms (Jackson labs) it caused full seizures in varying numbers.

DBA/2J	7/7	C58/J	5/6
C57BL/6J	0/7	LP/J	0/8
C3H/HeJ	0/9	CBA/J	5/7
A/J	3/7	MA/MyJ	0/8

These results suggest that BDZ receptor efficiency may be increased in the inverse agonist direction in those strains having seizures. DBA/2 mice have been found to be particularly sensitive to the effects of other convulsants acting at the benzodiazepine receptor (2). An increased sensitivity to FG 7142 has been observed in mice tolerant to the anticonvulsant effects of benzodiazepines (3).

In 2 strains from Charles River CD_{50} measurements were made (4). These were: DBA/2N 10 ± 1.4 (mg/kg ± 95% confidence limits), C57BL/6N 20 ± 1.5; No seizures were observed in NIH Swiss mice at doses up to 40 mg/kg.

To explore receptor function in these strains further FG 7142 was administered daily at a dose of 1/2 the CD_{50} (40 mg/kg for the N.I.H. Swiss). In all groups a progressive increase in the number of mice having full seizures was seen (chemical kindling).

This sensitization to the convulsant effect of FG 7142 persisted after a 3 week drug-free period, with increased effects being noted in the C57's. Despite the different responses to the acute dose all 3 strains kindled roughly equally suggesting that this phenomenon is independent of the acute sensitivity to inverse agonists.

1) Little, Nutt & Taylor (1984) Br. J. Pharmac. 83:951-8
2) Seale, Bolger and Skolnick (1985) Neurosci. Abs. 11:275
3) Nutt (1986) T.I.P.S. 7:457-60
4) Weil (1952) Biometrics 8:249-63

265.13 MIANSERINE, A NON-CONVULSANT ANTIDEPRESSANT, PARTIALLY REVERSES THE INHIBITORY EFFECT OF GABA ON ^{35}S-TBPS BINDING. R.F. Squires and E. Saederup.* The Nathan Kline Inst., Orangeburg, NY, 10962.

Most clinically used antidepressants can produce convulsions in overdosage and we have found that many of these antidepressants and/or their metabolites, reverse the inhibitory effect of GABA on ^{35}S-TBPS binding to rat brain membranes. Eleven clinically active antidepressants fully reverse 1 μM GABA, (Amoxapine, EC_{50} = 1.6 μM; SKF 10,810, 4.3 μM; Viqualine, 10 μM; Indalpine, 13 μM; Inkasan, 14 μM; Methylene Blue, 20 μM; Zimelidine, 24 μM; Dibenzepine, 54 μM; Minaprine, 44 μM; Viloxazine, 490 μM; and Deanol, 34 mM) and most of them are capable of producing convulsions. Imipramine, Amitriptyline, Nortriptyline,Trimipramine and Trazodone, although not GABA antagonists in this system, have metabolites that are: 2-hydroxy-imipramine, 10-hydroxy-amitriptyline, 10-hydroxy-nortriptyline, 2-hydroxy-trimipramine and 1-(m-chlorophenyl)-piperazine. In contrast, a group of 12 antidepressants only partially reverse the inhibitory effect of 1 μM GABA on ^{35}S-TBPS binding. (Mianserine, EC_{50} = 7.1 μM, 40% max. reversal; Fluotracene, 7.3 μM, 56%; Zometapine, 15 μM, 76%; Thiazesim, 16 μM, 79%; Quinupramine, 23 μM, 30%; Noxiptiline, 24 μM, 52%; Marplan, 29 μM, 23%; Doxepine, 34 μM, 31%; Citalopram, 140 μM, 22%; Tranylcypromine, 160 μM, 61%; MK 940, 330 μM, 53%; Fenmetozole (DH-524), 590 μM, 26%). Mianserine, the most potent of the partial reversers, is practically devoid of convulsant activity in humans, even after large overdosage (R.N. Brogden et al., Drugs, 16:273-301, 1978). Mianserine may, therefore, block a sub-set of GABA-A receptors, possibly involved in the clinical antidepressant effect, without blocking the GABA-A receptors involved in convulsions. Further, Mianserine has a pharmacological profile quite different from most other clinically effective antidepressants (R.M. Pinder and A.M.L. van Delft, Acta Psychiat. Scand., Suppl. 302, 59-71, 1983) (it is not a reserpine or tetrabenazine antagonist; it does not potentiate NE, DA or 5HT; it is not a muscarinic receptor blocker, and it is a strong 5HT antagonist), strengthening the hypothesis that it may exert its antidepressant effect by selectively blocking a sub-type of GABA-A receptors. Recently, 4 out of 5 novel partial reversers were found to act electrophysiologically as GABA mimetics on population spikes in the rat hippocampus, while one (pipazethate) was a potent GABA antagonist (Dalkara et al., Life Sci., 39:415-422, 1986). These results also tend to support the hypothesis of pharmacologically distinct GABA-A receptors with different distributions in the brain. It is proposed that some antidepressants may act by selectively blocking the inhibitory action of GABA on the brain's reward systems (J. Nazzaro and E.L. Gardner, Brain Res., 189:279-83 1980; H.C. Fibiger and A.G. Phillips, Science, 214:643-5, 1981).

265.14 **EFFECTS OF MEMBRANE FLUIDIZING TREATMENTS ON THE GABA-A RECEPTOR- CHLORIDE CHANNEL COMPLEX OF MOUSE BRAIN.** K.J. Johnson*, A.M. Allan, and R.A. Harris. Dept. Pharmacology, Univ. Colo. Hlth. Sci. Center, Denver, CO 80262; and VA Med. Res. Serv., Denver, CO 80222.

We found that the potencies of chemically diverse intoxicant - anesthetic drugs to inhibit [35S]TBPS binding and to enhance muscimol-dependent 36Cl uptake are correlated with their potencies as general anesthetics, suggesting that enhancement of GABA action is important for their anesthetic actions (Allan & Harris, JPET, in press). In the present experiments we investigated the ability of three diverse membrane fluidizing treatments to affect the GABA-A receptor-chloride channel complex.

ICR mice were sacrificed by decapitation and the whole brain was used in the preparation of membrane vesicles (microsacs). The microsacs were fluidized, as measured by the decreased polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH), by temperature (24-36°C), 2-(2-methoxyethoxy)-ethyl 8-(cis-2-N-octylcyclopropyl) -octanoate (A2C; 2-600 uM), or benzyl alcohol (0.1-50 mM). [35S]TBPS (4 nM) binding and basal and muscimol-dependent 36Cl uptake were measured as previously described (Life Sci. 39: 2005, 1985).

Our results revealed that each of the three membrane fluidizing treatments reduced [35S]TBPS binding in a dose-dependent manner. At the highest concentration tested, benzyl alcohol (50 mM), A2C (600 uM), and temperature (36°C) reduced [35S]TBPS binding by 100%, 60% and 20%, and decreased DPH polarization by 0.035, 0.038 and 0.040, respectively. Although each of the treatments inhibited [35S]TBPS binding, as did anesthetic agents, their ability to reduce [35S]TBPS binding was poorly correlated with their ability to decrease DPH polarization. No change in nonspecific binding was observed with any of the membrane fluidizing treatments.

The anesthetic agents investigated previously enhanced muscimol-dependent 36Cl uptake. However, we found that increasing temperature had no pronounced effect on muscimol-dependent 36Cl uptake and increased basal 36Cl uptake; benzyl alcohol and A2C decreased muscimol-dependent 36Cl uptake with little effect on basal 36Cl uptake.

We conclude that the effects of membrane fluidizing treatments on muscimol-dependent 36Cl uptake do not mimic those of anesthetic agents, and effects of drugs on [35S]TBPS binding are not closely related to membrane fluidization. We suggest that the actions of general anesthetics on the GABA-A receptor-chloride channel complex are not due to changes in bulk membrane fluidity. Supported by the VA and AA06399.

265.15 THE EFFECT OF Ca, K, AND GUANYL NUCLEOTIDES UPON $GABA_B$ BINDING IN ADULT RAT CORTICAL MEMBRANES, AND CULTURED CHICK CEREBRAL NEURONS. M.I. Al-Dahan*, M.H. Jalilian Tehrani, E. M. Barnes Jr., and R.H. Thalmann. Depts. of Cell Biology, Biochemistry and The Program in Neuroscience, Baylor College of Medicine, Houston, TX 77030.

We have examined several features of the $GABA_B$ receptor system in adult rat cortical neurons, and have begun to assay a culture of chick embryonic neurons for the same features. We have confirmed the findings of others that adult rat cortical neurons display $GABA_B$ binding in the presence of endogenous calcium only (15.1 ± 0.7 fmol/mg protein as inferred from exposure to 10nM [^{3}H]GABA in the presence of 100μM bicuculline). This binding was stimulated 2-3 fold in the presence of 2.5mM Ca (41.2 ± 6.2 fmol/mg). The GTP analogue GMPNP (0.5mM) reduced the binding of (^{3}H)GABA to membranes solubilized in 0.05% Triton X-100 (30.6 fmol/mg versus a control level of 79.6 ± 2 fmol/mg). Mg did not stimulate $GABA_B$ receptor binding to the same extent as did Ca (1.5-fold increase in the presence of 1.2-5mM Mg versus a 2.2-fold increase in the presence of 2.5mM Ca), suggesting that this stimulation of binding was to some degree related to an ion whose conductance is affected by $GABA_B$ receptors. However, we have been unable to find evidence for similar stimulation by another ion whose conductance is affected by $GABA_B$ receptors, namely, K. Indeed at K concentrations exceeding 5mM, binding was reduced by about 50%. The latter result may reflect the release of endogenous GABA by K. Cultures from embryonic chick cerebrum, cultured for 7-14 days, were found to have specific $GABA_B$ binding as assayed in the presence of 2.5mM Ca by [^{3}H] - GABA in the presence of 100μM bicuculline (11.8 ± 3.8 fmol/mg), or by [^{3}H] - baclofen (10.9 ± 5.1 fmol/mg). This binding density was 20-30% of that assayed under similar conditions in adult rat cortex or adult chicken cerebrum using [^{3}H] - GABA or [^{3}H] - baclofen. $GABA_B$ binding was stimulated about 2-fold by 2.5mM Ca with an EC 50 of less than 10μM, as in the case of adult rat cortical membranes. GMPNP also inhibited GABA binding. Thus far, then, several features of an adult mammalian $GABA_B$ receptor system appear to be expressed in the chick neuronal cultures, namely 1) the presence of $GABA_B$ receptors, 2) the regulation of these receptors by calcium and 3) evidence of $GABA_B$ linkage to a GTP-binding protein. Supported by NIH grants NS21713, DK17436 and NS11535.

265.16 SUBACUTE ADMINISTRATION OF BICUCULLINE ON GABA RECEPTOR CHARACTERISTICS IN RAT BRAIN. Yoshihisa Ito*, Dong Koo Lim*, Beth Hoskins* and Ing K. Ho. Dept. Pharmacol. & Toxicol., Univ. MS Med. Ctr., Jackson, MS 39216.

Effects of acute and subacute administration of bicuculline on [^{3}H]-muscimol binding to various brain regions of Sprague-Dawley rats were studied. In subacutely treated animals, rats received bicuculline, 2 mg/kg, ip, daily for 10 days. For acute treatment, rats received saline vehicle daily for 9 days and bicuculline, 2 mg/kg, ip, on the 10th day. The control group received saline vehicle once a day for 10 days. The volume of injection was .1 ml/100 g body weight. All animals were sacrificed 30 min after the last injection.

There was no significant change in growth rates in rats treated subacutely with bicuculline. However, in these rats there was a significantly increased [^{3}H]-muscimol binding in frontal cortex, cerebellum, striatum and substantia nigra. The increase in [^{3}H]-muscimol binding was 69% in frontal cortex, 38% in cerebellum, 16% in striatum and 49% in substantia nigra. Scatchard analysis of the binding isotherms revealed that the K_d of high affinity binding sites was significantly lower in rats treated subacutely with bicuculline as compared to that of the control animals in all the regions studied. A significant increase in the B_{max} of high affinity sites was observed in cerebella of rats treated subacutely with bicuculline as compared to that of control groups, with no change in frontal cortex, striatum and substantia nigra. As far as low affinity binding sites were concerned, a significantly lower K_d in frontal cortex and a significantly higher B_{max} in cerebellum were noted in rats treated subacutely with bicuculline.

Studies on the in vitro displacement curve of [^{3}H]-muscimol binding by bicuculline also revealed that the K_i of bicuculline was significantly lower in rats treated subacutely with bicuculline as compared with that of the control animals. The acute administration of bicuculline, however, affected neither K_d nor B_{max} of both high and low affinity binding sites of various regions studied.

These results suggest that $GABA_A$ receptors are upregulated after subacute administration of bicuculline. (This study was partially supported by grant 5/SO7 RR05386 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.)

265.17 MODULATION OF γ-HYDROXYBUTYRATE BINDING BY GABA RECEPTOR COMPLEX LIGANDS. A. C. Nichols and O.C. Snead, III, Neuropsychiatry Research Program and Pediatrics Department, Univ. of Alabama at Birmingham, Birmingham, AL 35233.

Gamma-hydroxybutyric acid (GHB) produces generalized absence seizures in rodents. Augumentation of GABAergic transmission appears to exacerbate this effect. In the present study, we explored what effects modulators of the GABA chloride ionophore have on the specific binding of ^{3}H-GHB in rat synaptosomal membrane preparations. It has already been shown that chloride and other anions that are permeable to the chloride ion channel inhibit binding of [^{3}H]-GHB (Snead,Epilepsia 25:671, 1984). Therefore the effect of various agonists, antagonists, and modulators of the gamma aminobutyric acid (GABA)-benzodiazepine - picrotoxin chloride ionophore on specific, high affinity ^{3}H-GHB binding in synaptosomal membranes was determined. These compounds included GABA, the $GABA_A$ agonist muscimol, picrotoxin, diazepam, and pentobarbital. In addition the $GABA_B$ agonist baclofen and the glycine antagonist strychnine were also tested to determine if these compounds affected [^{3}H]-GHB specific binding.

^{3}H-GHB was custom labeled from gamma-crotonolactone. After 3 months it was necessary to repurify the ^{3}H-GHB by TLC. Pentobarbital and picrotoxin strongly enhanced ^{3}H-GHB binding while diazepam produced a more modest enhancement of binding. The other compounds showed no effect. This binding enhancement of binding was seen both in the presence and absence of 100 mM chloride, and was not anion dependent. Our data suggests that the GHB binding site may in some way modulate the chloride ion channel.

Such a mechanism could explain the convulsive action of GHB, and account for the fact that GABA potentiates experimental generalized absence seizures (King, Neuropharmacology 18:47-55, 1979)

265.18 EMBRYONIC DEVELOPMENT OF TWO CLASSES OF BENZODIAZEPINE BINDING SITES IN THE CHICK. T.T. Gibbs*, L.A. Borden* and D.H. Farb (SPON: H.-S. Yin). SUNY Health Science Center, Brooklyn, NY 11203.

We have examined the embryonic development of 2 classes of benzodiazepine binding sites in the embryonic chick CNS. Binding was examined by competition and saturation, using as radioligands [^{3}H]flunitrazepam, a classical benzodiazepine anxiolytic, and [^{3}H]Ro5-4864, a convulsant benzodiazepine. High-affinity (K_d = 2.3 nM) [^{3}H]flunitrazepam binding sites (site-A) are present as early as embryonic day 5 (Hamburger and Hamilton stage 27) and increase throughout development (B_{max} = 0.3 and 1.3 pmol/mg protein in 7 and 20 d brain membranes, respectively). When either 7 or 20 d brain membranes are photoaffinity labeled with [^{3}H]flunitrazepam and ultraviolet light, the radioactivity migrates as 2 bands on SDS-PAGE (Mr 48000 and 51000), indicating that high affinity flunitrazepam binding sites are similar in early and late embryos. Potentiation of [^{3}H]flunitrazepam binding by GABA was evident at both 7 and 20 d of development, indicating that functional coupling between site-A and the GABA receptor is acquired early in development.

Importantly, we have also identified a novel site (site-B) that binds classical benzodiazepine agonists with low affinity (micromolar), but displays high affinity (K_d = 41 nM) for Ro5-5864. Site-B exhibits characteristics expected for a functional receptor, including stereospecificity and sensitivity to inactivation by heat or protease treatment. Saturation binding studies employing [^{3}H]Ro5-4864 indicate that levels of site-B are similar in 7 and 20 d brain (ca. 2.5 pmol/mg protein). The function of site-B is not known, but its early appearance in embryonic brain suggests that it may play a role in early embryonic development.

GABA AND BENZODIAZEPINE RECEPTORS: MOLECULAR CHARACTERIZATION

266.1 PREPARATION AND PROPERTIES OF PURIFIED PEPTIDE SUBUNITS OF THE HUMAN BRAIN GABA RECEPTOR COMPLEX. M.Bureau*, A.I.Dilber*, G.Smith* and R.W. Olsen (SPON:A.V.Delgado-Escueta). Dept. Pharmacology and Brain Research Institute, Univ.California, Los Angeles, CA 90024.

Peptide subunits from affinity column-purified GABA-benzodiazepine(BZ) receptor from human brain were prepared by preparative SDS-PAGE, identified by photoaffinity labelling and immunoblotting, and eluted for further chemical characterization. Frozen human brain (parietal,frontal cortex) obtained at autopsy from neurologically normal patients was provided by the Human Neurospecimen Bank, Wadsworth VAH, Los Angeles. GABA-BZ receptor binding properties in human brain membranes were similar to those of animal tissues. Triton X-100 solubilized receptor from 100 g brain was purified about 1000-fold on an immobilized BZ affinity column (Stauber et al.,Eur.J.Biochem. in press) and photoaffinity labeled (Deng et al., BBRC 138,1308,1986). Aliquots of 100 pmol [^{3}H]FLU binding sites were incubated for 60 min at 4°C with 10 nM [^{3}H]FLU, followed by UV irradiation for 10 min. Aliquots precipitated with TCA, centrifuged and washed, showed incorporation of large amounts of covalently incorporated protein-bound [^{3}H]FLU. SDS-PAGE, followed by radioactivity measurement on the sliced gel, revealed a major [^{3}H]-FLU labeled band at 52 kD and minor bands at 58 kD and 46 kD, corresponding to three of the major stained bands in the purified receptor. The major BZ binding subunit (52 kD) was similar to that observed in rat and cow brain preparations, recognized on immunoblots by a monoclonal antibody bd24 specific for the BZ-binding subunit of the GABA-BZ receptor (Schoch et al., Nature 314,168 1985). The 46 kD band was variable and appears to be a proteolytic break-down product of the 52 kD peptide. The 58 kD band lightly labeled with [^{3}H]FLU corresponds to the GABA-binding subunit photolabeled with [^{3}H]muscimol that binds Schoch antibody bd17. Two other stained peptides observed at 32 kD and 62 kD were not labeled with [^{3}H]FLU, [^{3}H]muscimol, or Schoch antibodies, and may represent contaminants, break-down products, true subunits, or cytoskeletal elements. Preparative gels were electrophoresed using mg amounts of purified receptor, and all 5 peptides were eluted from the gel for further chemical analysis and antibody production. The major subunits (52 and 57 kD) gave no N-terminal sequence, so are being fragmented and purified by HPLC for sequencing.

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266.2 PHOSPHORYLATION OF A SUBUNIT OF THE GABA/BENZODIAZEPINE RECEPTOR BY A RECEPTOR-ASSOCIATED PROTEIN KINASE. P.M. Sweetnam, J.F. Tallman, D.W. Gallager, and E. J. Nestler. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Changes observed in the functional state of the GABA/benzodiazepine receptor in response to GABA and benzodiazepine exposure suggest that the receptor undergoes reversible modification. Yet, the molecular basis of such changes remain unknown. In the present study we investigated whether the GABA/benzodiazepine receptor undergoes phosphorylation.

Partially purified preparations of GABA/benzodiazepine receptor were obtained from rat brain by use of benzodiazepine affinity chromatography. Such preparations contain a major protein of M_r 50 kD, designated the α subunit of the receptor, plus a number of other proteins of unknown identity. The β subunit of the receptor has not yet been identified. Aliquots of the receptor preparation were incubated in the presence of standard phosphorylating conditions for 15 min at room temperature. The phosphorylated samples were then analyzed by one dimensional SDS polyacrylamide gel electrophoresis or two dimensional electrophoresis with isoelectric focusing in the first dimension.

Analysis of the samples by one dimensional electrophoresis revealed that a number of proteins in the receptor preparation are phosphorylated by an endogenous protein kinase activity present at high levels in the preparation. A major, broad phosphorylated band was observed with a M_r of about 52-50 kD. Two dimensional electrophoresis was employed to determine whether phosphorylated 50 kD receptor subunit was contained in this band. By two dimensional electrophoresis, it was found that a major phosphoprotein band comigrated with the coomassie blue-stained purified 50 kD receptor subunit; this band also comigrated on accompanying gels with the photolabeled and immunolabeled 50 kD receptor subunit. Peptide mapping and immunoprecipitation will be used to definitively identify this comigrating phospoprotein as the receptor. Another major phosphoprotein band was a highly acidic protein of M_r 52 kD; the identity of this protein, which did not correspond to a coomassie-blue stained protein band in this preparation, remains unknown.

Initial attempts to characterize the endogenous protein kinase activity present in the receptor preparation showed that it was not altered by cyclic AMP, cyclic GMP, calcium plus calmodulin or phosphatidylserine, protein kinase inhibitor, trifluoperazine, or EGTA under the assay conditions used. In addition, the protein kinase activity did not appear to be affected by various GABA and benzodiazepine agonists and antagonists.

Further studies are needed to characterize receptor phosphorylation and the associated protein kinase activity, as well as to determine their physiological significance.

266.3 BIOCHEMICAL PROPERTIES OF THE BENZODIAZEPINE-GABA RECEPTOR PROTEIN FROM CODFISH BRAIN. L. Deng *, R.W. Olsen, and M. Nielsen[1]. Dept. Pharmacology and Brain Research Institute, Univ. California, Los Angeles, CA 90024, and [1]Psychopharmacology Lab., Sct. Hans Hospital, Roskilde, Denmark.

The molecular weight of benzodiazepine (BZ) and GABA receptor proteins from brains of the North Atlantic codfish were estimated by photoaffinity labeling and irradiation inactivation target size analysis. Crude codfish brain homogenates bound [^{3}H]flunitrazepam (FLU) and [^{3}H]Ro 15-1788 with affinity, quantity, and pharmacological specificity similar to mammalian brain central BZ receptors. Crude membranes and receptor purified several hundred-fold on a BZ affinity column were photoaffinity labeled with [^{3}H]FLU and analyzed by SDS-PAGE as described in Deng et al., BBRC 138, 1308 (1986). A major radioactive band was observed in both cases at 53 kD, corresponding to the [^{3}H]FLU-labeled peptide from rat brain run in an adjacent lane. A minor peak about 10% as big was observed at 47 kD; labeling of both was prevented by 10µM diazepam. Irradiation of frozen intact codfish brains, preparation of membranes, binding assays, and target size estimations were performed as described in Nielsen et al., Biochem. Pharmacol. 34, 3633 (1985). Scatchard plots (7 concentrations) were employed to determine Bmax values for several radiation doses (0-20 Mrad). In contrast to photolabeling studies, the radiation target size for BZ receptor ([^{3}H]Ro 15-1788 binding) was 31,400 ±4000 daltons (n=6) and for GABA receptor ([^{3}H]muscimol binding to Ag+-activated membranes or [^{3}H]GABA binding to frozen-washed membranes) was 71,500 ±10,000 daltons (n=4). This contrasts with target sizes of 50-55 kD for both activities in rat brain, consistent with the photolabeled peptide bands on SDS-PAGE. The contribution of different subunits to these binding activities, as well as possible effects of proteolysis, on the codfish BZ-GABA receptor protein is being investigated with affinity column-purified samples. Comparison of different animal species may allow interesting structural insight into the evolution of the receptor-chloride channel complex.

Supported by NIH Grant NS 22071.

266.4 BENZODIAZEPINE AGONISTS PROTECT A HISTIDINE RESIDUE FROM DIETHYL PYROCARBONATE MODIFICATION WHEREAS PROPYL BETA-CARBOLINE DOES NOT. B. Lambolez* and J. Rossier. Laboratoire de Physiologie Nerveuse, CNRS, 91190 Gif-sur-Yvette, France.

The binding sites for both benzodiazepines and beta-carbolines (two competitive ligands of the benzodiazepine receptor) were studied in membranes of rat cerebral cortex in order to determine whether they are partially or completely overlapping.

A 57% decrease of the binding of the benzodiazepine antagonist (^{3}H)Ro 15-1788 was observed between pH 7.5 and 5.5. This suggested that a histidine residue, which has a side chain pK_a in this pH range, may be important in benzodiazepine binding.

An Eadie-Hofstee analysis of (^{3}H)Ro 15-1788 binding showed a 46% decrease in the number of sites (B_{max}) at pH 5.5 as compared to pH 7.5, the affinity (K_d) remaining unchanged.

This indicates that the protonation of a histidine residue within or close to the benzodiazepine binding site blocks the benzodiazepine binding. This was confirmed using diethyl pyrocarbonate, a quite selective reagent of histidine residues, under the following conditions: 1 mM diethyl pyrocarbonate in 20 mM sodium phosphate buffer containing 0.2 M NaCl at pH 6 was found, in 15 min at 20°C, to block 67% of (^{3}H)Ro 15-1788 binding sites without changing the K_d as revealed by Eadie-Hofstee analysis.

In order to assess whether this histidine residue is located inside or adjacent to the benzodiazepine and beta-carboline binding sites, experiments were performed using either benzodiazepine or beta-carboline to protect (^{3}H)Ro 15-1788 binding against diethyl pyrocarbonate treatment. It was found that flunitrazepam, a benzodiazepine agonist, completely protects the benzodiazepine binding site from diethyl pyrocarbonate modification, whereas the propyl beta-carboline does not.

This histidine residue may be a part of the benzodiazepine agonists binding site which is distinct from the beta-carboline binding site. In this case, this histidine residue would be sterically protected when benzodiazepine agonists are bound.

Alternatively, this histidine residue may be allosterically hidden away from diethyl pyrocarbonate modification in a conformation of the benzodiazepine receptor macromolecule which would be stabilised by the binding of benzodiazepine agonists but not by the binding of antagonist propyl beta-carboline.

266.5 PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS: ISOLATION FROM OUTER MITOCHONDRIAL MEMBRANES: PORPHYRINS AS ENDOGENOUS LIGANDS: HORMONAL ASSOCIATIONS. A. Verma*, R.R. Trifiletti*, E.M. Michael*, S.H. Snyder. Departments of Neuroscience and Obstetrics and Gynecology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Besides interacting with "central type" receptors in the brain, benzodiazepines (BZ) bind with high affinity to receptors in peripheral tissues. Insight into their function derives from subcellular fractionation studies localizing the receptor to outer micochondrial membranes (Anholt et al. J. Biol. Chem. 261:576, 1986). After photoaffinity labeling with [^{3}H]flunitrazepam we have purified the peripheral receptors to apparent homogeneity and identified two SDS-PAGE bands at 30 KD and 35 KD. The 35 KD band appears identical to the voltage dependent anion channel of outer mitochondrial membranes, designated mitochondrial porin. In analyzing tissue extracts for substances competing for receptor binding, we have isolated porphyrins as endogenous ligands of high affinity (KI=15 nM for protoporphyrin IX) (Verma et al. PNAS 84:2256, 1987). The selective, intense localization of BZ receptors to Leydig cells of the testes and the glomerulosa cells of the adrenal cortex suggested a role in hormone synthesis and/or secretion (DeSouza et al., Endocrinol. 116:657, 1985). We now report autoradiographic localization of [^{3}H]PK11195 labeled BZ receptors in rat ovary selectively to theca interna cells and to the corpus luteum, which are steroidogenic, while granulosa cells of ovarian follicles lack receptors. Benzodiazepine and porphyrin effects on hormonal secretion may clarify receptor function.

266.6 PURIFICATION OF THE GABA/BENZODIAZEPINE RECEPTOR COMPLEX BY IMMUNOAFFINITY CHROMATOGRAPHY. D. Park*, J. Vitorica* and A.L. de Blas. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

The $GABA_A$ receptor (GABAR) is a membrane protein functionally associated to benzodiazepine receptor (BZDR), the Cl^- channel and a receptor for the barbiturates. We have purified the GABAR/BZDR complex from bovine brain by affinity chromatography on the immobilized benzodiazepine Ro 7-1986/1 according to the method of Sigel et al.[1]. Monoclonal antibodies (mAbs) and antisera to the affinity purified GABAR/BZDR complex have been produced[2]. We have used the mAb 62-3G1 for the purification of the GABAR/BZDR complex by immunoaffinity chromatography. The mAb 62-3G1 immunoprecipitated the [^{3}H]flunitrazepam (FNZ) and [^{3}H]muscimol binding activities of the affinity purified receptor complex. In addition, the mAb 62-3G1 immunoprecipitated the [^{35}S]TBPS binding activity from CHAPS-solubilized membranes. The mAb 62-3G1 also reacted with the affinity purified receptor complex in a solid phase radioimmunoassay. In immunoblots using either affinity purified receptor complex or crude rat brain membranes, the mAb 62-3G1 bound to the 57,000 Mr peptide but it did not react with the 51,000 Mr peptide.

For immunoaffinity chromatography, the mAb 62-3G1 was coupled to Affigel-10. The GABAR/BZDR complex, either photoaffinity labeled or not with [^{3}H]FNZ, was solubilized from rat brain membranes and applied to the 62-3G1 Affigel-10 column. After several washes, the bound receptor was eluted with 50 mM citrate (pH 3.0)/ 0.05 % Triton X-100. Under these conditions, between 50% and 80% of the [^{3}H]FNZ binding activity was retained by the column, and between 60% and 80% of the retained activity was eluted from the column in a single peak. Therefore the yield of this procedure was 3-4 fold higher than the yield of the ligand affinity chromatography. The SDS-PAGE and silver staining of the immunoaffinity purified receptor, revealed peptides of 51,000 and 57,000 Mr.

[1] Sigel, E., F.A. Stephenson, C. Mamalaki and E.A. Barnard.(1983) J. Biol. Chem. 258, 6965-6971.
[2] Vitorica, J., D. Park and A.L. de Blas.(1987) Eur. J. Pharmacol. (in press)

266.7 COMPARATIVE ANALYSIS OF GABA/BENZODIAZEPINE RECEPTOR PROTEINS PURIFIED FROM RAT CEREBELLUM, SPINAL CORD, ADULT AND EARLY POSTNATAL CORTEX. Thomas N. Sato*, Alessandro Guidotti and Joseph H. Neale. Department of Biology and Fidia-Georgetown Inst. for Neurosciences, Georgetown University, Washington D.C. 20057.

The GABA/benzodiazepine receptors have been characterized as type I and type II, based upon a higher affinity of type I for β-carbolines and the triazolopyridazine compound, Cl 218872. We have analyzed GABA/benzodiazepine receptor diversity associated with the pharmacologically defined type I and type II receptors with 2-D gel electrophoresis of the protein constituents of purified (up to 2000-fold) receptor preparations from cerebellum and spinal cord, well as from postnatal day 8 and adult rat cortex.

The Triton X-100 solubilized GABA/benzodiazepine receptor complex was purified by Ro7-1986/001-agarose affinity chromatography and elution with clorazepate. The benzodiazepine was removed by DEAE-Sephacel chromatography and the purified receptor preparations assayed for benzodiazepine and GABA binding. The receptors purified from cerebellum exhibited maximal sensitivity to Cl 218872, indicating a high concentration of type I receptor, while the sensitivity of the cortical preparation was consistent with the presence of a greater concentration of type II. Purified spinal cord receptor as well as that from 8 day postnatal rat cortex were relatively insensitive to Cl 219972, indicating the presence of nearly exclusive populations of type II receptors. In contrast to the pharmacological differences exhibited by the purified adult cortex and cerebellum receptors, they had very similar protein constituents, when analyzed by IEF-SDS PAGE. Two-dimensional analysis of flunitrazepam photolabeled proteins in the two purified preparations further supported the conclusion that they were very similar. When the purified neonatal and spinal cord receptors were analyzed by SDS-PAGE, they differed from the cerebellum and adult cortex in the mobility of major proteins. Data on the properties of receptor complex proteins, as determined by IEF-SDS PAGE, together with 2-D TLC peptide maps of individual proteins will be useful in formulating more precise models of receptor structure, relatedness, diversity and development. (supported by NIDA grant, DA 02297)

266.8 A QUANTITATIVE EVALUATION OF THE RELATIVE AMOUNT OF SPECIFIC GABA-BENZODIAZEPINE RECEPTOR mRNA AFTER INJECTION OF TOTAL mRNA INTO XENOPUS OOCYTES. E. Zyzek*, B. Vimort* and J. F. Pujol* (SPON : A. Calas) Lab. de Neuropharmacologie - Fac. Medecine Alexis Carrel - Rue Guillaume Paradin - 69372 Lyon Cédex 2 - France.

GABA-benzodiazepine receptor-chloride channel complexes could be detected by electrophysiological recordings in Xenopus oocytes previously injected with messenger RNA extracted either from optic lobes of chick embryos or adult rat hippocampus. The oocyte's ability to translate correctly exogenous messengers was used to develop a routine method which could allow a quantitative evaluation of the specific mRNA coding for the GABA-benzodiazepine receptor proteins among total mRNA injected. The conditions for the validation of this method were determined. According to the equation describing the dose-response curves of the electrophysiological response to GABA as a function of the total mRNA concentration injected in oocytes, we concluded that total mRNA extracted from the optic lobes of chick embryos were more enriched in specific GABA-receptor mRNA than total mRNA from rat hippocampus. This method could be extended to others tissues and/or receptors messengers quantification.

GABA AND BENZODIAZEPINE RECEPTORS: BEHAVIORAL STUDIES

267.1 ALTERATIONS OF THE BEHAVIORAL EFFECTS OF BENZODIAZEPINE RECEPTOR LIGANDS DURING CHRONIC DIAZEPAM ADMINISTRATION. C.A. Sannerud*, J.M. Cook and R.R. Griffiths* (SPON: S.E. Lukas). The Johns Hopkins Univ. Medical School, Baltimore, MD 21205 and the University of Wisconsin, Milwaukee, WI 53201

Chronic administration of benzodiazepines (BZ) produce a number of physiological alterations including physical dependence on the drug which is manifest by the appearance of a withdrawal syndrome upon termination of drug administration. In order to more fully understand the functional changes produced by chronic BZ administration, the behavioral effects of several BZ receptor ligands which appear to exist on a continuum of efficacy were assessed in BZ-dependent baboons chronically receiving 20 mg/kg/day diazepam. Dose-effect functions for the behavioral effects of a BZ agonist (midazolam maleate MDZ; 0.32-100 mg/kg), an antagonist (Ro15-1788 Ro; 1-32 mg/kg), and an inverse agonist (ß-CCE HCl; 0.32-100 mg/kg) were determined in dependent baboons by scoring behavioral signs of sedation and excitation for 1 hour after intramuscular injections. The behavioral effects of ß-CCE (0.32, 3.2, 32 mg/kg) in combination with Ro (3.2, 10 mg/kg) were also determined in dependent baboons.

Compared to the effects of these compounds observed in non-dependent baboons (Sannerud et al., Fed. Proc. 46:1301, 1987), qualitative and quantitative differences in the expression of these signs were found in dependent baboons. ß-CCE produced dose-dependent increases in abnormal postures, limb tremor, vomiting and jerks/convulsions in both the dependent and non-dependent baboons, however ß-CCE was 3-10 times less potent in dependent baboons. MDZ produced sedation and ataxia in non-dependent baboons, but produced no such effects in dependent baboons, even at doses 100-fold higher than those producing effects in non-dependent baboons. Although Ro produced no behavioral signs in the non-dependent baboon, Ro produced an inverse agonist-like profile of behavioral effects in the dependent baboons: a dose-dependent increase in limb tremor, abnormal postures and jerks/convulsions. The Ro precipitated withdrawal syndrome in dependent baboons was similar to the behavioral effects produced by ß-CCE in non-dependent baboons. When given in combination, Ro potentiated the effects of ß-CCE producing increases in abnormal postures, limb tremor, twitches/jerks and vomiting. Taken together, these data show that physical dependence may quantitatively and qualitatively change the effects of BZ ligands. If the rightward shift of the ß-CCE curve in dependent baboons is assumed to reflect competition with diazepam at the receptor site, the remaining observations suggest that physical dependence appears to shift the effects of BZ ligands along a continuum of efficacy in the direction of the inverse agonist. Supported by DA-01147.

267.2 AN INCREASE IN LOCOMOTOR ACTIVITY PRODUCED BY CENTRAL ADMINISTRATION OF BETA-CARBOLINE-3-CARBOXYLIC ACID ETHYL ESTER IN RATS. L. J. Wichlinski and R. A. Jensen. Biopsychology Laboratory, Department of Psychology, Southern Illinois University, Carbondale, IL 62901.

Recently we reported that beta-carboline-3-carboxylic acid ethyl ester (B-CCE) produces a suppression of locomotor activity in rats when given at a dose of 10.0 mg/kg i.p. (Wichlinski, L.J. & Jensen, R.A., Soc. Neurosci. Abstr., 12:660, 1986. This effect is attenuated by the benzodiazepine receptor antagonist Ro 15-1788 suggesting an involvement of benzodiazepine-receptor systems. However, we cannot be sure that the B-CCE-induced suppression of locomotor activity is due to activation of central benzodiazepine/B-CCE recognition sites. The discovery that benzodiazepine receptors exist in peripheral tissues (Kataoka, Y. et al., Proc. Nat. Acad. Sci., U.S.A., 81:3218, 1984) raises the possibility that this suppression of locomotor activity may be the result of a peripheral site of action for this compound.

To confirm that B-CCE is suppressing locomotor activity via initial activation of central benzodiazepine/B-CCE recognition sites, we administered B-CCE (5.0 and 10.0 ug) directly into the lateral ventricles. Locomotor activity was assessed using automatic activity monitors for the next 30 minutes. Preliminary data indicate that both doses of B-CCE increase locomotor activity in rats. This finding stands in contrast to the reduction in activity seen after peripheral administration of this compound. The elevation in activity level is most apparent during the latter half of the 30 minute period. While vehicle-treated rats habituate, B-CCE-treated rats fail to do so during the latter portion of the activity period. Testing with a wider dose range of B-CCE is necessary to determine whether this compound produces distinct effects on activity depending on the route of administration. In addition, further studies must be undertaken using benzodiazepine receptor antagonists to verify that this increase in activity after central administration is in fact mediated by benzodiazepine receptor systems.

We thank G. D. Searle and Co. for their contribution of the B-CCE and Ro 15-1788 (E-909) used in these experiments.

267.3 GABA AGONISTS MODULATE THE BEHAVIORAL EFFECTS OF LORAZEPAM IN SQUIRREL MONKEYS. Joseph G. Wettstein* and Roger D. Spealman* (SPON: Peter B. Dews). Harvard Medical School and New England Regional Primate Research Center, Southborough MA 01772.

Benzodiazepines (BZs) bind to recognition sites on protein complexes that also contain receptors for GABA. Previous studies have shown that GABA agonists can enhance the binding of BZ agonists in vitro, but little is known about the consequences of such interactions in vivo. The purpose of this study was to assess how pretreatment with the GABA agonists SL 75102 (4-{[(chlorophenyl)-5-fluoro-2-hydroxyphenyl)methylene]-amino}butyric acid) and THIP (4,5,6,7-tetrahydroisoxazolo-[5,4-c]-pyridin-3-ol) modify the behavioral effects of the prototypical BZ lorazepam. Comparison studies were conducted with SL 75102 and THIP combined with pentobarbital, which also appears to act at the GABA/BZ complex. Two groups of monkeys responded under a fixed-interval schedule of food presentation; in one group, responding was suppressed by a superimposed fixed-ratio schedule of response-produced electric shock. Dose-effect curves were determined by giving cumulative doses i.v. during timeout periods that preceded sequential components of the fixed-interval schedule.

When given alone, lorazepam (0.01-0.3 mg/kg) produced dose-dependent increases in the rates of both suppressed and nonsuppressed responding, effects characteristic for BZ antianxiety drugs. Pentobarbital, in contrast, had little effect except at the highest dose (5.6 mg/kg), which decreased responding. Neither SL 75102 (1.0-10.0 mg/kg) nor THIP (0.03-1.0 mg/kg) markedly altered rates of either suppressed or nonsuppressed responding. Pretreatment with SL 75102 (1.0 or 10.0 mg/kg) enhanced the rate-increasing effects of lorazepam on both suppressed and nonsuppressed responding, resulting in leftward shifts in the lorazepam dose-effect curves. Pretreatment with THIP (0.3 or 1.0 mg/kg) did not enhance the rate-increasing effects of lorazepam. Instead, the lorazepam dose-effect curves were either unchanged or shifted downward and rightward. The effects of pentobarbital were not altered systematically by pretreatment with either SL 75102 (10.0 mg/kg) or THIP (1.0 mg/kg).

The finding that SL 75102 enhanced the behavioral effects of lorazepam suggests a biologically significant counterpart to the interaction between GABA agonists and BZs at the receptor level. Enhancement of the behavioral effects of BZs does not appear to be a necessary consequence of pretreatment with GABA agonists, however, as THIP altered the effects of lorazepam in a complex manner without producing enhancement. There was no evidence that either GABA agonist altered the effects of pentobarbital. (USPHS support: DA02658, DA00088, RR00168.)

267.4 THE BENZODIAZEPINE RECEPTOR SYSTEM IN LS AND SS MICE: BEHAVIORAL AND BIOCHEMICAL STUDIES. B. J. Martin*, R. J. Marley*, A. Stinchcomb*, and J. M. Wehner. Instit. for Beh. Genetics and School of Pharm., Univ. of Colorado, Boulder, CO 80309.

Previous studies from our laboratory have demonstrated that long sleep (LS) and short sleep (SS) mice do not differ in the affinity or number of benzodiazepine (BZ) receptors in forebrain and cerebellum. They do, however, differ in GABA and ethanol enhancement of BZ binding in these regions (Alc. and Drug Res. 7: 25-32, 1986 and Alcohol, in press), as well as in behavioral sensitivity to anticonvulsant, hypothermic, and sedative properties of benzodiazepines (data submitted). In current studies the Plus elevated maze was used to measure the anxiolytic effects of diazepam and ethanol. LS mice were more sensitive than SS mice to the effects of diazepam at 1 mg/kg, exhibiting a greater percentage of entrances into and amount of time spent in the open arm vs. the closed arm of the maze ($P < .05$). LS mice also demonstrated a greater anxiolytic response due to ethanol (1 g/kg), as indicated by a significant difference between the two lines for entrances in the open arm ($P < .01$).

In order to determine possible mechanisms that underlie differences in enhancement of ^{3}H-flunitrazepam (FNZ) binding between LS and SS mice, we have examined ^{3}H-GABA binding and patterns of heat denaturation of ^{3}H-FNZ binding in forebrain and cerebellar tissue from LS and SS mice. The two lines did not differ in either the affinity or number of high and low affinity ^{3}H-GABA binding sites in either region. They also did not differ in the degree of bicuculline competition of ^{3}H-GABA binding. However, significant differences were observed between LS and SS mice in the patterns of heat denaturation of ^{3}H-FNZ binding in the forebrain region ($P < .001$), but not in the cerebellum. Differences were also observed in the degree to which GABA (0.1 mM) protected the BZ receptor from heat denaturation in both regions ($P < .001$). Analyses of these denaturation curves suggested that the observed differences in half-life ($t_{1/2}$) for denaturation may be due to variations in the molecular structure of one subtype of BZ receptor, whereas differences in the degree of protection by GABA may reflect differences in the relative proportions of BZ receptor subtypes.

From these data we conclude that differential sensitivity of LS and SS mice to benzodiazepines may be explained by molecular differences in the GABA/BZ receptor complex or intregal membrane constituents associated with the receptor complex that result in differential denaturation of the benzodiazepine receptor. (supported by AA-03527, and training grants HD-07289 and MH-16880)

267.5 THE EFFECTS OF Ro15-4513 ON GENERALIZED MOTOR ACTIVITY. L.T. Johnson,* H.L. June and M.J. Lewis. Dept. of Psychology, Howard University, Washington, D.C. 20059.

The imidazobenzodiazepine compound Ro15-4513 has been shown to block the behavioral effects of ethanol (ETOH). Published reports (Bonette, Burkard, Gabl, and Mohler, 1986) show that Ro15-4513 antagonizes both the sedation as well as the motor impairment induced by ETOH. More recently, consistent with the above findings, Suzdak, Glowa, Crawley,Schwartz, and Paul (1986) have reported that Ro15-4513 also blocks the anti-conflict and intoxicating effects of ETOH. While these preliminary reports address both the anti-conflict and intoxicating effects of Ro15-4513, the effect of Ro15-4513 on the depressive effects of ETOH on generalized locomotor activity has not been investigated. The purpose of this study was to investigate the effects of Ro15-4513 on generalized motor activity.

Generalized motor activity of 14 male Charles River rats was assessed by the Omnitech Digiscan activity monitor system. Daily sessions were 10 minutes each. Baseline activity measures were obtained for 3 days under drug-free conditions (4% tween-80 vehicle intraperitoneal injection). Each animal then received 2.5 and 1.25 mg/kg intraperitoneal injections of Ro15-4513 alone. Each was followed by at least 2 drug-free sessions. Animals were then divided into two groups. The first group (n=8) received 2.5 mg/kg Ro15-4513 i.p. Ten minutes later animals received .75 gm/kg ETOH i.p. The second group (n=6) received .75 gm/kg only. Neither dose of Ro15-4513 alone produced any significant difference in activity from baseline levels. Animals receiving both Ro15-4513 and ETOH also showed difference in activity from baseline. Only animals receiving ETOH, however, showed a significant depression in generalized locomotor activity. These results show that Ro15-4513 is effective in attenuating the depressive effects of ETOH on generalized locomotor activity. Furthermore, they are consistent with the previous reports of the effects of this compound on the behavioral effects of ETOH (Suzdak et al., 1986) in the literature.

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Supported in part by DHHS grant AA06263 and NSF grant RII8505430.

267.6 THE PARTIAL INVERSE BENZODIAZEPINE AGONIST RO 15-4513 POTENTIATES ETHANOL INDUCED SUPPRESSION OF WHEEL-RUNNING IN THE RAT. M.A. Bixler and M.J. Lewis. Dept. of Psychology, Howard University, Washington D.C. 20059.

Evidence suggests the imidazobenzodiazepine Ro 15-4513 blocks both the anticonflict and behavioral intoxicating effects of ethanol (ETOH) (Suzdak, P.D. et al., Science, 234: 1243, 1986). In a series of experiments, the ability of Ro 15-4513 to block acute ETOH-induced (0.75 g/kg; 30% v/v; i.p.) suppression of wheel-running was assessed.

ETOH administration alone, in combination with Ro 15-4513 (2.5mg/kg in 4% Tween 80/saline; i.p.), and Ro 15-4513 alone all produced an approximately 70% decrease in wheel-running.

Ro 15-4513 administered at 2.5, 1.25, 0.625, 0.312, 0.156, and 0.078 mg/kg doses revealed a dose-dependent suppression of wheel-running . All except the 0.078 mg/kg dose produced significant suppression. Pretreatment with this dose also failed to block ETOH suppression of wheel-running . Moreover, a trend in the direction of greater suppression in the pretreated subjects was noted. In a subsequent experiment, pretreatment with Ro 15-4513 (3.0 mg/kg) produced greater suppression of wheel-running performance (90%) than ETOH administration alone (53%).

These data suggest that wheel-running behavior is particularly sensitive to both the effects of ETOH and Ro 15-4513, and that in this task this inverse benzodiazepine agonist does not block ETOH induced suppression.

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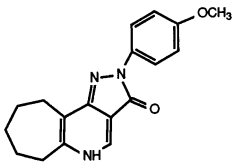
267.7 **CGS 20625: PHARMACOLOGICAL PROFILE OF A PYRAZOLOPYRIDINE ANXIOSELECTIVE ANXIOLYTIC WITH PARTIAL AGONIST/ANTAGONIST ACTIVITY.** M. Williams, D. A. Bennett, P. A. Loo*, A. F. Braunwalder*, C. L. Amrick, D. E. Wilson, N. Yokoyama* and J. W. F. Wasley*. Drug Discovery Division, Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ 07901.

CGS 20625 (2(4-Methoxyphenyl)-2,3,5,6,7,8,9,10-octahydrocyclohepta[b]-pyrazolo-[3,4-d]pyridin-3-one) is a potent and selective ligand at the central benzodiazepine (BZ) receptor (IC-50 =1.3 nM). The compound had a GABA ratio of 0.9 and stimulated TBPS binding to the chloride channel component of the BZ complex by 20%, a profile indicative of a partial agonist.



CGS 20625

In vivo, CGS 20625 blocked a pentylenetetrazol discriminative cue (ED-50 = 1.7 mg/kg p.o.) and selectively increased conflict responding with an MED of 0.3 mg/kg p.o. The compound generalized to both diazepam and CGS 9896 with ED-50 values of 9.7 and 0.9 mg/kg p.o. respectively. CGS 20625 had no effect on variable interval responding or rotorod performance, indicating a reduced tendency to cause muscle relaxation or sedation. The compound potentiated the actions of ethanol at a dose of 30 mg/kg, in contrast to diazepam which was active at 5.4 mg/kg p.o. CGS 20625 had no marked effects on rat locomotor activity and did not potentiate hexobarbital-induced sleep time until a dose of 100 mg/kg p.o. CGS 20625 antagonized diazepam-induced rotorod deficit (ED-50 = 7.2 mg/kg p.o.) indicating intrinsic antagonist properties.

CGS 20625 has a preclinical profile indicative of an anxioselective agent with minimal tendency for the side effects associated with the classical BZs. The partial agonist/antagonist profile of the compound is unique and may be responsible for its lack of side effects.

267.8 A COMPARISON OF THE DISCRIMINATIVE STIMULUS PROPERTIES OF CHLORDIAZEPOXIDE AND THE ß-CARBOLINE ZK 95 962.

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Classical benzodiazepines (BZ) are clinically effective anxiolytics, but the anxiolytic properties are confounded by unwanted sedative and muscle relaxant effects. Recently BZ-receptor ligands have been synthesised which possess only some BZ-like properties. The ß-carboline ZK 95 962 has been identified as a partial agonist at the BZ receptor. ZK 95 962 generalises to chlordiazepoxide (CDP) in drug discrimination procedures, has anxiolytic (Stephens, D.N. et al., Pharmacol. Biochem. Behav. in press) and antiepileptic properties but is non-sedative (Chapman, A.G. et al., Pharmacol. Biochem. Behav. in press).

Rats were trained to discriminate either CDP (5 mg/kg) from vehicle, or ZK 95 962 (10 mg/kg) from vehicle in a standard FR 10 food reinforced, 2-lever discrimination (Stephens, D.N. et al., Psychopharmacology 83 (1984) 233-239). Differences in the interoceptive properties of the two cues were investigated by comparing the effects of a range of BZ receptor ligands in generalising to, or antagonising the respective cues.

CDP and ZK 95 962 generalised to one another at similar doses (ED_{50}'s: CDP in CDP cue 2.5 mg/kg, CDP in ZK 95 962 cue 1.5 mg/kg; ZK 95 962 in CDP cue 1.6 mg/kg, ZK 95 962 in ZK 95 962 cue 1.5 mg/kg). However, there were striking differences in the dose effect curves for other BZ ligands. Ro 15-1788, a BZ antagonist with weak agonist properties, did not generalise to CDP in doses upto 40 mg/kg, but did generalise to ZK 95 962 (ED_{50} 2.3 mg/kg). CGS 9596, a weak partial agonist, generalised to CDP (2 mg/kg), but showed a bell-shaped response curve in the ZK 95 962 cue. The ß-carboline BZ receptor partial inverse agonist ZK 93 426 did not generalise to CDP but antagonised CDP stimulus effects (0.26 mg/kg) and generalised to the ZK 95 962 cue (20 mg/kg).

These results, as well as others to be presented, suggest that the stimulus properties generated in the two cues differ. The removal of sedative effects from a BZ ligand alters the discriminative stimulus in comparison to a classical BZ. These results may reflect the properties of different BZ receptor subtypes.

267.9 NO-05-0328: A new potent GABA-uptake inhibitor

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R-(-)-1-(4,4-(3-Methyl-2-thienyl)-3-butenyl)-3-piperidine carboxylic acid (NO-05-0328) was identified as a selective and potent inhibitor GABA-uptake (IC_{50} = 75 nM in a rat brain synaptosomal preparation). The compound did not inhibit the binding of ligands to a variety of conventional neurotransmitter receptors and uptake sites. NO-05-0328 exerted marked and long-lasting anticonvulsant activity in a number of model systems. For example, the ED_{50} was 0.4 mg/kg for inhibition of convulsions in DBA/2 mice (ip, 30 min; diazepam 0.17, SKF 100330A 4.0 and valproic acid 31 mg/kg). In mice given DMCM, a full inverse agonist at benzodiazepine receptors, NO-05-0328 inhibited convulsions with an ED_{50} of 1 mg/kg (diazepam = 2.5). In pentylenetetrazol-treated animals, convulsions were inhibited with an ED_{50} of 6 mg/kg. In tests for sedation, NO-05-0328 exhibited effects in doses higher than those producing anticonvulsant effects. For example, exploratory behavior was reduced with ED_{50}'s of 15 (rearing) and 40 mg/kg (locomotion) in rats (diazepam 1.3 and 0.9 mg/kg, respectively).

These results indicate that NO-05-0328 is a promising candidate for a novel class of antiepileptic drugs with a selective mechanism of action.

267.10 BACLOFEN AND SOMATOSENSORY CORTICAL RESPONSE PROPERTIES.

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Many response properties of cells in sensory cortex are influenced by synaptically released GABA. Recent evidence (Hicks and Dykes, Brain Res. 274: 160-164; 1983; Oka et al., Brain Res. 376: 194-198; 1986) from somatosensory experiments has described how GABA acts in controlling certain response attributes of functionally distinct cortical cell types. Baclofen has been said to be a selective agonist for a postulated class of receptor, termed $GABA_B$ receptors. Accordingly, we sought to ascertain whether baclofen exerts actions similar to GABA on cell excitations induced by a variety of means of activation — both physiological and non-physiological. Cells in S1 cortex were excited synaptically by electrical stimulation of the VPL nucleus and naturally by hand-held probes or by computer controlled air puffers directed to the receptive field (RF).

Baclofen suppressed cell responses elicited by all experimental paradigms. When contrasted with the suppressant effects of GABA, relatively few cells showed differences in effect by the two inhibitory agents: one cell being GABA sensitive but baclofen insensitive, another two responding with a considerable difference in potency. Both compounds exerted similar effects upon puffer-induced responses irrespective of the air jet position within the RF. However, when the suppressions of air puffer induced activity were challenged by bicuculline methiodide (BMI), on 2 of 5 cells where complete tests including recovery could be obtained baclofen exerted BMI resistant suppressions. With both of 2 cells tested for BMI enlargements of RF size (Hicks and Dykes, 1983), baclofen produced RF size decreases despite the presence of BMI. Synaptic driving invariably was reduced by baclofen; in the one case where these excitations were challenged by BMI, the response suppressions remained unaltered. Differences in effect upon spontaneous firing also were noted between the two classes of agonist.

The experiments reveal a close functional similarity in overall effect of baclofen and GABA regardless of the method of excitation employed although it appears that there are important differences as well, some of which only become apparent through the use of BMI. Thus, the very interesting possibility is raised that there may exist a multiplicity of sites sensitive to GABA, which differentially underlie the various response properties exhibited by the distinctive cell types of S1 cortex. These sites provide two mechanisms — possibly the $GABA_B$ and $GABA_A$ receptors — by which RF size and cell responsiveness can be controlled. It is expected that the development and future testing of new classes of pharmacologically specific agonist (eg., THIP) and antagonist (phaclofen; Kerr et al., Brain Res. 405: 150-154; 1987) of the different classes of GABA receptor reported to date, will advance further our understanding of the role of the diverse types of inhibition extant in primary sensory cortex.

Supported by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.

267.11 GABA IN THE NUCLEUS TRACTUS SOLITARIUS INCREASES BLOOD PRESSURE INDEPENDENT OF AN ACTION ON $GABA_A$ RECEPTORS. A.F. Sved and J.M. Catelli, Neurology Svc., VA Med. Ctr., East Orange, NJ 07019.

Previous studies in our laboratory have shown that ongoing GABAergic neurotransmission in the nucleus tractus solitarius (NTS) contributes to the maintenance of blood pressure (BP). Increasing the synaptic action of GABA by injection of a GABA uptake blocker (nipecotic acid; NIP) into the NTS increased BP. The current studies examined the GABA receptor subtypes involved in this process. These studies were carried out using chloralose-anesthetized (60 mg/kg iv), paralyzed (d-tubocurarine, 0.5 mg/kg iv), ventilated, Sprague Dawley rats. Microinjections (100 nl) of GABA agents were made into the right NTS at least 30 minutes following electrolytic lesion of the contralateral NTS. The pressor response to NIP injected into the NTS was not attenuated by pretreatment of the NTS with the selective $GABA_A$ receptor antagonist bicuculline (BIC). Injection of NIP (10 nmol) alone elevated BP 39±5 mmHg whereas when IP was injected immediately following BIC (10 pmol), BP increased 45±5 mmHg (n=6); these responses were not significantly different. In contrast, BIC (10 pmols) significantly attenuated the pressor response to injection of the GABA agonist muscimol (MUS), 2.5 pmol) into the NTS: BP increased 23±1 mmhg in response to MUS alone and 9±1 mmhg when MUS was injected following BIC (n=7, $p<0.01$). In addition, the pressor response to MUS in the NTS could be completely reversed by BIC: BP was increased 18±5 mmHg immediately following BIC injection (n=3). Selective stimulation of $GABA_B$ receptors with the specific $GABA_B$ agonist (-)baclofen (40 pmol) elicited a significant increase in BP (66±2 mmHg, n=4, $p<0.01$). These results demonstrate that the pressor response to enhanced synaptic action of GABA in the NTS can be produced independent of the involvement of $GABA_A$ receptors, and presumably is mediated via an action on $GABA_B$ receptors.

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267.12 **INTEROCEPTIVE STIMULUS PRODUCED BY DIAZEPAM WITHDRAWAL IS POTENTIATED BY PICROTOXININ.** S.O. Idemudia* and H. Lal.(SPON: Martha A. Mann). Dept. of Pharmacology, Texas Coll. of Osteopath. Med., Fort Worth, TX, 76107.

Withdrawal from diazepam produces anxiety in man and a pentylenetetrazol-like interoceptive discriminative stimulus (PTZ-like IDS) in rats (Emmett-Oglesby, et al. Eur. J. Pharmacol. 92:127, 1983). We(Idemudia, et al. Soc. Neurosci. Abs. 12:484, 1986) recently showed that the PTZ-like IDS produced by diazepam(DZ) withdrawal was enhanced by the GABA-A antagonist bicuculline, suggesting that the withdrawal stimulus may be due to a decrease in the activity of the chloride ionophore complex modulated by binding at GABA, benzodiazepine, and/or picrotoxin receptors. We therefore tested the ability of a picrotoxin receptor agonist to enhance DZ withdrawal stimulus. Rats were trained in a two-choice, food reinforced operant task to discriminate PTZ (20 mg/kg) from saline (1 ml/kg). Thereafter, rats selected the saline-appropriate lever following saline, Ro 15-1788, or DZ, while no more than 50% of them selected the PTZ-appropriate lever following picrotoxinin (PTX; 0.02 to 0.64 mg/kg) or PTZ (2.5 to 10 mg/kg). DZ (120 mg/kg) was then administered in a liquid diet (Benjamin, et al. Fed. Proc. 46:712, 1987) twice daily for 3, 6, or 12 days. At 12hr after DZ, Ro 15-1788 produced a PTZ-like IDS. At 84hr, the rats were in spontaneous withdrawal, as 33% of them selected the PTZ-appropriate lever after either saline or Ro 15-1788. PTX (0.02 to 0.16 mg/kg) or PTZ (2.5 to 10 mg/kg) increased the withdrawal stimulus dose-dependently. The increase was the sum of the effects of the drugs and the withdrawal(i.e. additivity) in the 3- and 6-day groups. In the 12-day group, the PTZ-appropriate lever selection increased from 33% to 70% following 0.04 mg/kg PTX. This was a supra-additive effect, as only 10% of control rats selected this lever following this dose. Thus, chronic DZ produced a PTZ-like IDS which was enhanced by the GABA antagonists PTZ and PTX. The degree of enhancement depended upon the length of DZ treatment. Therefore, these data support the hypothesis that the PTZ-like IDS produced by withdrawal from chronic DZ may be due to an increase in the sensitivity of receptors for GABA antagonists resulting in an overall decrease in GABAergic activity. (Supported by NIDA Grant # RO1 DA 03521).

267.13 CHANGES IN PRESYNAPTIC INHIBITION IN THE CUNEATE NUCLEUS OF THE RAT PRODUCED BY DIAZEPAM, THIP, AND SKF 89976A. P.S. Blum (SPON: R. Shank). Department of Biological Research, McNeil Pharmaceutical, Spring House, PA 19477.

Presynaptic inhibition in the cuneate nucleus is mediated by GABA (Polc, P and W. Haefley, Naunyn-Schmiedebergs Arch. Pharmacol., 294:121, 1976). The mechanism for presynaptic inhibition in the cuneate nucleus is the depolarization of primary afferent fibers, and this primary afferent depolarization (PAD) can be measured using the Wall method of excitability testing (J. Physiol., 142:1, 1958). In the present study, the measurement of PAD in the cuneate nucleus of the urethane anesthetized rat was used to assess GABAergic properties of three drugs. The compound antidromic action potential evoked by stimulation in the cuneate nucleus (single pulses 0.1 ms duration, 2-5 V intensity) was recorded using a bipolar hook electrode on the median nerve (test response). The amplitude of the test response was compared to the amplitude of the response when the cuneate stimulus was preceeded (4-160 ms) by a conditioning stimulus to the ulnar nerve (single pulse, 0.5 ms duration, 7-100 V intensity). Increases in the peak height caused by either conditioning stimulus or drug effects were taken as evidence of PAD. In the absence of drug, PAD evoked by ulnar stimulation was stable for up to 3 hrs. Diazepam (0.5 mg/kg i.v.) produced a 40% increase in ulnar-evoked PAD, without any change in the amplitude of the test response. THIP (3 mg/kg i.v.), a GABA receptor agonist, blocked ulnar-evoked PAD without changing the amplitude of the test response. SKF 89976A [N-(4, 4-diphenyl-3-butenyl)-nipecotic acid] (1 mg/kg i.v.), a GABA uptake inhibitor, produced a third effect. No change was seen in PAD evoked by ulnar stimulation, but there was a 35% increase in the amplitude of the test response. Diazepam, THIP, and SKF 89976A, three compounds that interact with different aspects of GABA function, also affected presynaptic inhibition. Each of these compounds, however, produced a different effect. These studies suggest that excitability testing of primary afferent fibers can be used to assay the in vivo effects of test compounds on GABA neurotransmission.

267.14 DOES CHRONIC, VARIED STRESS EFFECT PICROTOXIN-INDUCED SEIZURES? T.D. Wolinsky* and E.E. Coons* (SPON: M.S. Gizzi) Dept. of Psychology, New York University, New York, NY 10003.

Evidence is accumulating for the involvment of GABA in both the pharmacology and treatment of depression. After exposing rats to a protocol based on the animal model of depression of R.J. Katz (Neurosci. and Biobehav. Rev, 5(2) p.231) we evaluated central GABAergic activity by examining the severity of seizures induced by the GABA antagonist, picrotoxin.

Rats underwent a three week regimen of chronic, varied and unpredictable stress. One or two days later, they were injected with picrotoxin (7.5 mg/kg, i.p.). All seizures were video-taped for later analysis. The latency and duration for each episode of the following seizure stages were measured: myoclonic jerks and twitches, writhes, clonus that occurred alone and in association with writhes and tonic extension, tonic extension of fore- and hindlimbs, and bouts of status epilepticus characterized by prolonged periods of alternating tonic and clonic activity. The order of these categories represents progressive stages of seizure severity.

In Experiment 1, two groups of 6 rats were used. One was exposed to chronic stress; the other was not. It appeared that the control animals experienced more severe seizures than the stressed group. For example, this was evidenced by a greater incidence of tonic activity in the control group, suggesting that the chronic stress provided partial protection from this form of seizure activity. However, because the stress regimen involved periods of food or water deprivation, the two groups differed in mean body weight; the control animals weighed significantly more. Therefore, we could not eliminate the possiblity that the above findings were due to differences in weight.

Experiment 2 consisted of three groups of 10 animals: a chronic stress, an acute stress group which was exposed to the same stressor given on the last day of the chronic stress, and a food-yoked control group. Using this more appropriate control, we were unable to replicate the results of Experiment 1. Moreover, the seizure severity and mean weight of the acute group were not radically different in comparison to those of the chronically stressed animals.

These data suggest that: 1) chronic, varied stress does not effect picrotoxin-induced seizure severity as assessed by this method; 2) great caution must be excercised when interpreting between-group differnces that may be due to experimental artifact (in this case, weight differences); and 3) while it has been reported that weight does not influence latency to the first occurrence of a variety of convulsive endpoints, we have demonstrated that the examination of the entire seizure profile does reveal weight effects.

267.15 INJECTION OF BENZODIAZEPINES, BUT NOT GABA OR MUSCIMOL INTO SUBSTANTIA NIGRA PARS RETICULATA SUPPRESSES PENTYLENETETRAZOL SEIZURES. H. Zhang*, H.C. Rosenberg and E.I. Tietz (SPON: T.H. Chiu). Dept. of Pharmacol., Med. Coll. of Ohio, Toledo, OH 43699.

In an earlier study (Soc. Neurosci. Abst. 11:272) it was found that the benzodiazepine (BZ) midazolam injected into the pars reticulata of the substantia nigra (SNpr) can suppress seizures produced by pentylenetetrazol (PTZ). The present study was done to determine if PTZ seizures could also be suppressed by the water-soluble BZ flurazepam (FZP), GABA or muscimol.

Male, Sprague-Dawley rats (275-300 g) were deeply anesthetized and guide cannulas with close-fitting obturators were implanted. A week later, 1 µl FZP, GABA or muscimol was infused over 1 min, bilaterally. The injectors were left in place another min, then withdrawn slowly and replaced with the obturators. PTZ, 70 mg/kg was injected i.p. 5 min after the start of FZP or GABA infusions, or 20 min after muscimol infusion. These times were based on the time-course of the circling response reported after unilateral injection of these drugs into SNpr (Fed. Proc. 45:806). Control rats received 1 µl of saline 5 or 20 min before PTZ. All injection sites were verified histologically.

5 rats with no prior surgery were given PTZ; all had tonic-clonic (TC) seizures. Five rats were given PTZ a week after surgery, but with no intranigral injection; 4 had TC seizures. 14 rats received intranigral saline before PTZ; 8 of these had TC seizures. This suggested that the saline infusion itself had some anticonvulsant effect. FZP, 50 µg bilaterally, produced a clear anti-PTZ action; 0 of 5 rats had TC seizures. In contrast, GABA doses of 20 and 100 µg had no clear effect. After 20 µg, 3 out of 8 had TC seizures; after 100 µg, 4 of 5 had TC seizures. Muscimol also failed to suppress PTZ seizures; 4 of 9 rats had TC seizures after 25 ng, 5 of 7 had TC seizures after 50 ng, and 2 of 5 had TC seizures after 100 ng muscimol.

Intranigral injection of BZ's suppressed PTZ seizures. Neither GABA nor muscimol did, which is similar to the results found by Mirski et al (1986) using intranigral gamma-vinyl GABA. GABA and muscimol doses were as great as those reported to block various types of experimental seizures, and to cause behavioral responses. If only 1 injector was in the SNpr, all drugs caused circling behavior. 50 and 100 ng muscimol produced self-injurious behavior in some rats. Thus, GABA and muscimol were producing actions expected of GABA-A agonists. Their failure to block PTZ seizures suggests that the anti-PTZ effect of intranigral BZ's is not mediated through GABA-A receptor-linked mechanisms. The same conclusion was reached by Chweh et al (1985) using systemically administered drugs. The SNpr may be the site of anti-PTZ action of systemically administered BZ's.

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267.16 FLURAZEPAM TREATMENT CONFERS TOLERANCE TO THE ANTI-PENTYLENETETRAZOL ACTION OF SEVERAL, BUT NOT ALL, BENZODIAZEPINES. H.C. Rosenberg, J.M. Duggan*, E.I. Tietz and T.H. Chiu. Dept. of Pharmacology, Medical College of Ohio, Toledo, Ohio, 43699.

Benzodiazepines produce several pharmacologic effects by virtue of their actions at brain benzodiazepine (BZ) receptors. Studying the pharmacologic activity of a series of BZ agonists may reveal differing profiles that could be related to varying patterns of BZ receptor activation. The purpose of this experiment was to study patterns of tolerance and cross-tolerance among agonists of differing structures. Tolerance was studied in rats treated with a saccharin-flavored solution of the water-soluble BZ flurazepam, which was offered as the sole source of water. The concentration was adjusted so that rats could consume up to 100 mg/kg daily for 3 days, then up to 150 mg/kg daily for 4 days. Consumed in this fashion, the dose was sub-threshold for producing overt signs of intoxication. Flurazepam is quickly metabolized to active products, notably desalkyl-flurazepam, which is longer lasting and much more potent. Tolerance was sought by studying the activity of BZs against pentylenetetrazol (PTZ). Rats were tested 48 hrs after stopping the 1 week treatment. At this time, there is no measurable change in sensitivity to PTZ, and brain level of active flurazepam metabolites (as determined by radio-receptor assay) is very low. Benzodiazepines were administered before PTZ, 100 mg/kg, i.p. The injection interval was based on previous observations of the time-action characteristics of each BZ. Each rat was assigned a seizure score based on the most severe seizure observed: 0=none; 1=myoclonic jerks; 2= forelimb clonus; 3=clonus of all legs and loss of upright posture; 4=lethal tonic-clonic seizure. Results were evaluated by the Kruskal-Wallis test. Tolerance was seen, but only if the appropriate dose of BZ was chosen. Thus, too large a dose protected both control and 1 week treated rats against PTZ seizures. By adjusting doses accordingly, tolerance was observed for diazepam, clonazepam, desalkyl-flurazepam, and the 1,5-BZ clobazam. In contrast, tolerance was not seen with the imidazo-benzodiazepine, midazolam, even though a wide range of doses was examined. Furthermore, there was no tolerance to the anti-PTZ action of flurazepam. These data show that measuring tolerance, using this experimental design, can reveal differences between BZs that may be related to variations in their interactions with BZ receptors. The lack of tolerance to midazolam and flurazepam may be related to the imidazo ring in midazolam and the large N-containing side-chain of flurazepam.

Supported by DHHS grants DA02194, NS16595,and DA04075.

267.17 A COMPARISON OF THE ANTICONVULSANT EFFECTS OF 1,4 AND 1,5 BENZODIAZEPINES IN THE AMYGDALA-KINDLED RAT AND THEIR EFFECTS ON MOTOR IMPAIRMENT. E. I. Tietz, H. C. Rosenberg and Ted H. Chiu. Dept. of Pharmacology, Medical College of Ohio Toledo, OH 43699.

The benzodiazepines (BZ's) are effective for the treatment of a variety of epilepsies but their usefulness is limited by psychomotor impairment and the development of tolerance. Clinical and animal studies suggest that the anticonvulsant 1,5 BZ, clobazam (CBZ) produces minimal neurotoxicity. The anticonvulsant efficacy of CBZ, diazepam (DZP) and clonazepam (CZP) was evaluated in the kindling model and was compared by dose-response analysis to the motor impairment produced by these BZ's.

Male, Sprague-Dawley rats (n=25) were deeply anesthetized and implanted with amygdaloid and contralateral cortical electrodes. After 1 week recovery, afterdischarge (AD) thresholds (79.6 ±6.3 µA) were determined. Rats were stimulated once daily for 1 sec at 400 µA (60 Hz, biphasic symmetrical square wave, 2.0 msec total pulse duration) until a criterion of 5 consecutive Stage 5 seizures was reached. Baseline responses were averaged over the next 2 days. Drug effect was calculated as a percent of baseline for motor seizure latency (3.5 ±0.4 sec) and duration (94.3 ±3.5), forelimb clonus latency (9.5 ±1.4) and duration (65.8 ±2.6), amygdala AD duration (82.3 ±3.5), and cortical AD latency (2.6 ±0.5) and duration (81.5 ±3.7). Doses of CBZ (0.5-15.0 mg/kg), DZP (0.1-2.0 mg/kg) and CZP (0.02-1.5 mg/kg) or vehicle were administered, i.p., 30 min before test stimulations in a single-blind crossover design with 3 days between each test dose. Motor impairment and muscle relaxation were measured 5 min before stimulation by evaluating ability to stay on a vertical screen, impairment of gait, and abdominal and hindlimb muscle tone.

The relative order of potency for all actions was CZP > DZP > CBZ. Each drug limited seizure spread, suppressing motor and EEG measures of kindled seizures. Forelimb clonus duration was the most sensitive predictor. The slope of the dose-response curve of CZP for this measure was significantly shallower than that of DZP. Though the CBZ dose-response curves for tests of motor impairment appeared steeper, they were not significantly different from those of DZP. Comparing ED50's for suppression of forelimb clonus with those for motor impairment revealed differences among the drugs. CBZ was relatively less potent than the other drugs for causing ataxia, while CZP was relatively more potent for causing muscle relaxation. Both CBZ and CZP were more likely to impair vertical screen performance than equi-effective anticonvulsant doses of DZP. Quantitative description of the acute anticonvulsant actions of the 1,4 and 1,5 BZ's in the kindling model will serve as a stable baseline for the study of tolerance development during chronic treatment.

Supported by NINCDS Contract NO1-NS62310.

267.18 INTRACEREBROVENTRICULAR 4,5,6,7-TETRAHYDROISOXAZOLO[4,5-C]PYRIDIN-3-OL (THPO) PROTECTS AGAINST PENTYLENETETRAZOL SEIZURES IN RATS. S.F. Gonsalves, B. Twitchell*, A. Schousboe*, and R.E. Harbaugh*. Depts. of Surgery and Pharmacology, Dartmouth-Hitchcock Medical Center, Hanover, NH 03756 and Dept. of Biochemistry, Univ. of Copenhagen, Copenhagen, Denmark.

Previous studies suggest that at least some seizures may be ameliorated by enhancing GABAergic neurotransmission. THPO selectively inhibits high affinity uptake of GABA into astrocytes (Schousboe et al., Epilepsia 24:531, 1983). Theoretically, this action should increase the amount of GABA available for neuronal uptake. Evaluation of the anticonvulsant potential of this substance has been hampered by its inability to penetrate the blood-brain barrier. We assessed the anti-pentylenetetrazol (PTZ) activity of THPO following injection directly into the CSF. For comparison, the anticonvulsant activity of intracerebroventricular muscimol was also evaluated.

THPO (0.1-0.75 mg), muscimol (0.1-1.0 µg) or mock CSF was infused into the lateral ventricles of Sprague-Dawley rats 30 min to 3 hrs prior to PTZ administration. Maximal clonic-tonic seizures were elicited by injecting 25 mg/kg PTZ iv. Endpoints were tonic forelimb extension and death. To determine seizure threshold, PTZ was administered as a continuous iv infusion, and doses of PTZ producing various seizure components were calculated. THPO, at 0.3 mg and above, protected all animals (n=9) against a suprathreshold PTZ challenge for at least 1 hr. THPO did not elevate the seizure threshold dose. In contrast, muscimol (0.3 and 1.0 µg) protected against forelimb extension in only 33% of animals and against death in 58%. Low doses of muscimol did not affect seizure threshold; the threshold decreased following the 1.0-µg dose [TWITCH (mg/kg): Control=24.7±2.0; 1.0 µg muscimol=16.9±1.4, $p<0.05$]. Both THPO and muscimol exhibited neurotoxic effects at anticonvulsant doses. Initially, a reduction in locomotor activity and an impaired positional sense were observed. At the highest doses, effects included spontaneous twitches (THPO) and loss of righting reflex (muscimol). Our data suggest that THPO may protect against maximal PTZ seizures. Thus, glial GABA uptake inhibitors may suppress mechanisms involved in seizure spread. (Support: American Health Assistance Foundation and Hitchcock Foundation).

267.19 IN VIVO LABELING DEMONSTRATES BENZODIAZEPINE RECEPTOR MEDIATION OF RAT PUP ULTRASONIC ISOLATION CALLS. T.R. Insel, L.P. Miller*, and R.E. Gelhard*. Lab. of Clinical Science, NIMH, Poolesville, MD 20837.

Rat pups from 1-14 days of age emit ultrasonic (35-40 kh_z) calls when isolated. These isolation calls are a potent stimulus for maternal retrieval and may be a behavioral index of separation distress. Pups from a rat strain genetically selected for fearfulness show an increased rate of calling (Insel & Hill, Biol. Psychiatry, in press). The benzodiazepine (BZD) diazepam, in clinically anxiolytic doses, decreases the rate of calling whereas pentylenetetrazol, a drug with clinically anxiogenic properties, increases calling during a 2-minute separation test. (Insel et al., Pharm. Biochem. Behav. 24:1263, 1986). The BZD receptor antagonist, RO 15-1788, which generally lacks intrinsic effects was also found to decrease isolation calls at a dose of 5 mg/kg.

To investigate if the BZD receptor is involved in the mediation of these ultrasonic isolation calls, in vivo binding of ^{3}H-RO 15-1788 (3 μCi/pup) was compared in 10-day-old pups either (a) unseparated from their littermates, (b) separated for 5 minutes or (c) separated for 25 minutes. Twenty minutes after IP injection with ^{3}H-RO 15-1788, pups were sacrificed by decapitation, brains were immediately removed and either dissected for a regional analysis of binding or frozen for cryostat sectioning (24 μ) with subsequent autoradiography of slide-mounted sections (8 week exposure). Non-specific binding was assessed by preinjecting a subset of each group with diazepam (5 mg/kg).

Calling rate (calls/min.) was higher in both separated groups (unsep. = 1.9 ± 0.6, sep (5) = 41.5 ± 5.3, sep (25) = 28.0 ± 8.9). In three independent studies of macro-dissected tissue, sep (25) pups showed a 30% decrease in specific binding to cortex, but not to olfactory bulb, midbrain, or cerebellum. Autoradiographic binding in the separated groups was decreased significantly in the cingulate and pyriform cortex, increased in the inferior colliculus, but not affected in nine other brain regions analyzed.

These results are consistent with the hypothesis that the BZD receptor participates in the neural response to separation in the rat pup. Ongoing studies are investigating whether endogenous ligands for this receptor can alter the behavioral response to separation.

267.20 STUDIES OF CENTRAL NEUROTRANSMITTER FUNCTION IN EXPERIMENTAL HEPATIC ENCEPHALOPATHY. R.F. Butterworth, J.F. Giguère, A.M. Besnard*, H. Fournier*, G. Girard* and M. Bergeron*, Lab. of Neurochemistry, André-Viallet Clin. Res. Centre, Hôpital St. Luc, Montreal, Qué. H2X 3J4.

It has been repeatedly suggested that modifications of cerebral neurotransmitter function may be important in the pathogenesis of hepatic encephalopathy. Data to be presented is derived from an ongoing study in our laboratory, to evaluate this hypothesis. Four weeks following surgical construction of an end-to-side portocaval anastomosis, rats are chronically hyperammonemic and display neurological symptoms of encephalopathy. Measurement of cerebral amino acids by HPLC of their o-phthalaldehyde derivatives in shunted and sham-operated rats yielded the following:

(i) Region-selective increases in brain glutamine ranging from 1.7 times normal values in spinal cord to 3.5 times normal values in cerebral cortex.

(ii) Selectively decreased glutamate in cerebral cortex and brainstem.

(iii) No changes of brain GABA in any region studied.

Activities of the GABA nerve-terminal marker enzyme GAD were found to be within the normal range; activities of the cholinergic marker enzyme CAT were likewise unchanged. However measurement of the astrocytic enzyme glutamine synthetase gave values that were significantly decreased in several brain structures. High affinity binding of ^{3}H-GABA, ^{3}H-muscimol and ^{3}H-flunitrazapam to synaptic plasma membranes from cerebral cortex of shunted rats was unaltered. Taken together, this data does not support the much subscribed-to hypothesis that cerebral GABA dysfunction plays a pathogenetic role in hepatic encephalopathy. On the other hand, ^{3}H-glutamate binding was found to be significantly altered in cerebral cortex of shunted rats, the density of binding sites (B_{max}) being found to be increased from 9.2 to 14.6 pmol.per mg. protein ($p<0.01$) without changes in binding affinity (Kd). Such alterations of central glutamatergic function and the concomitant predominance of inhibitory (GABAergic) neurotransmission may be of pathophysiological importance in hepatic encephalopathy associated with chronic liver disease and portal-systemic shunting.

(Supported by the Medical Research Council of Canada and Fonds de la recherche en santé du Québec).

REGENERATION: LOWER FORMS

268.1 GASTROPOD GANGLION TRANSPLANTS: NEURON SURVIVAL IN SWAPS BETWEEN CLOSELY AND DISTANTLY RELATED SNAILS. S.B. Moffett. Dept. of Zoology, Washington State University, Pullman, WA 99164-4220.

In the pulmonate snail Melampus bidentatus, cerebral ganglia implanted into the hemocoel of host snails form connections with the host CNS and can induce the formation of supernumerary eyes or tentacles (Moffett and Austin, J. Exp. Zool. 216:321-325, 1981). An alternative approach used in this study has been to remove one of the host's cerebral ganglia and to implant another cerebral ganglion either from the same species or a different species in the same genus (Melampus species: M. bidentatus, M. castaneus, and M. olivaceus) or from a different genus in the same family (Ovatella myosotis).

Incorporation of implanted ganglia into the nervous system of the host can be evaluated using the animal's behavior, electrophysiological recordings, nerve and connective backfills, and immunohistochemistry. Snails with intraspecific swaps regain normal tentacle withdrawal responses much more rapidly than snails recovering from removal of a single cerebral ganglion (described in Moffett and Snyder, J. Neurobiol. 16:193-209, 1985). In many instances the implanted ganglion assumes the position and role of the excised ganglion, forming connections with the central ganglia and periphery. There are also regions of accessory neuropil development at the confluence of the host's cut nerves and connectives that secondarily contact the implant. Interspecific ganglion swaps within the genus Melampus also exhibit ganglion incorporation and support behavioral recovery. Whole mount immunofluorescence methods are being used to examine the fate of identified serotonergic cells and cell clusters in the implanted ganglia. Ganglion swaps between Melampus and Ovatella exhibit differential survival: ganglia from Melampus survive for up to 5 years in Ovatella and sometimes form connections with the host CNS or induce supernumerary sensory structures. In contrast, ganglia from Ovatella rarely survive for more than two weeks in the hemocoel of Melampus. These results suggest that whereas homologous neurons are able to find their places within the neural circuitry of closely related snails, phylogenetic closeness alone is unable to account for the regeneration success of implanted ganglia. (Supported by NIH grant RO1 NS 22896).

268.2 ALTERED SYNTHESIS OF SPECIFIC PROTEINS DURING REGENERATION OF AN IDENTIFIED APLYSIA NEURON IN VIVO. M.J. Savage and D.J. Goldberg. Department of Pharmacology, Columbia University College of P&S, New York, NY 10032.

After axotomy, neurons undergo many metabolic changes leading to either regeneration and synapse formation or death of the neuron. One change demonstrated to occur in a variety of regenerating nerve preparations is an altered synthesis of specific proteins. We have identified proteins whose synthesis is consistently increased or decreased in the identified giant cerebral neuron (GCN) of the marine slug, Aplysia californica during regeneration. With this cell we are investigating which aspects of axotomy or regeneration could act as signals for the changes in protein synthesis.

We have used one- and two-dimensional gel electrophoresis to visualize proteins in the cell bodies of GCNs which were labeled for 5 hours with ^{35}S-methionine. These cells had been axotomized and allowed to regenerate for one week in vivo. We identified three proteins with apparent molecular weights and isoelectric points of 66K,5.7, 116K,5.5, and 150K,5.5, whose incorporation of ^{35}S-methionine was significantly increased during regeneration. The latter two proteins are known to undergo fast axonal transport in other neurons of Aplysia. We found a second group of proteins whose incorporation of ^{35}S-methionine was reduced after one week of regeneration. This group consisted of six proteins: two proteins, 89K,4.9 and 104K,4.9, that are known to undergo fast axonal transport, one protein, 60K,5.6, that is thought to be a neurofilament subunit, another protein, 78K,6.1, whose synthesis has been shown to be synapse-dependent in another neuron of Aplysia, and two other proteins, 67K,5.5 and 66K,6.3.

Experiments are in progress to determine signaling events for the changes in synthesis of these proteins after axotomy. Using in vitro and in vivo methodology, we are assessing the importance of the loss of target cells, the type of target cell lost, and growth itself.

268.3 REPAIR OF BRAINSTEM-SPINAL PATHWAYS AFTER THORACIC SPINAL CORD TRANSECTION IN THE CHICK EMBRYO. B.H. Nelson* and J.D. Steeves. Dept. of Zoology, University of British Columbia (UBC), Vancouver, B.C., V6T 2A9.

The purpose of this study was to determine the exact stages during embryonic development in the chick when descending brainstem-spinal tracts lose the capacity for anatomical and functional repair after complete thoracic spinal cord transection. Transections and sham-operations were performed on embryonic days (E)3 through E13. The post-operative recovery period varied from 4 to 19 days, with some of the embryos being allowed to hatch.

The extent of anatomical repair was assessed by injecting 0.1 - 0.4 ul of a 10% solution of the retrograde tracer, wheat germ agglutinin-horseradish peroxidase (WGA-HRP), into the spinal cord, caudal to the transection site. The CNS tissue was then processed using a tetramethylbenzizdine (TMB) technique. The brainstem nuclei were examined for WGA-HRP positive cells and plotted using computerized camera lucida drawings .

Results indicate similar distributions of retrograde labelled cell bodies within the brainstems of both sham operated controls and embryos transected prior to E9. A few embryos transected on E3 actually hatched, and demonstrated complete restitution of motor function. In contrast, embryos transected on E9 through E13 never hatched and exhibited deficits in the labelling of certain supraspinal cell groups, including the reticulospinal, , rubrospinal, coeruleospinal and raphe-spinal nuclei.

The repair of spinal cord damage in early transected embryos may be due to either the regeneration of previously axotomized fibers, or to subsequent axonal projections from later developing supraspinal neurons. In an attempt to differentiate between these two possibilities, double labelling experiments, using two different retrograde tracers, injected immediately before and 4-7 days after spinal cord transection are currently in progress.

Supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada.

268.4 FURTHER CHARACTERIZATION OF A REGENERATION-SPECIFIC CYTOSOLIC PROTEIN IN THE GOLDFISH VISUAL SYSTEM. G.R. Wilmot*, T.H. Ford-Holevinski* and B.W. Agranoff. Neuroscience Lab Building, University of Michigan, Ann Arbor, MI 48104-1687.

The synthesis of a number of proteins is initiated or greatly increased following optic nerve crush in the goldfish. We have previously reported that incorporation of [^{35}S]Met into a retinal cytosolic protein doublet (68 and 70 kDa, pI 4.9 and 4.8, respectively) increases dramatically between 4 and 36 d postcrush (PC), and that these proteins reach the tectum in the newly regenerated nerve via slow axonal transport. Both the slow rate of transport and localization in a high speed supernatant fraction suggest strongly that the protein is associated with cytoskeletal elements. However, techniques for microtubule aggregation do not co-purify the doublet proteins, nor does preparation of neurofilaments by detergent sedimentation procedures. The doublet proteins have been partially purified by ion exchange and lectin affinity chromatography. While the doublet appears to be essentially absent from all non-neural tissues, a co-migrating doublet has been seen in silver stained gels of goldfish egg supernatant fraction. Following further purification of goldfish brain doublet via one- and two-dimensional PAGE gels, the electroblotted protein was administered to rabbits by subcutaneous implantation of nitrocellulose strips containing the doublet. The antibodies produced were used to probe Western blots of purified egg doublet, which indicated that the doublets from the two sources were immunologically cross-reactive. Further analysis by limited proteolysis within 1D SDS-PAGE gels (Cleveland mapping) confirmed that the egg and brain doublets are similar. The results also indicated considerable homology between the 68 and 70 kDa components. Because of the above-mentioned characteristics and presence in eggs and nervous tissue, we entertained the hypothesis that the 68-70 doublet is related to a 65-70 squid brain doublet thought to be associated with kinesin. Tubulin did not bind the 68-70 doublet in the presence of AMP-PNP and furthermore, our antibodies were not cross-reactive with a squid kinesin preparation. Efforts to sequence the goldfish doublet proteins thus far have been hampered by apparent NH_2-terminal blocks. (Supported by NEI Grant EY 05947.)

268.5 OBSERVATIONS ON ADULT APTERONOTUS SPINAL CORD FOLLOWING TRITIATED THYMIDINE INJECTIONS IN VIVO. M. J. Anderson[1], A. Reed[2], and S. G. Waxman[3]. [1]Dept. of Anatomy, Colorado State Univ., Fort Collins, CO 80523; [2] Dept. of Neurology, Stanford Univ. and VA Med. Ctr., Palo Alto, CA 94304; [3] Dept. of Neurology, Yale Med. Sch., New Haven, CT 06510.

Spinal cord of the adult teleost, Apteronotus albifrons, can regenerate new nerve cells bodies and fibers after injury. Normal adult Apteronotus cord also continues to grow in length and add new neurons in a small region at its caudalmost tip. It has been suggested that adult Apteronotus cord may continue to add neurons all along its length and that this low level of continued neurogenesis is the basis for the remarkable degree of regeneration possible in this species. We have therefore injected normal Apteronotus with ^{3}H-thymidine in order to look for addition of new neurons in spinal cord rostral to the caudalmost region of on-going growth.

Mature adult Apteronotus were injected I.P. with 0.5-1.0mCi (20μCi/g. body wgt) of ^{3}H-thymidine, sp. act. 78 Ci/mmol. Some fish were given three injections over the incubation period rather than just one injection. After an incubation period of 7 das. to 4 months, the fish were sacrificed and spinal cord was dissected out. Tissue was also taken from liver and stomach, as a control for tritium incorporation. Tissue was processed in two ways. Some tissue was fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer with 0.08M sucrose (pH7.3; 300 mOs), postfixed in 2% OsO_4, and embedded in epon-araldite. Other tissue was fixed in 10% buffered formalin and embedded in paraffin. Sections of tissue were mounted on glass slides, deparaffinized, dipped in Kodak NTB-2 emulsion (diluted 1:1), stored in light-tight boxes at 4°C for 7-30 days, then developed with D-19.

Examination of autoradiographs from 7 different fish revealed no incorporation of ^{3}H-thymidine into cells of the spinal cord. In each fish, sections of spinal cord were taken at 5 different levels along the length of the cord; all levels were rostral to the caudalmost (normally-growing) tip. Even in fish with repeated tritium injections and a 4-month incubation period, tritium labelling was not observed in any of the levels of rostral spinal cord examined. The present experiments also confirmed our previous work that there is not an increase in number of neurons in spinal cord of older Apteronotus, as has been observed in some other fish (J.Comp.Neur.179:13 and 194:291). The absence of ^{3}H-thymidine incorporation in rostral Apteronotus spinal cord has important implications. It suggests that rostral Apteronotus spinal cord is quiescent and not a site of on-going neurogenesis. Since neurogenesis can be elicited following injury to rostal regions of cord, the present results emphasize the importance of mechanisms that modulate neurogenesis in this species. Supported by the Veterans Administration and NS-15320.

268.6 SEROTONIN AXONS IN THE SPINAL CORD OF XENOPUS TADPOLES: DEVELOPMENT AND REACTION TO SPINAL CORD TRANSECTION. M.S. Beattie, J.C. Bresnahan, and G.L. Lopate. Depts. of Surgery and Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH. 43210.

Antibodies to serotonin (5-HT) were used to trace the development of selected brainstem spinal axons and their reaction to spinal transection in Xenopus laevis tadpoles.

5-HT-positive cells are observed as early as stage 29 in the ventral, medial brainstem. Others have recently reported such neurons as early as stage 25, about 28 hours after first cleavage (Van Meir et al., Int. J. Develop. Neurosci, in press). At stage 29, 5-HT positive axons are not yet seen in the spinal cord; they are present at about stage 32 in the lateral funiculuis of the rostral cord, and rapidly reach the most caudal levels.

Low thoracic spinal cord transections were made in tadpoles at stages 48-56. Animals were sacrificed at varying periods following the transection, including a number of cases which were allowed to proceed through metamorphic climax. These long-term survivals produced animals with apparently normal neurological status, and reconstituted spinal cords, as we have reported previously (Lopate et al., Neurosci. Absts, '85). In these post-metamorphic cases, 5-HT-positive fibers were frequently observed crossing the lesion site, which could be distinguished by its disorganized structure, reduced size, and often, the appearance of ependyma-lined cavities. In addition, a column of 5-HT-positive cells were found located around the central canal rostral to the lesion, and occasional, scattered cells caudal to the lesion.

The short-term response to transection was characterized by a clearing of the neuropil staining caudal to the lesion, 5-HT-positive axonal swellings at the lesion site, and the appearance of 5-HT positive cells in the cord rostral to the lesion site. Within 3-5 days, a bridge of tissue was present, and 5-HT axons with swellings and varicosities could be seen entering the region of the lesion. Within 11 days, axons could be traced well caudal to the lesion in the funiculi, and had begun reinnervating the spinal gray.

Some of the fibers which are able to bridge a spinal transection during metamorphic climax in Xenopus appear to contain 5-HT. Studies are currently underway to determine the development and reaction to injury of tyrosine hydroxylase immunopositive axons in this model of spinal cord regeneration. (Supported by NS-10165)

268.7 **THE EFFECT OF TEMPERATURE ON THE CHRONOLOGY OF THE RECOVERY OF BEHAVIOR FOLLOWING SPINAL CORD TRANSECTION IN METAMORPHOSING SEA LAMPREY.** C. Wilbur*, L. Margolin* and J. Ayers. (*Spon: Norman Boisse*). Dept. of Biology and Marine Science Center, Northeastern University, East Point, Nahant, MA 01908.

It is generally accepted that larval and juvenile animals recover from CNS injury much better than their adult counterparts. In a previous investigation (*Soc. Neurosci. Abstr. 12: 1574*), we found that ithe latency to criterion of recovery is significantly longer at lower temperatures (13°C) than in larvae which recovered at room temperature (21°). In the present experiments, we investigated how metamorphosis affects the chronology of recovery from spinal transection and whether development or temperature has a greater influence on the time course of recovery.

Complete spinal cord transections were performed on experimental animals at 25% of total body length. Specimens were then placed in individual aquaria where they were allowed to recover at either 13° or 21°C. Specimens were scored weekly for behavioral recovery. We have been able to identify five stages of functional recovery of swimming in adult lamprey. We have determined the latency from the time of the lesion to the achievement of criteria for each of these stages, as well as for crawling, burrowing and withdrawal behaviors.

There is little difference in the time to recovery between pre- and post-metamorphic specimens when held at the same temperature. In contrast reduced holding temperature (13° vs. 21°) causes a significant increase upon the time course of the recovery of swimming and other behaviors. In general, specimens held at 13° take about 30% longer to reach criterion than those held at room temperature.

Acutely transected adult lampreys are known to exhibit a behavior not displayed by acutely transected ammocoetes: spinal undulations (*Science 221: 1312*). We also examined influence of temperature upon the time course of spinal undulations. Higher holding temperatures hasten the onset of attached undulations and epochs of spontaneous undulations tend to alternate with quiescent periods more frequently than at lower temperatures. Temperature appears to have little no effect on the overall duration of the attached undulations. *Supported by NSF Grant BNS-8406880.*

268.8 REGENERATION OF AXONS AND ASSOCIATED NEUROPIL IN CLAWS OF DEVELOPING LOBSTERS. (HOMARUS AMERICANUS) P. Cauwenbergs and C.K. Govind, Life Sciences Division, Scarborough Campus, Univ. of Toronto, 1265 Military Trail, Scarborough, Ontario, Canada M1C 1A4.

Developing tissue is much more plastic than adult tissue, a phenomenon also evident during regeneration in crustaceans. In one intermolt after limb autotomy adult lobsters regenerate entire limbs which are smaller than original (intact) limbs. During subsequent intermolts these newly regenerated limbs hypertrophy to reach pristine proportions. However, early juvenile lobsters regenerate entire limbs of pristine proportions in one intermolt. Whether such robustness in regenerative capacity extends to nervous tissue was studied by comparing nerve roots and hemiganglia between regenerated and opposite claws in developing juvenile lobsters. Unilateral autotomy of a cheliped was performed at various stages in juvenile animals, and the nerve supply to the regenerate and original limbs was analysed after one intermolt.

In early juveniles axon numbers in regenerated nerve roots slightly exceeded that of original roots, while in later juveniles, axon numbers in regenerated nerves were fewer than in controls. This robust regenerative capacity in early juveniles resulted in a two fold increase in synaptic density in hemiganglia of the regenerated versus original side. No differences were noted between regenerated and original hemiganglia in early juveniles when either the types (excitatory, inhibitory and neurosecretory) or diameter of nerve terminals were compared. Thus, the regenerative capacity in early juveniles is very robust with a slight overproduction of axons and a dramatic overproduction of central synapses.

In a second series of experiments, regeneration was delayed over several intermolts with repeated removal of the regenerate. As these experimental animals aged, increasingly fewer axons were counted in regenerated versus original roots and a corresponding decrease in synaptic density in hemiganglia on the regenerated side was observed. Nerve terminals, however, were markedly larger in regenerated versus original ganglia. Such a hypertrophy denotes a compensatory mechanism by existing terminals to occupy available space in the neuropil in the absence of the normal complement of sensory axons from the periphery. Subsequent reinvasion of the ganglionic neuropil by regenerating sensory fibres must entail a down-scaling of existing terminals in order to accommodate the full complement of axons. Clearly the nerve roots and ganglionic neuropil undergo considerable remodelling during claw regeneration in juvenile lobsters.

(Supported by grants from NSERC and MDAC)

268.9 ACCELERATION OF SLOW TRANSPORT IN AXONS OF REGROWING NEWT LIMBS BY A NERVE CONDITIONING LESION MADE PRIOR TO AMPUTATION. C. Eberhardt Maier* and I.G. McQuarrie) Dept. of Devel. Genetics and Anat., Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

Forelimb regeneration in the newt (Notophthalmus viridescens) is a nerve-dependent phenomenon. After a limb is amputated, all the tissues of the replacement limb (except nerve axons) are formed from cells of the limb bud. Neurons innervate the new limb by regenerating axons that had been severed by amputation. Axonal sprouting and elongation is the result of the transport of cytoskeletal elements to the distal end of the axon. Previous studies have shown that the rate of axonal elongation coincides with the transport rate of the labeled cytomatrix proteins (e.g. actin, calmodulin, fodrin) and the fastest moving microtubules, representing Slow Component b (SCb) of axonal transport.

Studies were undertaken to determine whether a correlation exists between SCb and limb regrowth. The first requirement was to establish the rate of SCb in brachiospinal nerves. A laminectomy was performed to expose the cervical spinal cord. 35S-methionine was injected into the motor columns of spinal segments 3, 4, and 5. Injection-sacrifice intervals of 7, 14, 21, 28, and 42 days were employed, and serial 1 mm nerve segments were homogenized in SUB (0.5% SDS, 8M urea, 2% beta-mercaptoethanol). The supernatants were subjected to SDS-PAGE followed by fluorography. Fluorograms were used as templates to identify proteins to be removed from gels for solubilization and liquid scintillation counting. These included neurofilament proteins, microtubule-associated proteins, tubulins, actin, calmodulin, and fodrin. The peak of labeling for SCb proteins was found to advance at approximately 0.25 mm/d in normal nerves. The peak of labeling for neurofilament proteins, which identify the slowest moving rate component (SCa), advanced at less than 0.1 mm/d.

We have previously demonstrated that a conditioning lesion of newt brachiospinal nerves, made 2 weeks before amputation, will accelerate axonal sprouting and limb regrowth (Maier et al., J Exp Zool 232:181-6, 1984). We now show that the analysis of SCb transport patterns after amputation indicates a 2-fold increase in the rate of SCb during accelerated limb regrowth.

Supported by NINCDS grant RO1-18975 to I.G.McQ.

268.10 FRAGMENTS MADE FROM FULLY DIFFERENTIATED XENOPUS EYES REGENERATE TO FORM PATTERN DUPLICATED VISUO-TECTAL PROJECTIONS. L. Wunsh* and C.F. Ide. Dept. of Biology, Tulane University, New Orleans, LA 70118

In previous studies, removal of the temporal 2/3 of the stage 32 Xenopus eye bud resulted in restoration of an intact eye which formed pattern duplicated projections to the midbrain optic tectum. Restoration of the eyebud may have involved true regenerative growth, or merely a reassignment of uncommitted embryonic cells (e.g., optic stalk cells) to become part of the eye. To determine if Xenopus is capable of "true" retinal regenerative growth, we removed the temporal 2/3 of the retina of progressively older larval eyes (stages 38 and 48). Ten animals from each stage were injected with tritiated thymidine at one day post surgery and prepared for autoradiography one day later. Other animals were reared through metamorphosis and assayed for visuo-tectal projection type via electrophysiology techniques. More than 60% of eye fragments from both stages regenerated to form a normal sized eye.

Stage 38 fragments analyzed histologically at stage 43 showed a large mass of undifferentiated, heavily labeled cells in the naso-ventral region of the fragment. Similar fragments reared through metamorphosis showed pattern duplication of the visual projection to the medial tectum, the normal site of innervation of naso-ventral retinal cells. Thus, stage 38 fragments were similiar to embryonic stage 32 fragments with regard to healing mode, location of undifferentiated, labeled cells in the naso-ventral retina, and pattern duplication of naso-ventral map values. Stage 48 fragments on the other hand analyzed two days post surgery resembled normal, differentiated retina except for "ciliary margin-like" cells found throughout most of the dorsal part of the fragment which folded back upon themselves during healing and were heavily labeled with tritiated thymidine. Fragments mapped after metamorphosis showed partial duplication of the viso-tectal projection in the lateral tectum, the normal site of innervation from dorsal retina.

Stage 48 fragments represent a true "regenerating" system in that the retina is fully differentiated at the time of ablation and during subsequent regeneration. It may be, in these older fragments, that the remaining ciliary margin is the source of cells involved in retinal regeneration and that local growth in oddly juxtaposed regions of the margin correlate with partial pattern duplication of visuo-tectal map values. Supported by NSF Grant PCM-8316142.

268.11 REGENERATION AND NEURAL PLASTICITY FOLLOWING AXOTOMY OF AN IDENTIFIED GASTROPOD NEURON. R.P. Croll, M.W. Baker*, R.C. Swetnam* and R.Y.S. Lo*. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

The nervous systems of several gastropod molluscs have been shown to be capable of regeneration and plasticity following injury. This study examines the axonal regeneration of an identified, serotonergic cell within the cerebral ganglion following a crush of its 2-5 collateral axons within the cerebrobuccal connective (CBC). In vitro, axonal regeneration of a homologous cell in Helisoma has previously been reported (Murphy et al., 1985, J. Neurobiol. 16:137). Our study examined this phenomenon using an in vivo preparation. Use of a much larger snail, Achatina, facilitated surgery and resulted in survival rates of near 90%. Following paralysis by injection of succinyl choline, a dorsal incision in the body wall permitted access to the paired CBC's. A single CBC in each subject was then crushed near the buccal ganglion and after closure of the incision, the subjects were maintained for up to 120 days. Regeneration was examined following the lesion using histochemical localization of serotonin-like immunoreactivity (SLIR), intracellular dye injections and backfilling techniques. Within 2-3 days following crush, blebs appeared along all SLIR fibres in the CBC ipsilateral and distal to the crush and in all ipsilateral buccal roots. These blebs became separated from each other and then decreased in number over the first 7 days post-lesion leaving little or no ipsilateral SLIR distal to the lesion. Also within the first week a number of small neurites were seen sprouting from the several axons proximal to the lesion site. These small neurites increased in number during the next two weeks at which point the first fibres were seen traversing the lesion site. The number of neurites which regenerate past the lesion gradually increased and by 8-10 weeks hundreds of very small fibres reached the buccal ganglia and exit the ganglion via the buccal roots.

Use of this preparation has also proved advantageous in another regard. In Achatina the buccal projection of the serotonergic cerebral cell is mostly unilateral. Unilateral axonal crush therefore results in nearly complete depletion of serotonin from the buccal ganglion ipsilateral to lesion. The buccal ganglia were therefore examined for evidence of compensatory sprouting of the contralateral, uninjured cell into denervated area. Sprouting of new fibres across the buccal commissure and out contralateral buccal roots were detected within a week following axonal crush. The number of contralateral fibres appeared to remain stable for at least several weeks following the lesion.

This study was supported by an NSERC (Canada) grant to RPC.

NEURAL PLASTICITY IN ADULT ANIMALS: HIPPOCAMPUS

269.1 A FREQUENCY-DEPENDENT THRESHOLD-LIKE EFFECT CONTROLS BOTH LTP AND LTD. Barbara Burger & W.B Levy. Dept. of Neurosurgery, Univ. of Va., Charlottesville, Va 22908.

The accompanying abstract (Levy & Burger) shows that the number of active afferents can control the production of entorhinal cortex-dentate gyrus LTP and LTD through a highly nonlinear, presumably postsynaptic process. The same interpretation explains the results here where frequency of ipsilateral (ipsi) afferent activity is used to control the event permitting contralateral (contra) LTP and LTD.

6 rats, prepared similarly to Levy et al.'83, received a series of unilateral conditioning trains at frequencies varying from 50-400Hz (2-9 pulses/20ms). 9 rats, prepared similarly, received bilateral conditioning stimulation in which the ipsi pathway received conditioning stimulation at frequencies varying from 25-400Hz (2 pulses/40ms or 2-9 pulses/20ms) while, simultaneously, the converging contra pathway received 400Hz (9 pulses) conditioning stimulation. The contra response is tested alone for 10-15' after each conditioning set.

For the train lengths and intensities used, ipsi conditioning of 100Hz or less does not support significant contra LTP or LTD, regardless of the concurrent contra afferent activity. However, ipsi conditioning at 200Hz or more results in LTD or LTP, depending on the concurrent contra afferent activity. 127 trains of 200Hz ipsi conditioning stimulation alone depresses the unconditioned, converging contra response to 76% of its previous baseline; p=.04. 63 trains of 200Hz ipsi conditioning paired with trains of 400Hz contra conditioning, increases the contra response to 126% of its previous baseline; p=.01. Importantly, 200Hz ipsi conditioning requires only 3 trains (i.e. just 15 stimulation pulses) to induce significant LTD or LTP; ipsi conditioning at lower frequencies fails to produce significant changes after 63 or 127 trains (i.e. 254-635 pulses).

These results indicate that: (1) There is a nonlinear relationship between presynaptic activity, varied as frequency here, and the postsynaptic event that permits synaptic modification. (2) Postsynaptic cell firing is not sufficient for supporting synaptic modification since lower frequencies fail to support LTP or LTD even though conditioning stimulation evokes population spikes. (3) LTD and LTP can be viewed as two parts of a single modification process since equivalent ipsi conditioning stimulation permits either LTP or LTD. Supported by NIH NS 15488 and NIMH RSDA MH00622 to W.B Levy.

269.2 AN INTENSITY-DEPENDENT THRESHOLD-LIKE EFFECT CONTROLS BOTH LTP AND LTD. W. B Levy & Barbara Burger. Dept. of Neurosurgery, Univ. of Va., Charlottesville, Va 22908.

Although somatic discharge of the postsynaptic cell is neither necessary nor sufficient to produce long-term potentiation (LTP), there is clear evidence that induction of LTP requires a permissive event with threshold characteristics (McNaughton et al.'78; Wilson,'81) Using the monosynaptic, bilateral input from the entorhinal cortex to the dentate gyrus this report separates presynaptic requirements from the event permitting long-term modification. The results are consistent with a highly non-linear postsynaptic event that permits either contralateral (contra) LTP or contra long-term depression (LTD).

8 rats, prepared as in Levy et al.'83, receive 3 ipsilateral (ipsi) alone conditioning sets of different intensities, followed by 3 sets of bilateral conditioning in which the ipsi pathway receives different intensity conditionings while the converging contra pathway receives conditioning stimulation of constant intensity. The contra response is tested for 15' after each of the 6 conditioning sets. Though cell firing is neither necessary nor sufficient for LTP, there is often a good correlation between the existence of a population spike and induction of LTP. Thus, 3 ipsi conditioning intensities are used: a low intensity evoking a 1-2 mV ipsi response; a medium intensity evoking a 10-15 mV ipsi response without a population spike; and a high intensity evoking a 10-20 mV ipsi response with a population spike.

With unilateral conditioning, neither low nor medium intensity ipsi conditioning significantly depresses the contra response. However, high intensity conditioning significantly depresses the contra response to 73% of its preconditioning values; p=.02. Likewise, with bilateral conditioning, neither low nor medium intensity ipsi conditioning permits potentiation of the contra response. However, high intensity ipsi conditioning paired with contra conditioning induces significant LTP of the contra response (142% of its pre-bilateral conditioning value; p=.02).

These data suggest that active afferents sum to control a nonlinear, or perhaps, a threshold-like process which permits the induction of either LTP or LTD. The accompanying abstract, Burger and Levy, shows a similar result using frequency instead of intensity as the experimental variable.

Levy et al, Neurosci. 8, p799, 1983. McNaughton et al, Brain Res. 156, p277, 1978. Wilson, J. Neurophys. 46, p324, 1981.
Supported by NIH NS 15488 & NIMH RSDA MH00622 to W.B Levy.

269.3 CHANGES IN DENDRITIC SPINE DIMENSIONS CAN CONTRIBUTE TO LTP IN THE HIPPOCAMPAL DENTATE GYRUS. N. L Desmond, R. E. Williams*, and W. B Levy. Dept. Neurosurgery, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.

There is some controversy as to the role dendritic spines play in controlling excitatory synaptic drive. Some investigators, following Rall's '74 proposal, argue that changes in the dimensions of long, thin spine stems could significantly alter current flow from an activated spine synapse to the parent dendrite. That is, as a spine stem shortens and widens, its longitudinal resistance (R_{SS}) decreases. With this decrease, current flow into the parent dendrite would increase. Another group essentially argues that spine synapses are indistinguishable from shaft synapses because changes in stem dimensions have little or no effect on current flow from an activated spine synapse. The argument hinges on the actual spine stem dimensions and the resistance to ground (R_{BI}) at the intersection of the parent dendrite with the spine stem. Using morphometric values (Desmond and Levy, '82, '84, '85) from Golgi-impregnated granule cells of the dentate gyrus (DG) and a steady state model of the granule cell, this report finds that spine stem alterations can contribute significantly to LTP in the DG. A computer program solved the usual second order partial differential equation $[(\lambda^2\delta^2V/\delta X^2)-V=0$; Rall, '77] to calculate steady-state R_N (resistance to ground at the soma), R_{SS}, and R_{BI} values for 7 cells. The model used an R_M of 8000 ohm·cm^2 and an R_i of 70 ohm·cm. Values obtained for R_N (mean=83 Mohm) are somewhat higher than those reported from electrophysiological analyses (40-60 Mohm). The following example uses a dorsal leaf granule cell. A spine stem with a diameter of 0.1 um and a length of 1.2 um has an R_{SS} of roughly 107 Mohms. In the middle third of the molecular layer, R_{BI} ranges from 70-180 Mohms, with an average of 118 Mohm. Such a spine has a R_{SS}/R_{BI} ratio of 0.91 "before" LTP. Our LTP experiments find that spine stems shorten 15% and widen by 21%. If the exemplar spine stem dimensions are changed as suggested by these percentages, the dimensions become 0.12 um (diameter) and 1.02 um (length), producing an R_{SS} of 63 Mohms. With LTP then, the R_{SS}/R_{BI} ratio becomes 0.53, a decrease of 42% in the ratio of spine stem resistance to branch input resistance. This decrease produces a current increase of 24% (18-33% depending on R_{BI}). Thus, these data support Rall's original proposal so that the mechanisms underlying LTP may include spine stem modification as well as increased transmitter release (Dolphin et al., '82) and postsynaptic changes (Desmond & Levy, '86; by inference, more receptors). Supported by NS15488 and NIMH RSDA MH00622 to WBL.

269.4 INDUCTION OF HIPPOCAMPAL LONG TERM POTENTIATION IN THE AWAKE RAT USING PHYSIOLOGICALLY PATTERNED STIMULATION. D.M. Diamond and G.M.Rose. Medical Research Service, VAMC and Dept. of Pharmacology, UCHSC, Denver, CO 80262

Long term potentiation (LTP) has been described extensively as a mnemonic model. However, in most studies the stimulation required to induce LTP exceeds normal physiological activity. Recently, Rose and Dunwiddie (Neurosci. Lett., 69:244, 1986) reported that the threshold to induce LTP was reduced when the stimulation parameters more closely mimicked hippocampal discharge activity. They incorporated two well known characteristics of physiological activity in the hippocampus into a pattern of electrical stimulation: 1) hippocampal neurons discharge in a burst of activity, and 2) rhythmic activity at approximately 6 Hz (170 msec period) is observed during exploration (theta rhythm). Using the in vitro preparation, they stimulated the commissural input to CA1 with a single pulse, followed 170 msec later by a high frequency burst of 4 pulses (primed burst, PB). This pattern of stimulation, combining the timing of the theta rhythm with the bursting activity intrinsic to hippocampal neurons, resulted in a long term increase in the amplitude of the population spike (PB-LTP). In contrast, a high frequency train of 5 pulses (unprimed burst) did not induce long lasting effects. In this report, we have extended the findings of the in vitro study by using patterned stimulation to induce PB-LTP in the awake rat.

Data were obtained from 9 rats in 26 recording sessions. Under barbiturate anesthesia, the subjects were implanted with a stimulator in the hippocampal commissure. Contralateral to the stimulation site, a microdrive base was implanted over CA1. A miniature microdrive was then attached to the base after the subject recovered from the surgery. The removable microdrive allowed for accurate localization of the recording electrode in the CA1 cell body layer. Responses were recorded in CA1 following stimulation of the commissure. Population spike amplitude was just above threshold (.5-1 mV). The subjects were either asleep or in a quiet awake state during all baseline and post-high frequency recordings. Immediately prior to patterned stimulation (1+4 pulses), the subjects were awakened. Lasting increases (>20 min) in population spike amplitude occurred in 65% (17/26) of the recordings. In 13 sessions in which an initial EPSP was evident, increases in the slope occurred in 54% (7/13) of the recordings. There were no changes (0/17) in response to a train of 5 pulses.

Studies using patterned stimulation have provided an initial understanding of the relationship between endogenous rhythms and synaptic plasticity. By replicating the earlier in vitro work, we can now apply a two-tiered approach towards understanding both the mechanisms and behavioral basis of LTP.

This work was supported by the VA Medical Research Service.

269.5 CHARACTERISTICS OF CROSSED AND MULTISYNAPTIC PATHWAYS FROM THE ENTORHINAL CORTEX TO THE CONTRALATERAL DENTATE GYRUS IN UNANESTHETIZED RATS. R.L. Wilson*, K. Pang and G.M. Rose. Dept. of Pharmacology, UCHSC and Medical Research, VAMC, Denver, CO 80262

Previous studies have described a sparse monosynaptic connection from the entorhinal cortex to the contralateral dentate gyrus, termed the crossed temporodentate pathway (CTD). Since previous electrophysiological descriptions of this connection are from work in anesthetized animals, we re-examined this pathway in unanesthetized, behaving rats.

Sprague-Dawley rats were bilaterally implanted with stainless steel electrodes for chronic neurophysiological experiments. Recording electrodes were placed in the ilar region of the dentate gyrus and stimulating electrodes were placed in the angular bundle to allow activation of the entorhinal afferents. Input-output curves were constructed by stimulating an individual perforant path from 1 to 20 volts and recording the responses in the contralateral dentate gyrus. High frequency stimulation (HFS) consisting of 150 stimuli (10 trains of 15 pulses at 500 Hz; 5 sec. intertrain interval) was delivered to a single perforant path (HFS-PP).

Two evoked potentials were observed in response to perforant path stimulation which could be discriminated by differing onset latencies. The early (about 4.2 ms) potential corresponded to the previously described CTD response. The long latency (about 7.2 ms) of the second response suggested that it resulted from activation of a di-synaptic pathway (entorhinal->entorhinal->dentate; DS). Both responses, when recorded from the dentate hilus, displayed a positive field EPSP and a population spike. The CTD response was observed at higher stimulation voltages than the DS response.

After HFS, the following results were observed: 1) When recording ipsilateral to the HFS-PP, the direct perforant path-dentate (PP-DG) response was significantly increased. In contrast, the PP-DG response recorded contralateral to the HFS-PP was NOT increased. 2) The CTD response recorded ipsilateral to the HFS-PP was NOT significantly increased. However, a significant increase was seen in the CTD response recorded contralateral to the HFS-PP. 3) The DS responses recorded both ipsilateral and contralateral to the HFS-PP were significantly increased. In all cases where the response was elevated, it remained so for at least 24 hours.

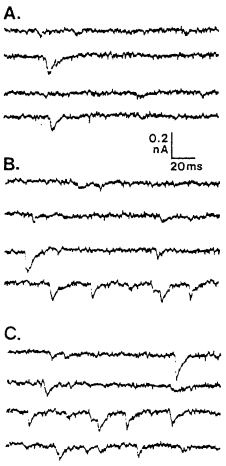
In conclusion: 1) A population spike was elicited in the CTD pathway in the absence of anesthesia. 2) A longer latency, presumably DS, response was also recorded following contralateral PP stimulation. 3) HFS to a single perforant path produced LTP of the CTD and DS responses. 4) LTP of the DS response resulted from the potentiation of the first synapse (entorhinal -> entorhinal) and/or the second synapse (entorhinal -> dentate). These results demonstrate that information from a single entorhinal cortex is transferred to both the ipsilateral and contralateral dentate gyrus.

This work was supported by the VA Medical Research Service.

269.6 SINGLE QUANTAL CURRENTS IN HIPPOCAMPAL NEURONS. X.-W. Rong*, C. L. Keenan, and T. H. Brown (SPON: E. Roberts). Div. of Neurosci., Beckman Res. Institute, City of Hope, Duarte, CA 91010.

Application of the single-microelectrode clamp (SEC) technique to synapses in hippocampal brain slices (Brown and Johnston, J. Neurophysiol. 50: 487, 1983) enabled studies of conductance mechanisms responsible for long-term synaptic potentiation (LTP) in this tissue (Barrionuevo, Kelso, Johnston and Brown, J. Neurophysiol. 55: 540, 1986). Further elucidation of the mechanism responsible for LTP expression in hippocampus requires a quantal analysis (Barrionuevo, et al, 1986). This is best done using voltage-clamp techniques under conditions in which the quantal currents are larger than the noise.

Single quantal events have been detected in CA3 pyramidal neurons under both current- and voltage-clamp conditions (Brown, Wong and Prince, Brain Res. 177: 194, 1979; Johnston and Brown, Brain Slices, Plenum, 1984). The purpose of the present study was to improve the signal-to-noise ratio in our measurements of single quantal currents in these cells using a better SEC.



Hippocampal slices were prepared and maintained in the usual fashion. To the normal bathing medium was added tetrodotoxin (1 μM, to block evoked release), picrotoxin (10 μM, to block inhibitory quanta--see Johnston and Brown, 1984), and CsCl (2 mM, to block anomalous rectification at hyperpolarized holding potentials). Microelectrodes (25 - 40 MΩ) were beveled and the switching rate of the SEC was 5 - 8 kHz. Spontaneous miniature excitatory postsynaptic currents (MEPSCs) were recorded from a holding potential of -120 mV. Illustrated MEPSCs were recorded at three different time periods (A - C) over the course of an hour. Assuming a reversal potential near 0 mV (Brown and Johnston, 1983; Johnston and Brown, 1984), the larger currents represent a measured conductance increase (see Barrionuevo, et al, 1986) of about 10^{-9} S.

If the larger MEPSCs are produced by the mossy-fiber inputs--a possibility that we are now testing--then these synapses should be suitable for performing a quantal analysis of LTP.

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269.7 MANIPULATION OF PITUITARY-ADRENAL ACTIVITY AFFECTS NEURAL PLASTICITY IN RODENT HIPPOCAMPUS. M.R. Foy, J.G. Banghart, S. Levine and R.F. Thompson. Departments of Psychology & Psychiatry, Stanford University, Stanford, CA 94305.

Stress can both improve or impair learning, and many of the hormones secreted during stress (ACTH, glucocorticoids, opioids, epinephrine, NE and vasopressin) can affect learning and memory processes. A marked impairment of long-term potentiation (LTP) in hippocampal explants taken from rats exposed to acute stress (inescapable tail-shock) has recently been reported (Foy et al., Behav. & Neural Bio., 1987, in press). In the present study, we examined the effects of acute stress (inescapable tail-shock) on LTP in rats given water or in rats pretreated with dexamethasone (dex, a synthetic glucocorticoid which blocks the pituitary-adrenal response to stress). We also examined the time-course of the stress response on LTP impairment.

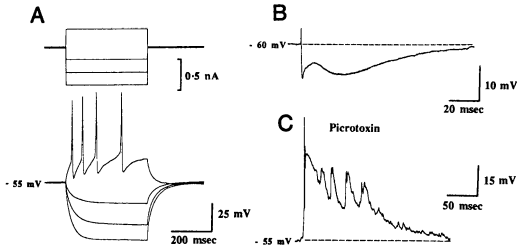
Adult male rats (300-450g) were pair-housed in climate controlled facilities on a 12 hr light/dark cycle with food and water available ad lib. One animal in each pair (water x stress) was placed in a restraining tube and received tail-shocks (1 mA, 1 sec) every minute for 30 min. The other animal in each pair (water x control) was taken directly from the home cage and received no restraint or tail-shock. Dexamethasone was administered in drinking water (20 ug/ml) for 48 hr prior to experimental conditions mentioned above, i.e., (dex x stress) and (dex x control). In vitro hippocampal slices (400 um) were then prepared from these animals according to standard methods. Extracellular recordings of the population field potential were taken from the CA1 cell body layer in response to stimulation (0.1 msec pulses) of Schaffer collateral afferents, before and after tetanus (100 Hz for 1 sec). A stable response potentiation following the decline from maximal potentiation (PTP) occurred sooner in the dexamethasone treated groups, regardless of experimental condition (stress vs control). However, a stress-induced impairment of LTP was observed, regardless of dexamethasone treatment, from 5-30 min post-tetanus (LTP).

Our data suggest that dexamethasone might facilitate the time course from PTP to LTP; some variable(s) in the temporal pattern of the PTP/LTP interaction may be modulated by dexamethasone. These data replicate our previous findings that stress impaires LTP in the in vitro hippocampal slice. In addition, manipulation of the pituitary-adrenal axis by dexamethasone treatment appears to influence temporal patterns in the PTP/LTP interaction.

Supported by NIMH (MH15147) to MRF; grant HD02881 from NICH & HD to SL; and by grants from ONR (N00014-83-K-0238), NSF (BNS-8106648), and the McKnight Foundation to RFT.

269.8 SYNAPTIC PLASTICITY AND THE ROLE OF NMDA RECEPTORS IN EPILEPTIFORM DISCHARGES IN TURTLE HIPPOCAMPUS *IN VITRO*. Linda J. Larson-Prior and N. Traverse Slater. Departments of Physiology and Psychiatry, Northwestern University Medical School, Chicago, IL 60611

The turtle telencephalon has provided a useful *in vitro* model for comparative studies of the physiology of the hippocampus and visual cortex due to the resistance of this tissue to anoxia and simplified intrinsic circuitry [1,2,3]. Intracellular recordings were made in pyramidal neurons of the turtle hippocampus (ventromedial cortex; VMC) identified by their characteristic firing properties (Fig 1A; refs 1&2). The intact VMC was maintained *in vitro* at 22 °C as previously described [1,2,3]. Stimulating electrodes were placed on the margin of the VMC adjacent to the lateral ventricle, and pyramidal neurons were impaled within 0.5 mm of the site of stimulation. Synaptic stimulation evoked either a monophasic or a biphasic ipsp (Fig 1B). Ipsps were transiently reduced by 1-2 Hz stimulation, and were irreversibly reduced (30-100%) by high frequency tetani (50 Hz, 2s), as observed in mammalian hippocampus [4,5]. Following the application of the GABA antagonist picrotoxin or 3-mercaptopropionic acid (3-MP) ipsps were reduced and synaptic stimulation produced epileptiform discharges (Fig 1C) which were not affected by the NMDA receptor antagonist ±AP5. In many cells 1 Hz stimulation or perfusion with magnesium-free medium revealed a slow epileptiform epsp which was reversibly blocked by the NMDA receptor antagonists ±AP5 and ketamine.



These results demonstrate a tetanus-induced plasticity of GABAergic inhibition in the turtle VMC which is similar to that described in the mammalian hippocampus [4,5]. However, the lack of sensitivity of epileptiform discharges induced by GABA antagonists to ±AP5 suggest a less prominent role is played by NMDA receptors in mediating epileptiform activity in the turtle VMC than has been previously demonstrated in the mammalian hippocampus *in vitro*.

[1] Shen & Kriegstein (1986)*J. Neurophysiol.* 56:1626.[2] Connors & Kriegstein (1986) *J. Neurosci.* 6:164. [3] Kriegstein & Connors (1986) *J. Neurosci.*, 6:178. [4] McCarren & Alger (1985) *J. Neurophysiol.*, 53:557. [5] Stelzer et al. (1987) *Nature*, 326:698.

269.9 LONG-TERM POTENTIATION IN CA3 NEURONS: CHANGES IN EFFICACY OF UNTETANIZED INPUTS. J. E. Bradler* and G. Barrionuevo. Departments of Behavioral Neuroscience, Psychiatry, and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Intracellular recordings were made from pyramidal neurons in the CA3b subfield of the in vitro rat hippocampal slice. EPSPs were reliably elicited by stimulation of three different sites in the slice: 1) the s. radiatum of the CA1 region; 2) the fimbria (Fi); and 3) the s. granulosum of the dentate gyrus to activate the mossy fibers (MF). Picrotoxin (8-15 M) was present in all experiments to block concomitant synaptic inhibition. During a control period the synaptic inputs were tested for 30 min at 0.16 Hz. If the EPSP amplitudes proved stable, tetanic stimulation was delivered to one of the stimulation sites to induce long-term potentiation (LTP). The tetanus consisted of eight 100 Hz 50 ms duration bursts, delivered at 0.16 Hz. LTP was defined as a nondecremental increase in EPSP amplitude of at least 25% 15 min posttetanus.

In experiments in which LTP was induced in the tetanized CA1-evoked EPSP (N=4), the amplitude of the untetanized MF-evoked EPSP underwent a depression (-20%) for the duration of the recording epoch. No consistent changes were observed upon the untetanized Fi-evoked EPSP. In experiments in which LTP was induced in the tetanized Fi-evoked EPSP (N=2), the untetanized CA1- and MF-evoked EPSPs were reduced in amplitude (-30%) during the posttetanus period. During the control period in experiments in which LTP was induced in the tetanized MF-evoked EPSP (N=6), stimulation of the MF site was simultaneously paired first with the CA1 site, and then with the Fi site. These pairings were designed to verify independence of the three inputs generating EPSPs in the impaled cell, and did not result in long term changes in the EPSPs. If independence was not confirmed, i.e., if the amplitude of the "paired" EPSP was smaller than the algebraic sum of the amplitudes of each individual EPSP, the data was not considered for analysis. In three neurons in which this criterion was fulfilled for the MF-and Fi-evoked EPSP, the untetanized Fi-evoked EPSP exhibited profound LTP (238%) considerably exceeding that induced in the tetanized MF-evoked EPSP (129%). In only one of these neurons was independence confirmed between the MF- and CA1-evoked EPSP. Upon induction of LTP in the MF-evoked EPSP, the untetanized CA1-evoked EPSP underwent LTP of considerable magnitude, reaching action potential threshold within 10 min posttetanus.

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269.10 EFFECTS OF NMDA RECEPTOR ANTAGONISTS ON NOREPINEPHRINE-INDUCED LONG-LASTING POTENTIATION (NELLP) OF POPULATION SPIKE AND EPSP IN RAT DENTATE GYRUS. E.C. Burgard*, G. Decker*, and J.M. Sarvey, Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

NELLP is a long-lasting increase in both population spike amplitude and EPSP slope induced in rat dentate gyrus granule cells by bath application of norepinephrine (NE) to the in vitro hippocampal slice (Stanton & Sarvey, Brain Res. Bull. 18:115, 1987). The role that N-methyl-D-Aspartate (NMDA) receptors play in this phenomenon is intriguing since it has been demonstrated that these receptors play a regulatory role in the induction of long-term potentiation in the hippocampal slice (Dingledine, TINS 9:47, 1986). Here we investigate the susceptibility of NELLP to two selective NMDA antagonists: D-2-amino-5-phosphonovaleric acid (APV) and 3-[(+)2carboxypiperazin-4-yl]-propyl-1-phosphonic acid (CPP).

Medial perforant path stimulation in the rat hippocampal slice evoked a population spike recorded in the granule cell layer of dentate gyrus along with a dendritic EPSP recorded 100-200 µm from the cell body layer. In control experiments, bath application of (-)NE (50 µM) for 30 minutes reliably induced NELLP of both population spike amplitude and initial negative slope of the EPSP. This potentiation persisted after a subsequent 30 minutes of drug-free wash. In other experiments, APV and CPP were administered via the bath 15 minutes prior to, during, and 15 minutes following NE application. The responses were then followed through a 30 to 60 minute wash with drug-free buffer.

Application of CPP (1 or 10 µM) reversibly depressed the population spike 3-55% as well as the EPSP slope 0-31% recorded 15 minutes following administration of the drug. D-(-)-APV (10 µM) also reversibly depressed the population spike 4-15% but did not affect the EPSP slope. All concentrations of both drugs completely blocked potentiation produced in the presence of NE as well as NELLP of both the EPSP and population spike.

We conclude that (1) NMDA receptors are involved in the regulation of NELLP of both population spike and EPSP in rat dentate gyrus, and (2) NMDA receptors may be involved in normal synaptic transmission in medial perforant path. (Supported by USUHS Grant No. C07514)

269.11 EFFECTS OF SELECTIVE FORNIX-FIMBRIA LESIONS ON SUBSTANCE P, SOMATOSTATIN, AND CHOLECYSTOKININ CONCENTRATIONS IN THE RAT HIPPOCAMPUS. U.E. Gasser*, A.R. Dravid and R.A. Siegel*. Preclinical Research, Sandoz Ltd., Basle, Switzerland and Dept. Pharmacology, New York Univ. Med. Ctr., New York, NY 10016, U.S.A.

The neuropeptides substance P (SP), somatostatin (SST), and cholecystokinin (CKK) are present in the mammalian Hippocampus (HI). The SP projection to the HI likely arises in the medial septum-diagonal band (MS). CKK neurons appear to be intrinsic to the HI whereas SST-positive cells and fibers have been reported in the HI and fornix-fimbria (FF), respectively. We determined the activity of choline acetyltransferase (ChAT), and SP, SST and CKK levels (RIA) in 3 septotemporal regions of the HI in intact rats and in rats with partial FF lesions (medial/lateral). In control rats, the three peptides, like ChAT, exhibited an ascending concentration gradient along the septotemporal axis of the HI. One week postlesion, the marked loss of SP manifested in a lesion- and HI region-specific manner; an almost complete loss of SP in the dorsal region after medial lesion, and a marked reduction in the ventral region after lateral lesion. Thus the mediolateral organization of SP efferents from the MS appears similar to that of cholinergic fibers. The contribution of the dorsal pathway to hippocampal SP-innervation was estimated to be about 65% (complete FF lesion). The complete recovery of SP levels in all HI regions, and an overshoot in dorsal and medial regions, 8 weeks after either lesion may be due to collateral sprouting and/or accumulation of SP in the spared afferents. The mechanism(s) involved in similar changes in the contralateral HI are unclear. The decrease of SP in the MS at 8 weeks may indicate axotomy-induced retrograde degeneration of SP neurons. The lack of lesion effect on SST content at 1 week suggests the absence of septo-hippocampal innervation whereas the decline in SST in both hippocampi at 8 weeks probably reflects retrograde degeneration of SST neurons in the HI whose efferents course through both ipsi- and contralateral dorsal pathway. CCK levels in HI were not influenced by either lesion supporting the view that CCK containing neurons are intrinsic to the hippocampus.

269.12 **POSSIBLE ROLE OF INHIBITION IN LTP INDUCTION.** G. J. Pacelli, F. Naghdi and S. R. Kelso. Dept of Biological Sciences and Committee on Neuroscience, University of Illinois at Chicago, Chicago, IL 60680.

Brief activation of afferent pathways to region CA1 of the hippocampus can lead to a long lasting increase in synaptic transmission that has been termed long-term potentiation (LTP). Several studies have shown that separate sets of afferent fibers may be used to associatively induce LTP, using paradigms that in some cases are analagous to classical conditioning paradigms. One recent study reported that optimal associative interactions occur when brief trains of stimulation (40 msec at 100 Hz) are delivered to two separate afferent inputs at about theta frequency, i.e., when stimulation of the two inputs is separated by 200 msec. These patterns of stimulation are also accompanied by a "priming" effect, in which the stimulation of one pathway causes a prolongation of the synaptic response elicited in the second pathway 200 msec later. We wished to examine the suggestion (Larson and Lynch, Science 232:985, 1986) that this could be due to a lack of inhibitory synaptic activation during the second, "primed", synaptic stimulation.

Hippocampal slices were prepared from male Sprague-Dawley rats and maintained in oxygenated nutrient medium at 33-36 ^{0}C. Bipolar wire stimulating electrodes were placed on either side of region CA1 to stimulate separate sets of afferent fibers in stratum radiatum. Electrophysiological recordings were then made from stratum radiatum and stratum pyramidale in CA1 one half way between the two stimulating electrodes.

Our results indicate that brief stimulation (40 msec at 100 Hz) that does not normally elicit LTP can be made to do so when preceeded by 200 msec with a single shock to a separate "priming" synaptic input. Similarly, a single priming shock can lead to a prolongation of the synaptic response of the second "primed" input. When slices are bathed in picrotoxin to block inhibitory synapses, the priming effect is blocked and synaptic responses appear prolonged even in the absence of a priming stimulus. In addition, the brief stimulation is capable of eliciting LTP without a preceeding priming pulse.

These data are consistent with the hypothesis that the priming effect is due to a use-dependent depression of inhibitory synapses (Mcarren and Alger, J.Neurophysiol. 53:557, 1985) that allows the later stimulation of the primed pathway to be more effective in depolarizing the postsynaptic cell and in turn, more effective in eliciting LTP (Kelso, Ganong and Brown, PNAS 83:5326, 1986).

Partially supported by NIH grant NS24591.

269.13 LOW-FREQUENCY SYNAPTIC DEPRESSION RECORDED INTRACELLULARLY FROM HIPPOCAMPAL GRANULE CELLS. P.C. Rinaldi, A.H. Ganong and T.H. Brown. Dept. of Surgery, Div. of Neurosurgery, Univ. California, Irvine, CA. 92717 and Div. of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA. 91010.

Low-frequency synaptic depression (LFD) may play an important role in modulating the flow of information through adaptive neural networks. Previous studies have used extracellular field potential recordings to demonstrate LFD (Teyler & Alger, Brain Res. 115, 413-25, 1976) in the perforant pathway synaptic inputs to granule cells. The parametric features of this response depression resemble those that characterize behavioral habituation (cf. Thompson & Spencer, Psyc. Rev., 173, 16-43, 1966). Little is known about the biophysical mechanisms for LFD in the hippocampal formation. As part of an effort to understand the underlying mechanisms, we have begun to study LFD using intracellular recording techniques.

Hippocampal slices from male rats were maintained in oxygenated, warmed, artificial CSF to which 10 μM picrotoxin was added to block synaptic inhibition. Bipolar electrodes were used to stimulate the perforant inputs. The postsynaptic responses were measured using 3 M KCl-filled intracellular micropipettes (50-100 M Ohms). During both the control and recovery periods, the synapses were stimulated at 30 sec. intervals. LFD was examined during the intervening period in which the synapses were stimulated at intervals of 2.5 or 5.0 sec.

Preliminary results suggest that the time-curve and magnitude of LFD observed in our intracellular measurement are similar to those previously reported based on field potential recordings. Within a restricted range of parametric values, the magnitude of LFD appeared to be inversely related to the stimulus intensity and directly related to the stimulus frequency. We are currently using the single-electrode voltage clamp (Brown & Johnston, J. Neurophys., 50, 487-505, 1983) to explain possible biophysical mechanisms of LFD.

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269.14 AUTORADIOGRAPHIC AND HISTOCHEMICAL ANALYSIS OF DENTATE GYRUS FOLLOWING UNILATERAL ENTORHINAL CORTEX LESIONS. J.W. Bekenstein*, C.A. Tansey*, G.F. Wooten, O. Steward (SPON: H.R. Brashear). Departments of Neurology and Neuroscience, University of Virginia, Charlottesville, VA 22908.

Binding of [^{3}H]hemicholinium-3 (HC-3), a presynaptic cholinergic terminal marker, and of [^{3}H]3-quinuclidinyl benzilate (QNB), a muscarinic receptor ligand, was analyzed with autoradiography to examine the time course and extent of putative sprouting of the cholinergic septohippocampal pathway following unilateral entorhinal cortical lesions in the rat. Electrolytic lesions were made, and HC-3 and QNB binding studies were performed 3, 9, and 15 days post lesion. Binding results were compared to acetylcholinesterase (AChE) histochemical staining patterns of unfixed tissue.

Histochemistry: AChE histochemistry demonstrated a dual banded pattern in the molecular layer of the dentate gyrus ipsilateral to the lesion at 9 and 15 days post lesion, but not at 3 days. This dual banding consisted of the normal band located immediately external to the granule cell layer and a second band of intensified staining in the denervated zone. The amount of shrinkage of the ipsilateral molecular layer of septal dentate gyrus was 0-6% at 3 days, 14% at 9 days, and 20% at 15 days post lesion. These results are consistent with previous studies (Matthews, et al., Brain Res. 115:1-21, 1976).

Autoradiography: Densitometric laminar analysis of QNB binding at 3, 9, and 15 days post lesion showed no change from the pattern observed in unoperated rats except for shrinkage of the molecular layer. Analysis of HC-3 binding at 9 and 15 days post lesion revealed a rearrangement in the pattern of binding, with an increase in binding in the outer 2/3 of the molecular layer ipsilateral to the lesion. The binding of HC-3 at 3 days post lesion was no different from controls. Shrinkage of the molecular layer comparable to that seen with QNB and AChE was observed at all time points with HC-3. With a conservative correction for shrinkage of the molecular layer (Steward and Vinsant, J. Comp. Neurol., 214:370-376, 1983), the increase in binding in the outer 2/3 of the molecular layer was 10-20% at both 9 and 15 days post lesion.

Because HC-3 binding reflects the distribution of cholinergic terminals, the present results suggest that a rearrangement of cholinergic terminals occurs in the molecular layer of the ipsilateral dentate gyrus following unilateral entorhinal cortical lesions. The HC-3 findings extend AChE and tract tracing studies by allowing quantitation of terminal redistribution. QNB results suggest that functional muscarinic synapses are not formed by 15 days post lesion.

269.15 **NOREPINEPHRINE (NE) MODULATION OF DENTATE GYRUS SYNAPTIC PLASTICITY: INTERACTIONS WITH N-METHYL-D-ASPARTATE (NMDA) RECEPTORS AND KINDLING-INDUCED ALTERATIONS IN NORADRENERGIC SENSITIVITY.** P.K. Stanton, I. Mody and U. Heinemann*, Dept. of Neurophysiology, Max Planck Institute for Psychiatry, Martinsried, West Germany.

Depleting brain norepinephrine (NE) impairs long-term potentiation (LTP) of perforant path (PP)-dentate gyrus (DG) synapses, promotes induction of kindled epilepsy, and NE elicits long-lasting potentiation of PP evoked potentials. However, mechanism(s) underlying these actions remain unclear. Therefore, we employed ion-selective microelectrodes, extra- and intracellular recordings from dentate granule neurons to examine actions of NE on granule cells, and changes in these actions after the induction of long-term kindling plasticity.

Bath applying NE (50 μM) to rat hippocampal slices markedly enhanced both Ca^{2+} influx and K^+ efflux (measured with ion-selective electrodes) evoked by high-frequency PP stimulation of a type which elicits LTP (20 Hz/ 10 s). The β_1-antagonist metoprolol blocked this action, as did the NMDA receptor antagonist 2-amino-5-phosphonovalerate (APV 30 μM). Experiments where extracellular [Ca^{2+}] was lowered to 0.1 mM to block synaptic transmission revealed that NE specifically enhances postsynaptic Ca^{2+} influx while not affecting presynaptic influx. Furthermore, NE potentiated Ca^{2+} influx induced by iontophoresing NMDA onto granule cells, but not influx induced by quisqualate.

In intracellular recordings, NE depolarized control granule cells with an increase in input resistance (R_N), blocked accomodation of firing to depolarizing current injection or synaptic tetanus, and blocked the late after-hyperpolarization - all actions antagonized by metoprolol. NE also produced an α_1-receptor mediated block of regenerative Ca^{2+} potentials (after injecting Cs^+ to block K^+ currents and QX314 to block Na^+ spikes). Interestingly, the depolarization and increase in R_N were both long-lasting after washout of NE, similar to long-lasting potentiation of PP evoked field potentials elicited by NE.

Additionally, we have found that the NMDA receptor antagonist APV (30 μM) specifically blocked the long-lasting phase of NE-induced potentiation of PP evoked population potentials, as well as the persistent phase of depolarization and R_N increase recorded intracellularly. Furthermore, after the induction of kindled epilepsy (a form of long-term neuronal plasticity which we have shown enhances NMDA receptor participation in dentate synaptic transmission), NE lost all actions on dentate granule cells.

These results show that NE likely modulates DG granule cell long-term plasticity via β_1-receptor action to activate normally quiescent NMDA receptors. Furthermore, kindling is associated with down-regulation of sensitivity to NE. Physiologically, this may represent a "write-protect" mechanism by which neuronal networks prevent over-writing of stored information, and changes in noradrenergic sensitivity may also contribute to epileptogenesis.

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BASAL GANGLIA: ELECTROPHYSIOLOGY AND BEHAVIOR

270.1 MOTOR FUNCTIONS OF THE CAIMAN BASAL GANGLIA. S.E. Brauth, Dept. of Psychology, Univ. of Maryland, College Park, MD 20742.

In the reptile Caiman crocodilus, the homologue of the mammalian corpus striatum is considered to be the ventrolateral area (VLA) of the telencephalon. In caiman, the VLA consists of two major cell fields, a small-celled field (VLA s.c.) containing a dense dopamingeric fiber plexus derived from the substantia nigra, and a large-celled field (VLA l.c.) in receipt of input from neurons located in the small-celled field, and which gives rise to an extensive fiber system, the ansa lenticularis (AL). On these bases, the VLA s.c. of the caiman has been compared to the caudate nucleus and putamen of mammals, while the VLA l.c. of the caiman has been compared to the globus pallidus of mammals.

Despite these histochemical and anatomical similarities, the pathways by which the reptilian and mammalian basal ganglia control motor functions differ in important ways. The principal targets of the AL in mammals are ventral tier thalamic nuclei which project back to motor and premotor cortices. In the caiman, the principal target of the AL is a pretectal nucleus which projects to the optic tectum. In order to gain insight into the behavioral functions of the caiman basal ganglia, the effects of ibotenic acid lesions placed within portions of the VLA l.c. (i.e., the globus pallidus) on prey-catching behaviors were assessed using computer quantification of videotaped behavioral sequences.

Juvenile caiman were videotaped while catching goldfish in a small enclosure. Prey-catching is fairly stereotyped in caiman. Normally, caiman orient toward their prey, then strike during which the jaws open and eyes close. Pallidal lesions result in excessive and poorly directed movements including inaccurate orientation movements. Strike accuracy was generally reduced from prelesion levels of 30-50% to between 10 and 30%. Many excess movements, including repetitive and apparently involuntary jaw movements were also observed. These excessive movements were most likely to occur if the lesions invaded the most ventral portion of the VLA, including the ventral pallidum.

These results are consistent with the view that motor functions of caiman basal ganglia involve modulation of tectal orientation and visually guided behaviors. In contrast to mammals, which are believed to have evolved from small burrowing insectivores and possess well developed somatosensory systems, many behavioral sequences such as prey-catching and nest building involve visually guided movements in birds and reptiles. The behavioral functions of the basal ganglia reflect these differences. Thus while the overall organization of the afferent and efferent pathways of the basal ganglia show many similarities among extant amniote species, the specific pathways by which motor functions are controlled, however, differ between vertebrate groups, reflecting the development of other sensory and motor systems as well.

270.2 RETICULAR AND CEREBELLAR STIMULATION MIMIC AMPHETAMINE ACTIONS ON AMPLITUDE AND TIMING OF FRONTAL CORTEX EVOKED NEOSTRIATAL RESPONSES IN RATS. Lawrence J. Ryan*, Stephen J. Young*, and Philip M. Groves. (SPON: Harry Klemfuss) Dept.Psychiatry, M-003,University of California San Diego, La Jolla, CA 92093.

Electrical stimulation of frontal cortex evokes a stereotyped sequence of extracellular potentials in rat neostriatum. Wave P1 corresponds temporally to the initial intracellularly recorded depolarization. The subsequent 80-150 ms duration hyperpolarization overlaps two extracellular waves: N2, which may reflect collateral inhibition, followed by P2, which may reflect thalamo-cortical disfacillitation. A late wave, N3, corresponds to rebound depolarization (Ryan, et al., 1986, Brain Res Bull 17: 751-758). Amphetamine (0.1-5.0 mg/kg, iv, d-amphetamine sulfate) reduces P1 amplitude and shortens the latency to N3 onset in a dose-dependent manner. These effects may be mediated extrastriatally, via noradrenergic mechanisms. First, these two effects of amphetamine are mimicked by repetitive stimulation (60 Hz, 0.1-0.3 mA square waves through 75u bipolar twisted teflon coated stainless steel electrodes) of the mesencephalic reticular formation and the deep cerebellar nuclei. Furthermore, mesencephalic reticular formation stimulation and amphetamine reduce the latency to the late rebound wave of cortico-cortical evoked responses. Second, kainic acid lesion of the medial thalamus, which eliminates wave N3, reduces or eliminates the amphetamine-induced reduction in P1 amplitude. Third, the beta noradrenergic drugs propranolol (5.0 mg/kg) and metoprolol (10.0 mg/kg) reverse these effects of amphetamine, whereas the dopamine antagonist, haloperidol (0.03 mg/kg) does not affect P1 amplitude and further shortens N3 latency. The alpha-2 antagonist yohimbine (1.0 mg/kg) strongly magnifies the amphetamine-induced reduction in N3 latency, but, paradoxically, reverses the reduction in P1 amplitude. The alpha agonist, clonidine (0.01-0.10 mg/kg) had inconsistent effects on P1 amplitude and N3 latency. Lastly, direct infusion of amphetamine (5uM) unilaterally into the contralateral deep cerebellar nuclei (N=2) reduced N3 latency. Taken together, these results suggest amphetamine strikingly alters cortico-striatal interactions via noradrenergic mechanisms acting on the medial thalamus and its afferents. Thus, one function of thalamostriatal projections may be to modulate corticostriatal relations.

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270.3 RETICULAR FORMATION STIMULATION MODIFIES CORTICALLY EVOKED INTRACELLULAR POTENTIALS IN NEOSTRIATUM OF RAT. L.J. FISHER, S.J. YOUNG*, J.M. TEPPER AND P.M. GROVES. Dept. Psychiatry, UCSD, San Diego, La Jolla CA 92093.

We have previously demonstrated a correspondence between components of the neostriatal extracellular field potential and intracellular events elicited by stimulation of cortical white matter (Ryan, et al., 1986, Brain Res Bull:751-758). An initial intracellular depolarization occurs concurrent with an initial positive wave (P1) in the field potential, while the subsequent hyperpolarization parallels waves N2 and P2 which may reflect collateral inhibition followed by cortico-thalamic disfacilitation. A late wave, N3, occurs during the intracellularly observed late rebound depolarization.

In another abstract (Ryan, et al.), we report that systemic administration of d-amphetamine sulfate reduces the amplitude of P1 and decreases the latency to N3. These effects also occur with high frequency stimulation of the mesencephalic reticular formation (RF) or deep cerebellar nuclei. The present study was designed to examine the intracellular events associated with these effects.

Intracellular recordings were obtained from neostriatum in urethane anesthetized rats. A field potential electrode was placed within 1mm of the intracellular recording site. Bipolar stimulating electrodes were positioned in cortical white matter, RF and within the deep cerebellar nuclei.

Preliminary results indicate that high frequency stimulation (20-60 Hz) of RF consistently resulted in a reduction of the peak amplitude of the cortically evoked initial polarization and an exaggeration of an inflection observed during the rising phase of this potential. Additionally, there was an apparent reduction in the magnitude and duration of the after-hyperpolarization. These effects persisted for up to 1 minute following RF stimulation and were accompanied by a decrease in resting membrane potential.

These results suggest that alterations in field potentials associated with RF stimulation are mirrored by temporally corresponding intracellular events. Reticular activation may modify the level of thalamic or cortical input to the striatum to effect both tonic and phasic aspects of striatal processing.

This research was supported by grants from the ONR and DA-02854 and DA-00079 from NIDA to P.M.G.

270.4 MEASUREMENT OF LOCOMOTOR PATTERNS IN THE RAT: EFFECTS OF AGE AND N-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP). M.E. Melnick, L.D. Ford*, and M.K. Shellenberger. Departments of Physical Therapy Education and Pharmacology, The University of Kansas Medical Center, Kansas City, Kansas 66103.

We have reported that MPTP causes alterations in the motor activity and gait pattern of rodents. (Melnick, M.E. et al in MPTP: A Neurotoxin Producing a Parkinsonian Syndrome, 1986; M.K. Shellenberger and M.E. Melnick, Neurosci Abst., 1984) However, it has been difficult to demonstrate a significant persistence of these effects in the rat for more than 10 days after cessation of injections. Therefore, we have been considering techniques for a more sensitive measure of the effects of MPTP on locomotion and muscle tone. We had noted that MPTP-treated rats tended to walk with the entire foot-pad in contact with the supporting surface (i.e. a flat-footed gait) in contrast to the more typical pattern of weight supported on the metatarsals only.

We have used the Zeiss Interactive Digital Analysis System to assess the perimeter of the foot-pad in contact with the surface as well as the area of the footprint. The footprints of young adult (6-month-old), 1-year old and 18-month-old animals were analyzed after receiving either solvent or MPTP treatment. The data indicate that the aging process produces a significant increase in the area of the foot in contact with the supporting surface during locomotion. Furthermore MPTP-treatment causes an increase in this area in the young adult and year-old animals which makes the footprints of these animals appear to be like those of the 18-month-old rat. Because of the natural tendency towards a flat-footed gait with aging, it is more difficult to utilize this technique to assess MPTP-induced motor impairment in older animals.

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270.5 DOPAMINERGIC INVOLVEMENT IN STRIATAL NEURONAL ACTIVITY DURING LOCOMOTOR BEHAVIOR AND ITS ENHANCEMENT BY COCAINE. N. Shimizu*, M.O. West, R.-S. Lee, J.K. Chapin, and D.J. Woodward. (SPON: G.A. Mihailoff) *Dept. of Cell Biology and Anatomy, The University of Texas Health Science Center at Dallas, TX 75235.*

Our previous studies demonstrated that a substantial population of the striatal neurons , considered to be important in motor function, increased their firing rate during locomotion. Also cocaine has been reported to elevate monoamines by inhibition of uptake. The objective of the present study was to clarify the following: (1) whether dopamine (DA) is involved in the excitatory response of striatal neurons during locomotor behavior, (2) whether cocaine modulates the striatal neuronal response to locomotion.

Adult Long-Evans hooded rats were prepared for chronic recording with a detachable microdrive positioned over the striatum. Four-barrel micropipettes were used for recording extracellularly the spontaneous activity of striatal neurons and for applying drugs. Single neuron activity was recorded through one of the barrels which was filled with a carbon fiber (7 μm in diameter, extending 20 μm beyond the tip of the glass capillary). Animals were trained to walk on a treadmill (TM) (30 sec on / 30 sec off) and a tone (800 Hz, 0.2 sec, 60 dB) served as a cue (0.5 sec before TM-onset) for the onset of TM locomotion.

Sixty-five percent of striatal units (n=185 neurons) increased firing rate during locomotion (185.8±26.7% increase, during locomotion vs. resting). Iontophoretic application of trifluoperazine (DA receptor blocking agent) significantly attenuated the locomotion-evoked excitatory response. Alpha-methyl-para-tyrosine (an inhibitor of tyrosine hydroxylase) blocked the TM-response 3-h post-injection (200 mg/kg, i.p.) in conjunction with the prolongation of the reaction time (latency to the first footfall after treadmill onset). In contrast, cocaine (1 mg/kg, i.p.) augmented the TM-response by 90±20% compared to the pre-injection values. This effect was observed within 10 min and reached to the maximum level at 30 min after injection. The response recovered within 1-h. The spontaneous firing rate did not alter, and little observable behavioral change occurred during resting at this dose. Our view is that the enhancement of the response may be elicited by the augmentation of dopaminergic neurotransmission. Furthermore, iontophoretically applied DA increased the spontaneous firing rate in 50 % of the neurons tested in the striatum (n=60), while inhibited 23 % of the neurons. Out of 60 neurons tested for DA sensitivity, 29 neurons were also analyzed for their response to TM-locomotion. Eighty percent of the neurons which increased the firing rate during locomotion were also excited by DA (significant $P \leq 0.05$, χ^2 test).

These observations suggest that DA participates in neural processes in striatum during locomotion, and its effect is excitatory in nature either through direct or modulatory actions. *(Supported by grants AA-5901, DA-02888 to DJW and awards from the Biological Humanics Foundation, and the RJ Reynolds Tobacco Co.)*

270.6 SINGLE UNIT CORRELATIONS WITH SPECIFIC MOVEMENTS IN LATERAL STRIATUM OF FREELY MOVING RATS. M. West, R, Carelli*, N. Shimizu, J. Chapin and D. Woodward. Dept. Psychology, Rutgers University, New Brunswick, NJ and Dept. Cell Biology & Anat., Univ. Texas Health Science Ctr., Dallas TX.

The objective of these experiments was to determine whether phasic patterns of neuronal activity correlated with discrete sensory and motor events are compartmentalized in the striatum, resulting in a functional "map." Long-Evans (hooded) rats of either sex, aged 90-120 days, were surgically prepared for chronic single-unit recording via a detachable, miniature microdrive positioned above specific areas of the striatum. Microelectrodes were advanced daily along vertical recording tracks through the striatum. Stereotaxic coordinates were referenced to Bregma, using a level skull. Videotape recordings of motor behavior were synchronized with computer-acquisition of neuronal firing patterns. Using stop-frame video analysis (30 frames/sec), raster displays and peri-event histograms (PEH) depicting striatal unit activity could be constructed around any observable movement. As previously reported (West et al, Neurosci. Abstr. 12:652, 1986), in central and medial regions of the striatum (A-P +0.2 to -0.2, M-L 2.5 to 3.5, D-V 3.0 to 7.0 mm), units showed no correlations with specific movements. Approximately 70% of these units significantly increased their firing rates during whole-body movements such as locomotion on a treadmill (TM). Over half the units exhibited short-latency (20 msec) sensory responses to a conditioned auditory tone stimulus signalling the onset of TM locomotion. We now report that highly specific correlations with discrete limb movements were exhibited by single units in the most lateral regions of the striatum (A-P 0.0, M-L 3.5 to 4.5, D-V 3.0 to 4.0 mm). Robust, short-duration (50-150 msec) bursts of activity were correlated with particular portions of the stance or swing phases of locomotion. In preliminary studies, these firing patterns were present during rhythmic, straight-ahead locomotion but not during backwards locomotion nor during exploratory locomotion composed of rapid shifts in posture and direction. Some units responded to passive manipulation of the limb with which they were correlated, while others fired only during active (voluntary) movement. Thus, variables related strictly to motor behavior did not appear to fully account for the magnitude of variations in unit activity observed under all conditions. The significance of these results is that they provide important neural substrates in a freely moving animal for evaluating factors such as behavioral context in studying striatal involvement in sensorimotor integration. Supported by PHS RR 07058-21, DA 02338, NIAAA 3901, and the Biological Humanics Foundation.

270.7 DIFFERENTIAL INVOLVEMENT OF THE STRIATUM AND ITS MAJOR EFFERENTS IN A REACTION TIME PERFORMANCE IN RATS.

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A major function of the dopamine in the striatum is to control the activity of its efferent systems which contains primarly GABAergic neurons. The purpose of the present study was to examine 1) the effects of a dopamine receptor blocker (haloperidol) in the striatum and 2) the effects of a GABA receptor agonist (muscimol) injected in the globus pallidus (GP) or in the substantia nigra reticulata (SNr) in an operant reaction time task in rats.

Rats were trained to depress a lever, to hold it until the presentation of a visual conditioned stimulus (CS) then to release it within 500 ms. Responses within 500 ms were reinforced by a food pellet. Daily sessions ended after 100 trials (for details, see Amalric and Koob 1987). Results were expressed as a percentage of correct responses (CR) or incorrect ones: either premature responses (PR: release of the lever before the CS) or long responses (LR: over 500ms). Reaction time (RT) was measured from the CS to the lever release.

Haloperidol (25 and 50 ug) injected intraperitoneally impaired reaction time performance. For the highest dose, rats stopped pressing the lever after 10 to 40 trials. When injected bilateraly in the striatum, haloperidol (2.5 and 5.0 ug) disrupted the performance by increasing reaction time. Percentage of PR was significantly reduced as compared to pretest controls. Percentage of correct responses were also decreased after perfusion of muscimol (2.5, 5.0 and 10.0 ng) in the SNr or the GP. Interestingly the two structures were differentially affected by the drug. Rats perfused with muscimol in the SNr showed an increase in the number of premature movements. Reaction time were shortened with no change in the number of longer RT. In contrary, muscimol in the GP did not induce any change in the number of PR but did increase the number of long RT.

These results show that blockade of the dopamine receptors in the striatum increased response duration, while stimulation of the GABA receptors either shortened reaction time and movement in the SN, or increased RT in the GP. Interactions between DA and GABAergic efferent neurons seem to be essential to trigger a reaction time motor task. Informations on the motor programm could be processed differently at the different levels of the basal ganglia.

270.8 EFFECTS OF GLOBUS PALLIDUS LESIONS IN RATS ON SUSTAINED, RESTRICTED FORELIMB FORCE EMISSION. J.V. Harrell, E.S. Hall*, and R.C. Hicks*. Dept. of Psychology, Hampden-Sydney College., Hampden-Sydney, VA 23943

We have examined the effects of globus pallidus lesions in rats on ability to perform a force-band/duration task with their forelimbs. The task required rats to reach through an opening in a Skinner box and press with their forepaw upon an isometric force transducer with a force between 20 and 50 g and to hold that limited force for 1.5 sec in order to receive water reinforcers. Five rats received bilateral, electrolytic lesions of the globus pallidus (GP), two rats received bilateral sensorimotor cortex lesions (SC), one rat received a bilateral sham lesion (SL), and two rats were unoperated. These last three groups served as contols.

Rats were trained on the required task for approximately four months. A baseline of 20 days data was then collected. Surgeries were performed and on the following day preoperative criterion testing resumed. The initial postoperative testing period lasted approximately three weeks. Thereafter followed, in order, a two week hiatus, five testing days, a two week hiatus, and five testing days. A total of 68 days elapsed from initial surgeries to the conclusion of the experiment.

Four of the five GP rats showed extensive effects of the lesions. These effects were characterized by an inability in the immediate postoperative period to initiate responses. Only with prompting were these rats able to be retrained to preoperative criteria. The number of reinforced responses decreased markedly for these rats. Strip chart recordings revealed a dramatic change in force emission characteristics, indicating a more variable pattern of force emission. Moreover, stereotyped behaviors (sniffing, head movement, exploration) also increased. Controls showed no effects of lesions or sham procedures.

Results, overall, are in agreement with those of a previous study which showed essentially the same effects from caudate nucleus lesions. Together, these data strongly support the notion that the basal ganglia are important contributors to fine motor control, particularly of the type that requires significant sensory feedback and or sustained performance. (Supported in part by a grant from the Gwathmey Foundation in Virtinia.)

270.9 DOUBLE DISSOCIATION OF ASSOCIATIVE AND MOTOR FUNCTIONS IN THE ROSTRAL STRIATUM OF THE RAT. M. Pisa. Dept. Neurosciences, McMaster University, Hamilton, Ont., Canada L8N 3Z5.

The aim of this study was to examine the hypothesis of regional specificity of both motor functions and modality-specific associative functions in the rostral striatum of rat. Wistar rats were assigned to groups (N=16) for bilateral control injections (.4 ul PBS) or ibotenate injections (6ug) into either the medial, dorsolateral or ventrolateral regions of the rostral striatum. Postoperatively, half the rats of each surgical group were assigned to turn discrimination (left vs. right) and the other half to brightness discrimination (white vs. black) in a cross maze, for the motive of food. After reaching the learning criterion of 90% correct on the original discrimination, the animals were tested for discrimination reversal (intradimensional shift). Then, the rats were trained to retrieve food pellets from a feeding tube, and their forelimb reaching performance was evaluated in terms of number of reaching attempts made before retrieving the pellets. At the end of testing, the sizes and locations of the ibotenate-induced, somatodendritic lesions were verified histologically. The results were as follows: 1) None of the regional striatal lesions reliably affected original learning of the discriminations or reversal of the brightness discrimination; 2) medial striatal lesions significantly impaired spatial discrimination reversal but not forelimb reaching; 3) lateral striatal lesions significantly impaired forelimb reaching but not spatial discrimination reversal; 4) lateral striatal lesions with attendant, substantial damage to the adjacent cortex impaired both spatial discrimination reversal and forelimb reaching. These results indicate selective roles of the rostromedial striatum in flexible use of spatial cues and of the lateral striatum in forelimb motor control. However, the results also indicate that the selectivity of regional effects on behavior can be lost if the cortex is also damaged. The demonstration of double behavioral dissociation is fresh evidence for a topographically organized and task-specific role of the rostral striatum in both motor programming and cognition.

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270.10 ATTENTIONAL VERSUS INTENTIONAL NEGLECT: CONTRASTING EFFECTS OF UNILATERAL STRIATAL AND CORTICAL LESIONS IN A VISUAL REACTION TIME TASK. V.Brown*, G.Mittleman* and T.W.Robbins*(SPON:E.Wong). Dept. of Exp. Psychology, University of Cambridge, Cambridge, CB2 3EB, UK.

Unilateral lesions of the cortex and the striatum produce syndromes of 'contralateral neglect' which may result from disruption of different processes. A paradigm for studying visual neglect has been developed for rats, which varies the response requirements for reporting the detection of brief visual stimuli in order to separate response related effects from those due to possible sensory or attentional deficits. Carli et al (Nature, 313;679, 1985) showed that unilateral dopamine (DA) depletion of the striatum impairs the capacity to initiate responses contralateral to the side of the lesion and enhances the tendency to respond ipsilaterally, regardless of the side of stimulus presentation. The present experiments compared the effects of hemidecortication and ibotenic acid (0.06M/lul) lesions (IBO) of the striatum in three visual reaction time tasks, in order to clarify the nature of the neglect syndrome associated with these lesions.

Rats were trained to make a sustained nose-poke in a centrally located, lit hole, until a brief (200ms), temporally unpredictable, visual stimulus was presented to either side of the head. Different groups were trained to report the detection of the stimulus by withdrawing from the central hole (measured as Reaction Time- RT) and completing the response by making a lateralised head movement either towards (Discrimination 1), or away from (Discrimination 2) the stimulus, or by opening a panel located at the rear of the test chamber (Discrimination 3).

The effects of the lesions on performance in Discriminations 1 and 2 were qualitatively similar to those following unilateral DA depletion of the striatum. There was a significant lengthening of RT in both cases when a contralateral response was required and an enhanced ipsilateral response bias. However, there were qualitative differences between the groups in their performance on Discrimination 3. Following hemi-decortication, rats showed a significant impairment in both accuracy and latency to report the presence of contralateral stimuli. Their performance on trials in which one or other of the stimulus lights was occluded is also consistent with the interpretation that they were failing to use the contralateral stimulus to mediate the discrimination. In contrast, the IBO group continued to use both stimulus lights postoperatively, as confirmed by tests of occlusion of the stimuli. In this group there was a general decline in the accuracy of detection but, if anything, this was less pronounced on the contralateral side.

270.11 SPONTANEOUS ACTIVITY OF TYPE II BUT NOT TYPE I STRIATAL NEURONS IS CORRELATED WITH RECOVERY OF BEHAVIORAL FUNCTION AFTER DOPAMINE-DEPLETING BRAIN LESIONS. E.S. Nisenbaum, M.J. Zigmond, E.M. Stricker, and T.W. Berger. Departments of Behavioral Neuroscience and Psychiatry and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Two types of extracellular waveforms, Type I and Type II, can be recorded from the rat striatum (Skirboll and Bunney, Life Sci., 25, 1979). Previously they have been shown to represent two functionally distinct subpopulations of neurons (Nisenbaum et al., Soc. Neurosci. Abstr., 12, 1986). One week after near-total destruction of the dopaminergic (DA) innervation to the striatum produced by intraventricular (ivt) administration of 6-hydroxydopamine (6-HDA), both Type I and Type II neurons exhibit increased spontaneous firing rates in association with severe ingestive and motor deficits (Orr et al., Soc. Neurosci. Abstr., 12, 1986). Despite the permanent loss of greater than 90% of striatal DA, animals can gradually recover from these behavioral dysfunctions. In association with behavioral recovery, the spontaneous activity of Type II neurons has been shown to return to pre-lesion levels (Nisenbaum et al., Brain Res., 398, 1986). The present study examined whether, similar to Type II neurons, the spontaneous firing of Type I neurons has returned to control levels in animals that have recovered from the effects of a 6-HDA-induced lesion.

Rats were injected ivt with 200 ug of 6-HDA, dissolved in 0.9% NaCl and 0.1% ascorbic acid, and extracellular single unit activity of Type I striatal neurons was recorded 4-6 weeks post-lesion from animals exhibiting behavioral recovery. A control group of animals was injected ivt with just the vehicle solution, and Type I firing rates were recorded 4-6 weeks later. Tissue punches subsequently were taken from the striatum and analyzed for DA content.

The spontaneous firing rates of Type I neurons recorded 4-6 weeks post-lesion from animals with striatal DA depletions of greater than 90% were significantly elevated relative to control levels. Thus, although Type I neurons exhibit increased activity in association with behavioral deficits after a 6-HDA-induced lesion, firing rates of Type I neurons do not return to normal levels in recovered animals. These results demonstrate that only the activity of Type II striatal neurons is positively correlated with behavioral recovery, and provide further evidence demonstrating the existence of two functionally distinct subpopulations of striatal neurons.

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270.12 SINGLE UNIT NEURONAL ACTIVITY IN PUTAMEN: ROLE IN THE TERMINAL PHASES OF MOVEMENT

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Earlier notions regarding the role of the basal ganglia in motor control emphasized a feedforward or "pre-programing" mechanism where motor programs are generated in the basal ganglia then relayed to the motor cortex for execution. However, single unit studies have failed to show changes in basal ganglia activity prior to that of the motor cortex or movement onset.

Of 111 units studied in the putamen of a monkey performing wrist extension and flexion movements, 19 had neuronal activity changes associated with the tasks. Tasks included fast step or slow tracking movements between two mechanical stops separated by 90 degrees either in response to a go signal or self-initiated. Neuronal discharges were temporally correlated with the go signal, movement onset and attainment of final target.

Most of the units which changed their activity with the tasks did so at or after the time of movement onset. Only one unit showed an increase in neuronal activity prior to any EMG or movement. Only 2 units showed a more consistent relationship to the go signal, usually for the fast tasks. Two units demonstrated a more consistent relationship to either flexion or extension. Only one unit was related most strongly to the pattern of muscle involvement.

In 8 of the 19 putamenal units, the most consistent relationships were those associated with attainment of the final target. This was most evident for the fast tasks and consisted of an increase in neuronal firing. For 6 of the 8 units the increased activity occurred from 100 to 400 msec before reaching the final target. For example, one unit changed from a tonic to a bursting pattern of discharge approximately 190 msec after the go signal and 300 msec before attainment of the final target for a fast flexion task. When trials were lined up on the attainment of the final target, there was a synchronization of the bursting pattern.

While the amount of data in this continuing study is small, there is a clear tendency of putamenal units to be better related to the final phases of movement rather than to the go signal or movement initiation. Even so, the time of onset of change in neuronal activity before attainment of the final target, suggests a role in anticipation of task termination and preserves the notion of a feedforward "pre-programing" mechanism. Thus, disorders of the basal ganglia may have greater effect on task termination and be reflected in abnormalities of movement time or duration rather than reaction times from go signal to movement onset. Studies in humans afflicted with Parkinson's disease and various experimental lesions of the basal ganglia in animals have shown normal reaction times but prolonged movement times. It is unclear whether this role is involved in the stopping of movement or in continuing the movement to its proper termination at the proper pace, although the latter is more likely the case.

270.13 CROSS-CORRELATION ANALYSIS OF GLOBUS PALLIDUS AND ENTOPEDUNCULAR UNIT ACTIVITY IN THE AWAKE CAT AND THE EFFECTS OF LESIONING NEOSTRIATUM. D. Jaeger, R.N.S. Sachdev*, G.W. Dauth, S. Gilman and J.W. Aldridge. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48104

The cross-correlation technique was used to assess the functional interactions between pairs of simultaneously recorded neurons in cat globus pallidus (GP) and entopeduncular nucleus (EP). Recordings were obtained from awake, quietly sitting cats (N=4) before and after ibotenic acid lesions of ipsilateral neostriatum. Spontaneous extracellular spike activity of 2-3 units was discriminated from the signal of a single microelectrode by using peak amplitudes and peak to peak time as discrimination parameters. Cross - correlograms were constructed from spike trains and significant interactions were determined by visual inspection.

In GP 31 unit pairs from controls and 49 unit pairs from lesioned cats were analyzed. Overall 49% of unit pairs showed significant functional interactions. In 44% of these interactions a 2-3 ms increase in the probability of activity of one unit 1-3 ms **either** before **or** after the second unit of the pair fired was seen (unilateral peak in correlogram). All other interactions (46%) consisted of an increase in the activity of units before **and** after the other unit fired (bilateral peak in correlogram). Both types of interactions were present in lesioned and control cats but the proportion of unilateral peak correlograms was significantly **increased** after the lesion (chi square test $p < 0.01$).

Similar interactions were seen in EP where 27 unit pairs before and 27 unit pairs after lesioning were analyzed. Here 30% of unit pairs had a significant interaction. The proportion of bilateral peak correlograms was unchanged compared to GP but there were significantly fewer correlograms with a unilateral peak ($p < 0.05$). In lesioned cats the proportion of significant interactions was higher. A significant increase ($p < 0.01$) in the proportion of unilateral peak correlations was most prominent.

The finding of unilateral peak correlations in GP and EP suggests a non - reciprocal functional excitatory interaction between neurons in these structures. An excitatory interaction between GABA'ergic neurons might be caused by a phasic rebound of spike activity after a short period of inhibition in tonically active neurons. Indeed our data show evidence of a short inhibitory interaction before the onset of excitation. The bilateral peak correlations we saw were quite symmetrical and on each side closely resembled the time course of unilateral peak correlations. This suggests a similar mechanism of generation which could be a reciprocal functional excitatory connection. Unilateral and bilateral peak correlations were also observed in GP but not putamen of quietly sitting awake monkeys. This suggests to us that these interactions are typical of mammalian GP. The finding of an increased number of correlations in GP and EP after removal of a major inhibitory input can be explained by a number of mechanisms. For example, the efficacy of remaining terminals could increase or new terminals could be formed through collateral sprouting.

Supported by the Hereditary Disease Foundation.

270.14 EFFECT OF STRIATAL LESIONS ON SPONTANEOUS UNIT ACTIVITY RECORDED FROM THE GLOBUS PALLIDUS, ENTOPEDUNCULAR NUCLEUS AND VENTRAL PALLIDUM. R.N.S.Sachdev*, J.W.Aldridge, Dept. of Neurology, Univ. of Mich., Neuroscience Lab Bldg., 1103 E. Huron, Ann Arbor, 48104

The goal of this study was to determine the effects of unilateral striatal lesions on unit activity in the ipsilateral pallidum. Lesioning striatal inhibitory GABAergic projections to the globus pallidus (GP), entopeduncular nucleus (EP), and ventral pallidum (VP) might be expected to increase firing rate of neurons in these structures. Spontaneous unit activity was recorded from awake and quietly sitting cats (N=4). Two cats were studied both before and after the lesion. In two other cats one had no lesion, and the other cat was studied in all tracks after the lesion. Lesions were produced by injecting ibotenic acid into the caudate and putamen. Data collection resumed 10 days after injections and lasted for up to one year. Single units were discriminated and analyzed off-line and spike trains were scanned for bursts using a quantitative procedure developed by Legendy and Salcman (J. Neurophysiol. 53:4, 1985). Data were collected from 139 GP control, 172 GP post lesion, 65 EP control, 62 EP post lesion, 52 VP control and 31 VP post lesion units.

The lesion resulted in a significant decrease in the mean interspike interval duration (increased firing rate) in GP alone: GP 59ms to 33ms, EP 47ms to 39ms, and VP 53ms to 42ms. The median interspike interval durations also decreased significantly in GP, but not in VP or EP: GP 34ms to 25ms, EP 28ms to 30ms and VP 37ms to 32ms. The coefficient of variation changed significantly only in EP: GP 1.33 to 1.03, EP 1.43 to 0.79 and VP 1.17 to 0.86. The classification of units by burst properties established that all segments of the pallidum have units which exhibit bursts. The percentage of units which exhibit bursts decreases significantly after the lesion in GP and EP but not VP: GP 24% to 14%, VP 27% to 6%, and EP 40% to 9%.

This study demonstrates that striatal lesions can affect the firing rate and pattern in post synaptic pallidal neurons. Although all pallidal structures exhibited qualitatively similar changes, only GP had a significant change in both rate and pattern of activity. EP was affected in bursting but not rate. No changes were significant in VP. The differential effects on the pallidal structures after the striatal lesion could be related to the proportion of afferents lost and/or the characteristic pattern of striatal peptidergic projections.

Supported by the Hereditary Disease Foundation.

270.15 RESPONSES OF MONKEY GLOBUS PALLIDUS CELL S DURING TARGETED REACHING MOVEMENTS. R. S. Turner* and M. E. Anderson (SPON: June L. DeVito) Dept. of Physiol. and Biophysics, University of Washington, Seattle, WA. 98195

Neurons in the internal pallidal segment (GPi) and reticular portion of the substantia nigra (SNr) are the output neurons of the basal ganglia. In awake monkeys, cells in both nuclei have high tonic firing rates in the absence of movement and they exert an inhibitory synaptic action on their target neurons. Nigral neurons typically exhibit a simple decrease in firing rate in association with saccadic eye movements in a specific direction (Hikosaka and Wurtz '83 J. Neurophysiol. 49:1230)

Pallidal neurons, however can show increases and/or decreases in firing rate with movements of the arm or leg (DeLong, Crutcher & Georgopoulus '85 J. Neurophysiol. 53:530). The limb movements used in these studies of the GP however, have always been unidimensional push-pull, side-to-side or reach-retract movements.

In order to better study the relationship between pallidal cell firing and limb movement we have examined the discharge of pallidal neurons in a monkey during horizontal arm reaching movements of 3 amplitudes (1", 2", & 3") made in one of 8 radial directions from a center start position. Under these conditions, of pallidal neurons showing a significant change in firing associated with the task, the majority of them (17/22) showed a response that changed with the direction of the movement performed.

Few of these cells showed a simple modulation in response magnitude depending on movement direction. Most directionally sensitive cells had responses composed of complex modulations in firing rate that changed with direction of movement. Few cells showed a simple reciprocal response relationship with movements in opposite directions.

Most behaviorally related neurons, however, (16/22) showed a decrease in firing rate as a first response with movements in at least one direction. These responses had an average onset time of ~100ms before movement initiation. The directional sensitivity of some cell reponses changed with the amplitude of the movements performed. However, no responses were found to have a linear relationship with movement amplitude.

Quantification of these results will allow a comparison of cell response with measured limb movement under different behavioral conditions.

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270.16 THE EFFECT OF PALLIDAL INACTIVATION ON TRAINED WRIST MOVEMENTS IN THE MONKEY. J.W. Mink and W.T. Thach. Departments of Anatomy & Neurobiology and Neurology & Neurosurgery and The McDonnell Center for the Study of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110

What aspects of movement do the basal ganglia control? Theories include 1) initiation of stimulus-triggered or 2) self-paced movement, 3) scaling of velocity and/or amplitude and/or 4) agonist EMG, and 5) control of maintained posture as distinct from volitional movement.

Previous studies had shown that most pallidal units changed after the earliest EMG activity (Mink and Thach, Soc. Neurosci. Abst., 1986). Most pallidal neurons (> 90%) were best related to step-tracking (Mink and Thach, Soc. Neurosci. Abst., 1985). Some of these (50-75%) were slightly related to ramp- and sinusoid-tracking but with peak changes occurring at the initial step-like movement in ramp-tracking and at the direction change (stop-start) in sinusoid-tracking. A smaller fraction (25%) were related, albeit poorly, to self-paced rapid alternation.

This study was designed to further test the above hypotheses by inactivating the globus pallidus in monkeys during these tasks. Movement direction and muscle activity were dissociated with torque loads in such a way that, e.g., a flexion movement could be made by turning on loaded flexor muscles or by turning off loaded extensor muscles.

Following pallidal inactivation with muscimol or ablation with kainic acid, step-tracking reaction time was normal but movement time was slower by 50-300 msec. Ramp-tracking reaction time was normal, but the velocity of the initial step component of ramp-tracking was slower. Visually guided sine-tracking was near normal, but there was tonic agonist-antagonist cocontraction with a flexor positional bias. Self-paced rapid alternation was also performed from a flexed posture with tonic cocontraction and was further hindered by phasic cocontraction. Movement was invariably slower (by 35-50%) when made by decreasing activity of the loaded antagonist than when increasing activity of the loaded agonist.

Because of in normal animals 1) the late onset of unit activity, and in pallidal inactivated animals 2) the normal reaction time but prolonged movement time, 3) the agonist-antagonist cocontraction and 4) the inability to turn off antagonist muscles, these data suggest that the inhibitory pallidal output acts to reduce existing postural or other maintained muscle activity that would interfere with voluntary limb movement initiated and controlled by other neural mechanisms. (This work was supported by NIH grant 2 RO1 NS12777-12 and The McDonnell Center.)

270.17 EVIDENCE FOR SELF-INHIBITION OF NIGROSTRIATAL DOPAMINE NEURONS MEDIATED BY ANTIDROMICALLY ELICITED SOMADENDRITIC AND INITIAL SEGMENT SPIKES. R.F. Gariano*, S.J. Young* and P.M. Groves. Depts. Neuroscience and Psychiatry, Univ. California, San Diego, La Jolla CA 92093.

Antidromic stimulation of nigrostriatal dopamine (DA) terminals in the striatum typically elicits an initial segment (IS) response at the cell body, though occasionally the full somadendritic spike (FS) is seen. We have utilized these distinctive antidromic responses to study the nature of the inhibition of DA cell activity that follows electrical stimulation of the neostriatum.

Single-unit extracellular recordings were obtained from electrophysiologically identified nigrostriatal DA neurons in the substantia nigra of urethane-anesthetized rats. Monophasic square wave stimuli were delivered to the dorsolateral striatum at 0.6 Hz with a constant current (30-500 μs, 1-3 mA), adjusted to elicit antidromic responses on approximately 50-80% of stimulus trials. Post-stimulus time histograms of impulse events were separately constructed for stimuli that resulted in either a FS antidromic response, an IS response, or no antidromic response.

Striatal stimulation was followed in all three cases by a post-stimulus inhibition (PSI). For all cells tested (n=10), the PSI was longest following an FS antidromic response, intermediate for the IS response, and least when no antidromic response was elicited. The greater inhibition following the FS may be secondary to FS-dependent afterhyperpolarization and/or somadendritic autoreceptor activation by FS-dependent dendritic release of DA. The greater PSI for the IS response than for the case of no antidromic response may be secondary to DA release following electrotonic spread of the IS spike to adjacent dendritic membrane, electrical coupling of DA neurons, or some unknown factors. Since inhibition due to activation of striatonigral fibers or to antidromic activation of neighboring DA cells should be equivalent in the three cases, the greater PSI for FS and IS responses implies self-inhibition by individual DA neurons subsequent to both FS and IS responses. Preliminary evidence using the DA receptor antagonist haloperidol indicates that a portion of the PSI for the FS and possibly the IS cases is mediated by DA receptors. The role of DA autoreceptors in mediating PSI will be further examined in rats pretreated with alphamethylparatyrosine to deplete endogenous DA.

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270.18 ELECTROPHYSIOLOGICAL EFFECTS OF NICOTINE AND GLUTAMATE INJECTIONS INTO THE TARGET REGIONS OF MIDBRAIN DOPAMINE NEURONS. K.W.P. Yoon*, G.P. Mereu and T.C. Westfall (SPON: S. Horenstein). Departments of Pharmacology and Neurosurgery, St. Louis University School of Medicine, St. Louis, MO 63104.

We have previously observed that the systemic administration of nicotine produces a dose-dependent increase in the firing rate of nigral pars compacta dopamine cells (A9) as well as ventral tegmental area dopamine cells (A10) of the rat (Soc. Neurosci. Abs. 12:1515, 1986). The purpose of the present study was to further investigate the effects of nicotine on midbrain dopamine neurons.

The changes in the action potential firing frequency and pattern of neurons in the substantia nigra dopaminergic (A9), pars reticulata (SNR), ventral tegmental area dopaminergic (A10), and ventral tegmental area non-dopaminergic cells of rats were recorded by extracellular single units during the microinjection of glutamate and nicotine into the respective striatal target regions of the dopaminergic projections: caudate nucleus for A9 and nucleus accumbens for A10.

Glutamate injections into the caudate nucleus most consistently caused a cessation of firing of SNR neurons and increased the firing rate of the A9 neurons. Nicotine injections into the caudate nucleus also caused a momentary cessation of the firing of SNR neurons but were frequently followed by excitation. The effect of nicotine injections into the caudate on A9 neurons was excitatory but in spite of the subsequent excitation of SNR neurons, the firing rate usually returned to the baseline.

Glutamate injections into the nucleus accumbens were most consistently followed by cessation of firing of ventral tegmental area non-dopaminergic neurons and excitation of the A10 cells. Nicotine injections into the nucleus accumbens also caused inhibition of ventral tegmental area non-dopaminergic neurons and excitation of the A10 cells. However, in contrast to the SNR neurons, no post-inhibitory excitation of the non-dopaminergic neurons was observed.

The results obtained suggest that the pharmacological stimulation of striatonigral or nucleus accumbens ventral tegmental area output cells by glutamate or nicotine influences dopaminergic neurons by removing the tonic inhibitory influence from the adjacent non-dopaminergic neurons. In spite of the well demonstrated inhibitory striatonigral (Precht, W. and Yoshida, M., Brain Res., 32:229, 1971) or nucleus accumbens-ventral tegmental area (Wolf, P., et al., Experientia, 34:72, 1978) outputs that directly inhibit the dopaminergic neurons, the overall excitation of the striatal regions caused excitation of the dopaminergic neurons. These results are consistent with the concept that nicotine produces multiple excitatory effects on midbrain dopamine neurons.

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270.19 EFFECTS OF ADENOSINE 3', 5' MONOPHOSPHATE (cAMP) ON RAT NEOSTRIATAL NEURONS: INTRACELLULAR STUDY IN AN *IN VITRO* SLICE PREPARATION. T. Kita, H. Kita and S. T. Kitai. Dep. of Anatomy and Neurobiology, Univ. of Tenn. Sch. of Medicine, Memphis, TN 38163

It is well known that a number of neuroactive substances (e.g. dopamine) stimulate adenylate cyclase in the neostriatum (Str). The neuropharmacological actions of cAMP on Str, however, are not well understood. We studied the effects of cAMP and forskolin, an adenylate cyclase activator, on rat Str neurons in an *in vitro* slice preparation. Brain removed from decapitated rats were blocked to contain Str and were sectioned (400μm) in a parasagittal plane using a Vibratome. The slices were placed in a recording chamber and continuously superfused with oxygenated Krebs solution (composition in mM: NaCl 124, KCl 5.0, $MgSO_4$ 2.0, KH_2PO_4 1.25, $CaCl_2$ 2.0, $NaHCO_3$ 26 and glucose 10, pH 7.2-7.4). Local stimulation was applied through bipolar electrodes placed on the surface of the slice. Glass pipettes filled with 2 M K-methylsulfate were used for intracellular recordings. 8-bromo cAMP (10^{-7} - 10^{-4} M) and forskolin (10^{-7} -10^{-4} M) were applied to the superfusing solution. In experiment using intracellular injection of cAMP, the microelectrodes were filled with 10mM cAMP in either 2 M K-methylsulfate or 2 M K-acetate.

Extracellular applications of 8-bromo cAMP or forskolin and intracellular cAMP injections consistently resulted in a membrane depolarization, a decrease of the membrane conductance, and a decrease of the anomalous rectification in a dose-dependent manner. The responses were not affected by the addition of TTX in the superfusing medium. However, they were attenuated when recorded neurons were injected with TEA prior to the application of 8-bromo cAMP or forskolin. Moreover, the application of 8-bromo cAMP at higher concentration (10^{-4}M) or intracellular injection of cAMP resulted in an increase in the spike threshold and a decrease the spike afterhyperpolarization. These results suggest that elevation of cAMP in Str neurons result in (1) a decrease of potassium conductance and (2) a suppression of sodium spike generation.

Application of cAMP had complex effects on the synaptic responses (i.e., fast EPSP, IPSP and slow EPSP) to local stimulation. The details of these effect are currently under investigation. Preliminary observations show, however, that (1) generation of sodium spikes from fast EPSPs is decreased and (2) the duration and amplitude of slow EPSP are increased. These results indicate that activation of adenylate cyclase may act primarily to modify voltage dependent conductance in Str neurons.

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SUBCORTICAL SOMATOSENSORY PATHWAYS

271.1 SOMATOSENSORY AND VISUAL REPRESENTATIONS IN THE CLAUSTRUM: TOPOGRAPHY AND COLLATERALIZATION OF CLAUSTRO-CORTICAL CELLS IN THE CAT. D. Minciacchi*, A. Granato*, A. Antonini* and G. Tassinari* (SPON: M. Bentivoglio). Inst. of Neurology and Inst. of Anatomy, Catholic Univ., Rome and Inst. of Physiology, Univ. of Verona, Italy.

The wide cortical distribution of claustral projections and the existence of sensory maps in the claustrum points out that this structure is a highly organized source of cortical input. After previous studies on the topography and collateralization of claustral projections to different cortical areas (Macchi et al., J. Comp. Neurol., 215, 121, 1983; Minciacchi et al., Neurosci., 16, 557, 1985) the present study was aimed at analyzing the organization of claustral projections to a single cortical area. A multiple retrograde fluorescence strategy utilizing Fast Blue, Diamidino Yellow, Evans Blue and Fluoro Gold was here employed. In all of the experiments these four tracers were injected under electrophysiological control in four cortical regions: the forepaw (S1fp) and face (S1fa) representation fields of the first somatic sensory area, the vertical meridian (V1vm) and periphery (V1pe) representations of the first visual area.

The claustral cell populations labeled from the S1 injections were located in the anterodorsal part of the claustrum while those labeled from the V1 injections were placed in its posterodorsal part. Thus, the claustral projections to S1 and V1 were mainly segregated and no claustral cells simultaneously projecting to S1 and V1 were found. Within the claustral region projecting to S1, the cell populations labeled from S1fa and S1fp were intermingled with a certain degree of topographical arrangement: the S1fa labeled neurons were located ventrally, partially surrounding the more dorsal S1fp labeled cells. Approximately 5% of the entire S1-projecting claustral population was simultaneously labeled from both the S1 injections. As for the visual projecting population, cells labeled from the V1pe and V1vm injections were clearly segregated: the cells labeled from V1pe were located dorsally, surrounding a central core of neurons labeled from V1vm. Cells simultaneously labeled from both V1 injections were also present (approximately 10% of the entire V1 projecting cell population).

Altogether, these data provide the first anatomical demonstration of a somatotopic arrangement of the claustro-S1 projections and confirm the retinotopical organization of the claustro-V1 projections in the cat. Furthermore, these results indicate that a certain degree of collateralization is present in the claustral projections to different fields within primary sensory areas, whereas no collateralization is present in the claustral projections to different primary sensory areas.

271.2 GABA-ergic INNERVATION OF IDENTIFIED THALAMO-CORTICAL NEURONS IN THE VENTROBASAL COMPLEX OF THE CAT. C. N. Honda, H. D. Schwark, and E. G. Jones. Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

A confluence of functional, morphological and immunocytochemical studies suggests that many of the inhibitory interactions in the dorsal thalamus are mediated by the putative neurotransmitter gamma aminobutyric acid (GABA). In the cat ventrobasal complex GABA is generally believed to derive from two sources: (1) presynaptic dendrites and axon terminals of intrinisic neurons, and (2) axonal projections of neurons located in the somatic sensory regions of the nucleus reticularis. The present study combines immunocytochemical and intracellular recording and staining techniques to demonstrate GABA-ergic innervation of individual thalamocortical neurons in the ventral posterolateral (VPL) nucleus.

In the anesthetized cat, antidromically identified thalamocortical neurons in VPL were functionally characterized then intracellularly injected with horseradish peroxidase (HRP). Following fixation with 3% paraformaldehyde / 0.25% glutaraldehyde, frozen or Vibratome sections were processed for HRP using cobalt-intensified DAB to yield a black reaction product. Sections containing portions of intracellularly stained neurons were then processed for GABA immunoreactivity using standard immunoperoxidase techniques to yield an amber-colored reaction product. Under oil immersion (1000X) HRP labeled neurons (black) and GABA-containing elements (amber) were clearly distinguishable in the same section. Appositions between GABA-containing terminals and HRP-labeled neurons were judged to be "contacts" when no gap appeared between the two elements, and when both lay in the same plane of focus.

Small GABA-positive perikarya were found homogeneously distributed in VPL and intermingled among unlabeled large neurons. GABA-containing profiles resembling both *en passant* and terminal axonal enlargements were densely distributed throughout VPL. GABA contacts were found to occur on all portions of thalamocortical neurons, but were predominantly localized to primary and secondary dendrites. The GABA contacts identified to date were found mostly on dendritic shafts and not on spinous processes. At the present time, it is not possible to distinguish between GABA-containing axonal terminals and presynaptic dendrites. Hence, we cannot yet assess the relative contribution of GABA-ergic inputs originating from intrinsic or reticular nucleus sources.

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271.3 ELECTROPHYSIOLOGICAL CHARACTERIZATION OF SINGLE NEURONS OF THE NUCLEUS RETICULARIS THALAMI (RTN) ON RAT SLICES. G.Avanzini*, M. De Curtis*, R.Spreafico* (Spon. E.Parati), Ist. Neurol. C.Besta 20133 Milano, Italy.

RTN is the most lateral nucleus interposed between the external medullari lamina and the internal capsule.It is known to receive collaterals both from cortico-thalamic and thalamo-cortical fibers arising from the dorsal thalamic nuclei. Immunocytochemical studies demonstrated the presence of GABA-positive terminals contacting cell bodies and dendrites of GABAergic RTN neurons. Aim of the present work is to investigate the physiological properties of RTN neurons. Experiments were performed on 350-400 um thick diencephalic orizontal slices of the rat, maintained in vitro. RTN neurons recorded with 4 M K acetate filled electrodes were activated by stimuli from the ventrobasal complex and from the internal capsule. All of the recorded neurons showed common electrophysiological properties: in resting conditions a single spike response followed by a short lasting hyperpolarizing potential was activated by synaptic or direct stimulations. When an hyperpolarizing steady current was injected intracellularly the response pattern changed to an all-or-none burst firing on a slow depolarizing potential, activated by both synaptic and direct stimuli; this response was followed by an afterhyperpolarizing potential showing a reverse potential around -80 mV. A non synaptic K^+-dependent nature of this hyperpolarizing potential is supported by the following facts. The block of synaptic transmission by lowering (Ca^{2+}) from 2 mM to 0,2 mM did not affect its amplitude. The selective block of GABAergic transmission by bicuculline mathiodide (10^{-5M}) mixed to perfusion fluid induced a gradual hyperpolarization of the membrane potential with a parallel transformation from single spike to burst response. In these conditions however a post-spike after hyperpolarization could still be detected by depolarizing the membrane potential to the resting level, thus restoring the single spike response pattern. The increase of the (K^+) from 3.5 to 7.5 mM decreased the amplitude of the hyperpolarizing potential and shifted its reversing point in depolarizing direction.

271.4 RESPONSE CHARACTERISTICS AND MORPHOLOGY OF VIBRISSA-SENSITIVE NEURONS IN THE VENTRAL POSTEROMEDIAL, THALAMIC RETICULAR AND POSTERIOR NUCLEI OF THE RAT. G.R. Belford, H.P. Killackey, N.L. Chiaia and R.W. Rhoades (SPON: R.K. Josephson). Dept. of Psychobiology, University of California, Irvine, CA 92717 and Dept. of Anatomy, University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine and Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Many diencephalic nuclei receive input from the trigeminal (V) brainstem complex and a number of thalamic structures have been shown to contain vibrissa-sensitive neurons. We have employed intracellular recording and horseradish peroxidase (HRP) injection techniques in an effort to relate differences in the responses of vibrissa-related neurons in three portions of the thalamus, the ventral posteromedial nucleus (VPM), the thalamic reticular nucleus (TRN) and the posterior nuclear group (PO), to the morphology of these cells.

Thus far, we have recovered 31 VPM cells, 8 TRN cells and 4 PO neurons that were responsive to vibrissa stimulation. Five VPM cells, 4 TRN cells, and one PO neuron have been subjected to detailed anatomical analysis. Vibrissa-sensitive cells in VPM were usually excited by deflection of only one or two vibrissae, but several cells that we have recovered responded to as many as four whiskers. These neurons had either bitufted or multipolar dendritic trees. The average soma area was 205 μm^2, the total dendritic arbor averaged 7,234 µm in length and the cross-sectional area encompassed by the dendritic field was 62,761 μm^2. The TRN cells were also generally responsive to only one or a few vibrissae, but three of these neurons had responses that were qualitatively different from those of VPM cells. They had high and regular spontaneous discharges that were suppressed by vibrissa deflection or electrical stimulation of the V brainstem complex. This suppression was associated with a long-lasting ipsp. The somas of the TRN cells had an average diameter of 228 μm^2. These neurons were essentially bipolar and their dendrites followed the contours of the nucleus. The average dendritic length for these cells was 4,118 µm and the average cross-sectional area of the dendritic tree was 26,675 μm^2. All of the PO cells we recovered were excited by deflection of >10 vibrissae. These neurons had sparse, but widespread dendritic arbors. The one cell that has been completely analyzed had a soma area of 278 μm^2 and a total dendritic length of 5,651 µm. The cross-sectional area of the dendritic tree was 110,381 μm^2.

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271.5 THE RESPONSE PROPERTIES OF MEDIAL THALAMIC NEURONS TO TECTAL AND SENSORY STIMULATION BEFORE AND AFTER LESIONS OF THE SUPERIOR COLLICULUS. B. Grunwerg* and G. M. Krauthamer. Department. of Anatomy, UMDNJ-Robert Wood Johnson Medical School at Rutgers, Piscataway, NJ 08854

The medial thalamus receives a massive projection from the deeper layers of the superior colliculus. In previous studies we determined the peripheral response properties of tectothalamic projection neurons. In the present study we examined the sensory responses of medial thalamic neurons activated by deep tectal stimulation (0.5 msec. pulses, 250-500 uA) in chloral hydrate anesthetized, flaxedil immobilized rats. Thalamic unit activity was recorded extracellularly with tungsten microelectrodes.

Results indicated distinct differences in the response properties of anterior neurons, largely confined to paralamellar MD and posteroventral CL, and neurons located more posteriorly in Pf. Anterior neurons responsive to tectal stimulation were spontaneously active and tended to have complex peripheral response properties, responding to light tapping, noxious stimuli and auditory stimulation. Receptive fields were large and bilateral but responses were more intense to contralateral inputs. More posteriorly, in Pf, complex units were rarely encountered; most units responded exclusively to tail pinch or other painful stimuli and spontaneous activity was low.

Following unilateral destruction of tectal neurons with kainic acid (3.5 nM/0.5 ul) the rats displayed typical ipsiversive circling. Thalamic neuronal activity, recorded 2-3 days later, was strikingly altered. On the side ipsilateral to the tectal lesion, no spontaneous activity was seen whereas spontaneous activity remained high on the intact side. Responses to peripheral stimuli could no longer be elicited on either side.

These results indicate that the excitability level of medial thalamic neurons is regulated by tectothalamic projection neurons. The absence of sensory responses on both sides may reflect changes in bilateral intrathalamic information processing.

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271.6 SOMATOSENSORY-VISUAL NEURONS IN CAT SUPERIOR COLLICULUS SHOW PARALLEL SOMATOSENSORY AND VISUAL VELOCITY TUNING. H.R. Clemo, M.A. Meredith and B.E. Stein. Depts. Physiol. & Anat., Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

There are many parallels between the somatosensory and visual representations in the deep layers of the superior colliculus (SC), both in their physiological properties and their involvement in localizing and orienting behaviors. However, it is not known whether there is a correspondence between functional properties of these modalities that may underlie such behaviors. Velocity of stimulus movement influences neuronal responsiveness and various velocities are optimal among SC neurons of either modality. Since the majority of somatosensory neurons are also responsive to other sensory stimuli (visual and/or auditory), we sought to determine the relationship of velocity tuning for each modality in somatosensory-visual neurons.

Cats (n=5), prepared 1 week prior to the initial recording session, were anesthetized (ketamine HCl), paralysed and respired with a mixture of N20 and O2. The somatosensory and visual receptive fields of 23 bimodal neurons in the intermediate layers were mapped and their responses to a variety of stimulus velocities for each modality were recorded. A mechanical probe was used to deliver stimuli in the cutaneous receptive field at different velocities (5-450 mm/s) and a galvanometer-driven mirror was used to move a bar of light across the visual receptive field at velocities ranging from 1-550°/s.

Of the neurons that showed velocity tuning (18/23; 78%), the majority (16/18; 89%) showed corresponding velocity tuning in both modalities. Thus, neurons (11/18; 61%) that were optimally activated (greatest number of spikes) by high velocity hair or skin displacement were optimally activated by high velocity visual stimuli. Similarly, those neurons (5/18; 28%) that responded best to slowly-moving somatosensory stimuli were also best activated by slowly-moving visual stimuli and the 5 neurons that showed no, or very broad, velocity tuning did so for both modalities.

To investigate the possible behavioral significance of this somatosensory-visual correspondence, stimulating electrodes were placed in the tecto-spinal tract of 3 cats. Forty percent (9/23) of all somatosensory-visual neurons were antidromically activated but no (0/27) unimodal somatosensory neurons could be activated in this way. It seems likely, then, that the correspondence of velocity sensitivity across somatosensory and visual modalities reflects parallel organizational schemes that underlie the behaviors mediated by the tectospinal pathway.

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271.7 SOMATOSENSORY PROJECTIONS FROM SIV AND ADJACENT CORTEX TO THE THALAMUS AND SUPERIOR COLLICULUS IN THE NEWBORN CAT.
J.G. McHaffie, L. Kruger, H.R. Clemo and B.E. Stein. Dept. of Physiol., Med. Coll. of VA., Richmond, VA 23298 and Dept. of Anatomy, UCLA Ctr. Health Sci., Los Angeles, CA 90024.

A fourth somatosensory representation (SIV) has been described in the anterior ectosylvian sulcus (AES) of the cat (Clemo & Stein, Brain Res. 235:162, 1982). Unlike SI-SIII, SIV sends a direct projection to the superior colliculus (SC) while its thalamic projection skirts the ventrobasal complex to terminate in the posterior complex (Stein et al., J. Neurophysiol. 50:896, 1983). Since fetal and neonatal SC neurons respond to tactile stimuli (Stein et al., J. Neurophysiol. 36:667, 1973), we sought to determine if these corticofugal projections from AES also were present in the newborn cat.

Corticofugal projections from the AES were studied with autoradiography and retrograde HRP. When the injection was restricted to the walls and fundus of the AES, heavy ipsilateral thalamic label was seen in the medial subdivision of the posterior complex, the suprageniculate nucleus, and the external medullary lamina. No label was seen in the contralateral thalamus although the homotopic cortex was labeled. In the ventrobasal complex (VB) dense axonal label was seen traversing VB, but only sparse label was observed in VB proper. However, in those cases with marked spread of tracer into adjacent SII, terminal label in VB was much more pronounced. Thus, the corticothalamic projection in neonates is consistent with observations in the adult.

Rostral AES injections produced predominantly ipsilateral terminal label in the SC that was distributed in two tiers: a discontinuous band in the intermediate gray lamina and a second, more diffuse band in deep gray lamina. Caudally, dense terminal label was seen in the intercollicular zone and the dorsolateral periaqueductal gray. After injections of HRP into the SC, retrogradely labeled neurons were found throughout the ipsilateral AES. The cytoarchitecture characteristic of the adult SII-SIV region was apparent in the newborn. Retrogradely labeled neurons were found in SIV but never in SII. Thus, the corticotectal projection in neonates also parallels that seen in the adult.

These data show that the elaboration of a major descending somatosensory pathway to the thalamus and midbrain is largely a prenatal event. The in utero anatomical maturation of descending corticofugal projections from SIV cortex to the SC contrasts with the protracted postnatal development of the corticotrigeminal projection from SI cortex (Tolbert et al., J. Comp. Neurol. 228:478, 1984) but is consistent with the mature anatomical state of ascending trigeminotectal projections (McHaffie et al., J. Comp. Neurol. 249:411, 1986). Supported by NIH Grant EY05554.

271.8 RESPONSE PROPERTIES AND ORGANIZATION OF NOCICEPTIVE NEURONS IN THE RAT SUPERIOR COLLICULUS.
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Numerous somatosensory neurons are present in the deep laminae of the superior colliculus (SC) and are topographically organized. Generally, both the response properties and spatial organization of such neurons are remarkably similar among species. Recently, we have quantitatively evaluated the response properties of nociceptive neurons in the hamster's SC (Larson, M.L. et al, J. Neurosci. 2:547, 1987). However, neither their properties nor their distributions in other species are known. The present study was an attempt to determine (a) whether nociceptive neurons are present in the SC of rat, if so, (b) whether they can be further catoragorized into nociceptive subtypes, and (c) whether they have any systematic organization.

Single- and multiunit activity was recorded in urethane anesthetised hooded rats. Neurons were first qualitatively characterized with manually presented innocuous (brushes, von Frey hairs, air puff) and noxious (pinch, glowing ember) stimuli. The stimulus-response relationships of nociceptive neurons were then evaluated quantitatively with an electronically controlled contact thermode placed on the skin.

In addition to those activated by low threshold (LT) tactile stimuli, many somatosensory neurons in the SC of rat responded preferentially, or soley, to frankly noxious stimulus. Two subtypes of nociceptive neurons were found: wide dynamic range (WDR- responsive optimally to noxious stimuli but also responsive to LT stimuli) and nociceptive specific (NS- responsive solely to noxious stimuli). The stimulus-response relationships of both WDR and NS neurons to noxious thermal stimuli appeared to be a positively accelerating power function. WDR receptive fields had two subregions: one in which LT and noxious stimuli were both effective and another, surrounding the first, in which noxious stimuli were more effective. NS receptive fields were significantly smaller than WDR receptive fields.

Several organizational features were evident. Regardless of subtype, nociceptive neurons had their heaviest concentration in the rostral one-half of the SC, and many had receptive fields on, or including, the face. A general dorsal to ventral segregation of somatosensory neurons was also noted, such that in a given electrode penetration, LT neurons were usually the most superficial, WDR neurons were just below these, and NS neurons were deepest of all.

These data indicate that nociceptive neurons are not a peculiarity of the hamster SC and that they have well-defined receptive field properties that are selectively distributed within the structure. Supported by NIH Grant EY05554.

271.9 SYNAPTIC ORGANIZATION OF THE VENTROBASAL COMPLEX IN THE PRIMATE.
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Most mammalian thalamic nuclei have a common organization and, in some cases, particular morphological features have been correlated to functional parameters. This paper is concerned with delineating the features of the synaptic organization of the monkey ventrobasal complex (VB) in order to provide a basis for experimental studies of the primate somatosensory thalamus.

Adult Macaca fascicularis were perfused under deep barbiturate anaesthesia with 2% paraformaldehyde/ 2% glutaraldehyde in 0.1M phosphate buffer pH 7.6. The thalamus was sectioned on a vibratome at 50um and the slices osmicated, stained with uranyl acetate, dehydrated and embedded in Epon. Serial thin sections were cut through different regions of the VB and examined with the electron microscope.

Terminal types and the contacts they establish are similar to those found in other thalamic sensory nuclei. Four vesicle containing profiles were identified. Large axon terminals (RL-type) containing spherical synaptic vesicles and establishing asymmetric synaptic contacts. Small terminals with densely packed spherical synaptic vesicles and establishing asymmetric synaptic contacts (RS-type). Terminals containing moderately packed flattened or cylindrical synaptic vesicles and establishing symmetrical synaptic contacts (F-type). Finally, vesicle filled dendritic appendages (PSD) containing pleomorphic synaptic vesicles with synaptic contacts intermediate between symmetrical and asymmetrical.

In the extra-glomerular neuropil RL terminals contact larger dendrites; RS terminals contact small dendrites; F terminals contact neuronal cell bodies and large or medium size dendrites. PSDs are presynaptic to neuronal cell bodies, dendrites and other PSDs and are postsynaptic to RS, F and PSD profiles. Within glomeruli, dendrites, RL, PSD and F terminals are present and triadic arrangements involving dendrites, RL terminals and PSDs are common. Three dimensional computer-aided reconstructions of glomeruli have been made (program supplied by the High voltage Electron Microscopy Laboratory, Boulder Colorado) to analyze the organization of components within the glomeruli and the number of synaptic contacts.
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271.10 THE PROJECTION OF THE DORSAL COLUMN NUCLEI AND THE SPINAL CORD TO NEURONS OF THE PRIMATE VENTROBASAL (VB) THALAMUS.
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The ventrobasal thalamus (VB) of the monkey is the major region of the thalamus concerned with somatic sensation. Some VB neurons have been found to respond specifically to a particular somatic stimulus; others respond to both noxious and nonnoxious stimuli. That region of VB representing the contralateral body receives projections from the dorsal column nuclei (DCN) and the spinal cord via the spinothalamic tract (STT). This study examines the two projection systems to determine the nature of their terminations upon VB neurons.

Macaca fascicularis monkeys were anesthetized and monitored during the entire surgical procedure. Using sterile neurosurgical techniques, the dorsal column nuclei and rostral cervical spinal cord were exposed. Unilateral lesions of the DCN were made, and microinjections of 0.1 ul of 10% WGA-HRP were made into the dorsal horn of the cervical enlargment on the same side as the DCN lesion, and into the contralateral DCN. The wound was closed, the animals survived for 2 or 3 days, then were reanesthetized and perfused intravascularly with aldehyde solutions. Thalamus, DCN and cord were cut in serial sections with a vibratome and alternate sections reacted for light microscopy using TMB-nitroprusside at pH 3.3, or for electron microscopy using TMB-ammonium molybdate at pH 6.6 (Olucha et al. J. Neurosci. Meth. 13:131,'85) followed by slow osmication at pH 5.0 (Henry et al. J. Histochem. Cytochem. 33:1256, '85). In some cases, DCN lesions were combined with GABA immunocytochemical studies to examine DCN projections to interneurons.

The molybdate-TMB/slow osmication method provides an excellent retention of reaction product combined with good quality fixation. Both DCN and STT projections to VB end as large profiles with round synaptic vesicles (RL), the DCN terminations being the most numerous, even in zones of overlap of the two projections. Both projections contact large dendrites in the extraglomerular regions, and medium dendrites and dendrites with vesicles (presynaptic dendrites - PSD) within glomeruli. In some cases, both DCN and STT-RL profiles contacted the same dendrite, indicating a convergent input of the 2 systems on single VB neurons. DCN projections to PSD's were more commonly found to contact PSDs than were STT projections, suggesting different degrees of inhibitory mechanisms for the 2 systems. Combined degeneration and immunocytochemical techniques demonstrated that the DCN projects to GABAergic neurons. (Supported by NS23347 and NS21445; and INSERM).

271.11 TERMINAL ARBORS OF INDIVIDUAL G HAIR FIBERS IN THE CAT CUNEATE NUCLEUS VISUALIZED WITH INTRAAXONAL HRP. J. Pierce, R. Weinberg, and A. Rustioni. Departments of Anatomy and Physiology, University of North Carolina, Chapel Hill, NC 27514.

Neurons in the dorsal column nuclei typically exhibit small, modality-specific receptive fields. Fibers innervating G hairs comprise a major part of the forelimb cutaneous input to the cuneate nucleus (CN). This study examined the pattern of collateral arborization of individual G hair fibers in the CN, stained by the intraaxonal iontophoresis of HRP; these findings bear upon the mechanisms that account for place- and modality-specificity in the lemniscal system. Cats were anesthetized with pentobarbital and α-chloralose. Microelectrodes containing HRP (Sigma Grade 1, 10% solution in TRIS-buffered KCl) were advanced through the dorsal columns, using stimulating cuff electrodes placed on the superficial radial and median nerves to provide a hunting stimulus. The modalities and receptive fields of cutaneous units were characterized with handheld probes. When a G hair fiber was identified, intra-axonal penetration and HRP injection were attempted. At the end of each experiment, animals were perfused with 2.5% glutaraldehyde/0.5% paraformaldehyde in phosphate buffer. Serial Vibratome-cut sections were reacted with DAB, and mounted for light microscopic examination. Fifteen collaterals from four identified G hair fibers were reconstructed in the transverse plane, using an immersion objective and a camera lucida attachment. In one case, the reconstruction of a series of collaterals was projected onto the longitudinal plane. Collaterals were spaced an average of 600 µm apart, with the closest spacing found between obex and 4 mm caudal to obex. In this region, adjacent collaterals tended to overlap, forming a relatively continuous rostrocaudal tube of terminations. In the transverse plane, the terminal fields had an oval shape, occupying on average 55,000 μm^2. Primary collateral branches would often spread out, and then bend back into the region in which most of the boutons were found. Arrangement of the terminal fields was generally consistent with cuneate comatotopic mapping studies. However, the large cross-sectional area of individual terminal fields suggest a high level of convergence. From published data one can calculate that roughly 5000 G hair fiber terminal fields could fit in the middle CN. The degree of anatomical convergence suggested by these experiments implies that intrinsic mechanisms are involved in generating place- and modality-specificity in the CN.

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271.12 INTRACELLULAR STAINING OF INTERNEURONS IN CAT CUNEATE NUCLEUS. R.J. Weinberg, C.Y. Wen, R.E.W. Fyffe*, R. Giuffrida*, and A. Rustioni. Depts. of Anatomy and Physiology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

Prior work from this laboratory has demonstrated a population of GABAergic interneurons in the cuneate nucleus. In the present study, interneurons from the cuneate core region have been intracellularly recorded and stained to further characterize them electrophysiologically and morphologically. Cats anesthetized with pentobarbital and α-chloralose were used. An array of stimulating electrodes was placed in the medial lemniscus at the level of the inferior colliculus. Cuneo-thalamic projecting neurons were identified by antidromic stimulation from this array, as shown by a short (<2 msec) constant-latency response. Previous work indicates that only few, if any, neurons in the cuneate core project to non-thalamic targets; therefore, cuneate neurons failing to respond antidromically were tentatively identified as interneurons and selected for further study. Modality and receptive fields were identified, as well as responses to stimulation of three major forearm nerves. Intracellular penetration was then attempted; following successful penetration, the neuron was intracellularly stained with HRP (Sigma Grade 1, 10% solution in TRIS-buffered KCl). Following intracardiac perfusion with 2.5% glutaraldehyde and 0.5% paraformaldehyde in phosphate buffer, Vibratome sections of the medulla were reacted with DAB and mounted for microscopic examination. In favorable cases, sections were osmicated and wafer-embedded in epon-Spurr resin for electron microscopy.

To date, five neurons have been recovered that a) match electrophysiological criteria for identification as cuneate interneurons, and b) correspond in size and location to cuneate neurons that are labeled by GAD immunocytochemistry (Rustioni et al., 1984). These neurons are all small (cell body 10 x 20µm diameter), with only a thin rim of cytoplasm surrounding the nucleus. They tend to lie at the periphery of cell clusters. Their dendrites extended several hundred µm from the cell body and branch only sparsely. In no case, could a clearly-defined axon be identified. The best characterized neurons respond to hair deflection, and exhibit short-latency excitatory responses to stimulation of more than one peripheral nerve. Immunocytochemical and electronmicroscopic study of this material is now in progress. Several other neurons of heterogeneous morphologies and not resembling the cuneo-thalamic neurons previously described by Fyffe et al. (1986), have also been recovered.

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271.13 MORPHOLOGICAL CHANGES IN THE RAT DORSAL COLUMN NUCLEI FOLLOWING PRENATAL LIMB REMOVAL. J.R. Norris, D.R. Dawson, and H.P. Killackey, Dept. of Psychobiology, University of California, Irvine, CA. 92717

Tangential sections of the primary somatosensory cortex of the rat stained for succinic dehydrogenase (SDH) activity exhibit an anatomical map of the entire body surface. Recently, it has been shown in the rat that, following forelimb amputations on embryonic day 16 or 17, the hindlimb portion of the SDH staining pattern exhibits an increase in size of as much as 100%. The extent to which these cortical changes reflect morphological changes in the subcortical somatosensory nuclei is not known. In the present study, cytoarchitectural and tract-tracing methods were used to analyze the morphological organization of the dorsal column nuclei (DCN) following prenatal limb amputations.

Forelimb or hindlimb amputations were performed in utero between embryonic day 16.5 and 19.5. Natural birth occurred on day 22 or 23. Animals were weaned after three weeks and raised until early adulthood. Some animals with forelimb amputations received complete lower thoracic spinal transections, were sacrificed and processed for Fink-Heimer staining. Some animals received bilateral injections of WGA-HRP, were sacrificed and reacted with TMB. Some animals were sacrificed and processed using Nissl staining methods.

In the DCN of adult rats which received limb amputations prenatally, three anatomical changes were observed. First, the ipsilateral nucleus associated with the amputated limb is reduced in size and is less distinct than the contralateral equivalent, despite a relatively normal location and shape. The reduction in size is most noticable with earlier amputations but is present in each of the animals observed. Second, the border between the two ipsilateral dorsal column nuclei is blurred in regions where the nuclei are in close approximation. Third, the termination pattern of the afferent projections to the ipsilateral DCN (associated with the intact limb) exhibits a slight increase in size which corresponds in location to the blurred border regions between the two ipsilatersal dorsal column nuclei.

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271.14 PROJECTIONS FROM GLABROUS SKIN OF SINGLE FOREPAW DIGITS TO THE CUNEATE NUCLEUS IN THE RACCOON. D.D. Rasmusson, Dept. of Physiology & Biophysics, Dalhousie University, Halifax, N.S., CANADA B3H 4H7

The anatomical and physiological organization of the cuneate nucleus in the raccoon has been described by Johnson et al. (J. Comp. Neurol. 132:1-44, 1968). The middle, cluster region of the cuneate is characterized by enlarged representations of the forepaw digits. These digit "columns" are elongated rostrocaudally and separated by dense myelin bundles. The purpose of the present experiment was to determine if the anatomical projection pattern from single digits corresponds precisely to these boundaries. Alternately, afferents from adjacent digits might overlap, requiring filtering to produce the restricted receptive fields seen at the single unit level.

Transganglionic transport of HRP was used to produce labeling of primary afferent terminals. A total of eleven digits were studied in 8 raccoons. HRP (20%) or WGA-HRP (2%) was injected into both ventral nerves of the digit (about 95% of the innervation of the glabrous skin travels in the ventral nerves: Somatosen. Res., 4:43-62, 1986). The animals were perfused three days after the injections and 50 µm thick horizontal sections of the medulla were taken. The tissue was reacted with TMB and in some cases counterstained with neutral red. Alternate sections were stained with thionin.

The projection patterns in every case consisted of dense labeling in long rostro-caudal columns extending throughout the cluster region of the cuneate with the 5th digit represented medially and successive digits more laterally. Within such a column, the labeling was dense over clusters of cells and considerably lighter between clusters. The mediolateral extent of the labeling was restricted to the somatotopically appropriate column and did not overlap into adjacent digit columns. This pattern broke down in the non-cluster regions (both rostral and caudal cuneate) where the labeling was more diffuse.

This pattern of projection indicates that the primary afferents are strictly segregated in the cluster region of the cuneate nucleus according to the digit of origin. The restricted terminal labelling seen here makes it possible to test the idea that these primary afferent terminals might sprout into adjacent digital regions of the cuneate nucleus after peripheral denervation.

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271.15 CLUSTERS OF AFFERENT TERMINALS FROM DIGITS AND PADS OF THE HAND RELATE TO DISCRETE CLUSTERS OF CELLS IN THE CUNEATE NUCLEUS OF MACAQUE MONKEYS AND PROBABLY HUMANS. S. L. Florence, J. T. Wall, and J. H. Kaas. Dept. of Psychology, Vanderbilt Univ., Nashville, TN 37240.

The present experiments demonstrate that clusters of primary afferents from specific digits and pads of the hand of macaque monkeys terminate in discrete clusters of cells in the cuneate nucleus that react densely for cytochrome oxidase and are outlined by myelinated axons.

To demonstrate the termination pattern of afferents, small subcutaneous injections of WGA-HRP or cholera toxin sub-unit B-HRP were made into one or more localized foci of the palm or digits of the hands of five macaque monkeys (Macaca fascicularis). Following survivals of 3-4 days, the brain stem of each perfused monkey was cut coronally and alternate sections were processed with tetramethylbenzidine, cytochrome oxidase (CO), myelin stain, or Nissl stain.

The middle two thirds of the cuneate nucleus (pars rotunda) was found to be parcellated into numerous CO-dark regions separated by CO-light septa-like zones. Careful alignment of adjacent brain sections revealed that CO-dark regions are relatively free of myelinated axons and correspond to cell clusters, while intervening CO-light septa correspond to bundles of myelinated fibers. The rostral and caudal poles of the cuneate nucleus and the pars triangularis were found to be structurally more homogeneous. The terminal fields of primary afferent fibers from individual digits were concentrated primarily within individual CO-dense regions of the pars rotunda and a CO-dense region could be related to each digit. Injections of the palm often involved more than one palmar pad and a group of CO-dense patches were usually associated with these injections. At the rostral and caudal poles of the nucleus, afferent fibers terminated more diffusely. A comparison of the brain sections from macaque monkeys and myelin-stained brain sections from humans revealed a parcellation pattern in the pars rotunda of the cuneate nucleus of humans that was very similar to that found in monkeys.

We conclude that the pars rotunda of the cuneate nucleus of macaques, and probably other primates including humans, has a somatotopic map that is anatomically apparent and precisely organized in a manner comparable to the "barrel field" of rats. The results also suggest that additional, less discrete maps exist at the rostral and caudal poles of the cuneate nucleus and perhaps also in the pars triangularis. The separate maps may relate to different submodalities.

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271.16 DORSAL COLUMN STIMULATION PRODUCES PRIMARY AFFERENT DEPOLARIZATION IN NUCLEUS CAUDALIS VIA A BRAINSTEM LOOP. N.E. Saadé, S.F. Atweh# and S.J. Jabbur#. Fac. of Sci., Lebanese Univ., Hadath-Beirut and #Fac. of Med., Amer. Univ. of Beirut, Beirut, Lebanon.

Dorsal column stimulation inhibits n. caudalis activity through a brainstem loop (Brain Res. 348:401, 1985). The inhibition begins 15-20 ms after stimulation, peaks at 35-50 ms, and lasts about 200 ms. A similar inhibition of dorsal horn activity through a similar loop is, in part at least, presynaptic (Brain Res. 310:180, 1984). We now show that primary afferent depolarization is produced in n. caudalis through a dorsal column-brainstem loop.

Anesthetized decerebrate/decerebellate cats were used, in which the ventral and lateral spinal funiculi were sectioned bilaterally at C1/C2 and C3/C4 levels. The presence of primary afferent depolarization was ascertained by changes in the positive wave recorded from the surface of n. caudalis in response to test stimulation of infraorbital nerve and changes recorded from infraorbital nerve to test stimulation in n. caudalis. A 30 ms train of shocks at 300 Hz was applied to the ipsilateral dorsal columns rostral to the cuts, and both forepaws were stimulated with single shocks. The areas of the positive wave of n. caudalis and the two components comprising the nerve response were measured with a waveform calculator.

Dorsal column stimulation depressed the positive wave evoked in n. caudalis by infraorbital nerve stimulation, the peak depression occurring 10-30 ms after stimulation and lasting about 150 ms. The positive wave decreased to 30% of control after the dorsal column stimulus and to 60% of control after contralateral forepaw shock. The nerve response consisted of an inital spike due to activation of infraorbital nerve terminals and a later complex spike related to the dorsal root reflex and due to antidromic activation of the nerve fibers by interneuron activity in n. caudalis producing presynaptic inhibition. Dorsal column stimulation increased the first spike to 150-180% of control, but decreased the later spike to 40-50% of control, both effects peaking at 30-50 ms and lasting about 100 ms. These changes, and their time course, provide the evidence that the inhibition of n. caudalis activity produced by the dorsal column-brainstem route is mediated, in part at least, by a presynaptic mechanism.

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271.17 SPINOCERVICAL TRACT NEURONS RESPONSIVE TO LIGHT TACTILE STIMULATION OF THE RACCOON FOREPAW. Harumitsu Hirata, Sherri L. Provencal*, Cecilia H. Yu*, and Benjamin H. Pubols Jr. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97209.

Response properties of single neurons activated by light mechanical stimulation of the glabrous surfaces of the forepaw have been extensively studied at the several levels of the raccoon dorsal column-medial lemniscal system (Pubols & Warren, 1985). The present investigation was undertaken in order to compare these findings with data obtained from the cells of origin of the raccoon spinocervical tract (SCT). Stainless steel microelectrodes were used to detect the extracellular electrical activity of SCT neurons in raccoons anesthetized with pentobarbital sodium.

A total of 29 antidromically identified SCT neurons with low threshold excitatory receptive fields (RFs) restricted to the glabrous surfaces of the ipsilateral forepaw have been studied. Of 8 neurons tested, none displayed enhanced response to noxious stimuli. All 29 neurons were histologically verified as falling within Rexed's laminae III-IV of dorsal horn segments C_7-C_8. Conduction velocities ranged between 8.3 and 56.8 $m.s^{-1}$.

Units were classified according to their response to a maintained mechanical stimulus as either rapidly adapting (RA; N = 25), or slowly adapting (SA; N = 4). The proportions of RA and SA do not differ significantly from those found in the clusters region of the main cuneate nucleus (MCN; Rowinski, Haring, & Pubols, 1985).

RF areas are significantly smaller ($P < .05$) on digits (N = 12; range = 0.4-45.0 mm^2) than on palm pads (N = 6; range = 8.8-56.4 mm^2). These values are slightly less than those previously seen in the MCN (Rowinski, et al., 1981). The median digital RF area (6.6 mm^2) is approximately 27 X that for primary afferents of the median nerve (Pubols, Pubols, & Munger, 1971), while the median palmar RF area (19.7 mm^2) is approximately 56 X that for median nerve fibers.

For 5 RA units, the range of exponents of the power function relating instantaneous spike frequency during displacement ramp stimulation to ramp velocity was .51-1.08. These values are comparable to those previously observed in the MCN (Rowinski, et al., 1985), In addition, 2 RA units displayed a discontinuous, or step, function, previously seen only in raccoon RA neurons of the thalamic ventrobasal complex (Warren, Kelahan, & Pubols, 1986).

Thus, the ability of the raccoon SCT to convey information from the glabrous skin of the forepaw regarding submodality, spatial, and dynamic characteristics of light mechanical stimuli appears to be at least as precise as that of the dorsal column-medial lemniscal system. (Supported in part by research grant NS-19486, USPHS, and by the Coleman H. Wheeler Jr. Memorial Fellowship Program of the Neurological Sciences Institute.)

272.1 INHIBITORY EFFECTS OF ANTERIOR PRETECTAL NUCLEUS STIMULATION ON JAW-OPENING REFLEX AND TRIGEMINAL (V) BRAINSTEM NEURONAL RESPONSES IN RATS. C.Y. Chiang*, I.C. Chen*, J.O. Dostrovsky and B.J. Sessle, Dept. of Physiology, Fac. of Medicine, and Fac. of Dentistry, Univ. of Toronto, Toronto, Ontario.

It has been recently reported that low-intensity stimulation of the anterior pretectal nucleus (APT) of the rat results in an increased tail-flick latency lasting for more than 45 min without causing escape behaviour or motor deficits (Roberts & Rees, Pain 25:83, 1986). The present study aimed to test the possible modulatory role of APT on the jaw-opening reflex (JOR) and neuronal responses in the trigeminal (V) brainstem sensory complex.

Experiments were performed on male Wistar rats anaesthetized with chloralose-urethane. Digastric activity was recorded intramuscularly with a pair of fine insulated stainless-steel wires. The JOR was elicited by test stimulation of the maxillary skin (0.1-0.2 ms, 0.5 Hz). In the experiments in which V neuronal activity was recorded, the animals were paralysed and artificially ventilated and the field potential (FP) evoked by facial skin (test) stimulation and the activity of single neurones in subnucleus caudalis of the V complex were recorded with tungsten microelectrodes (10-20 Mohms). The same type of electrode was also used for stimulation (8 pulses, 0.2 ms, 400 Hz, cathodal current) in APT (P:-4.3 to -4.8; L:1.6 to 1.8; V:5.0 to 6.0; with reference to Bregma) to test for conditioning effects on the JOR and caudalis activity at conditioning-test (C-T) intervals of 30 - 800 msec.

Conditioning stimulation of APT had an inhibitory effect on the JOR. This was manifested as a reduction of EMG amplitude, decreased incidence of single motor unit evoked discharges, and lengthening of JOR latency. The most effective stimulating loci were in the ventral APT, and currents as low as 35 uA often induced a 25 - 50% reduction in JOR amplitude. The APT-induced inhibition was usually noted at C-T intervals of 30 - 200 msec, and this period of inhibition was often preceded and followed by a period of facilitation. In addition, APT stimulation produced a 10-20% reduction in the caudalis FP and inhibited the evoked activity of low-threshold mechanoreceptive neurones and nociceptive neurones. APT stimulation inhibited the JOR and the neuronal responses bilaterally, but was more effective ipsilaterally.

These findings indicate that the APT exerts a modulatory influence on the JOR that is predominantly inhibitory in nature. Our findings of inhibitory effects also on V brainstem neuronal activity suggest that the inhibitory effect on the JOR may be at least partly due to modulatory influences on neurones in parts of the V brainstem sensory complex that subserve reflex and somatosensory functions. (supported by NIH)

272.2 MICROINJECTION OF GLUTAMATE INTO THE NUCLEUS TRACTUS SOLITARIUS PRODUCES ANALGESIA IN THE RAT. M. M. Morgan, J.-H. Sohn and J. C. Liebeskind. Department of Psychology, UCLA, Los Angeles, CA 90024-1563.

The nucleus tractus solitarius (NTS) is a medullary structure known to contain high levels of opioid peptides and receptors. Recently, Lewis and co-workers (in press) demonstrated that electrical stimulation of the nucleus tractus solitarius (NTS) could produce naloxone-reversible analgesia. In the present study, microinjections of glutamate, an excitatory amino acid, were made into the NTS to determine if activation of cell bodies of the NTS mediate this analgesic effect as opposed to fibers of passage.

Male Sprague-Dawley rats (200-400 g) were anesthetized with pentobarbital (55 mg/kg) and a guide cannula was stereotaxically positioned just dorsal to the obex. A monopolar electrode was inserted through the guide, extending 1 mm ventrally. The threshold for stimulation-produced analgesia was then determined using the tail-flick test to assess pain sensitivity. Once the threshold was determined, the electrode was removed and an injection cannula filled with glutamate (30 or 60 mM) or saline was inserted in its place. All injections were made in a volume of 0.5 µl over a 90 s period. Tail-flick tests were carried out 30 s after the end of the injection and at 1 min intervals thereafter for 5 min. If analgesia persisted, additional tail-flick tests were performed at 5 min intervals until response latencies returned to baseline values. All electrode sites were histologically verified following completion of the experiment.

Microinjections of glutamate into the NTS produced analgesia lasting approximately 5 to 10 min. Injections outside the NTS, or injections of saline into the NTS were ineffective in producing analgesia. In contrast, many sites outside the NTS supported analgesia during electrical stimulation, although the threshold for SPA was generally higher. This analgesia could be mediated by current spread activating NTS neurons or by activation of fibers of passage arising from rostral pain inhibition centers. Our glutamate microinjection data demonstrate that activation of cell bodies in the NTS can produce analgesia. (Supported by NIH grant NS07628).

272.3 EFFECTS OF MONOAMINE DEPLETION ON ANALGESIA PRODUCED BY ELECTRICAL STIMULATION OF THE NUCLEUS TRACTUS SOLITARIUS. A.M. Lohof, M.M. Morgan, and J.C. Liebeskind, Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024-1563

Electrical stimulation of the nucleus tractus solitarius (NTS) causes analgesia in the rat. The NTS is rich in opioid peptides and their receptors, and stimulation-produced analgesia (SPA) from the NTS is attenuated by naloxone. The role of other transmitter systems has not been studied. Akil & Liebeskind (1975) showed that depletion of monoamines by tetrabenazine (TBZ) attenuated SPA from sites in and around the dorsal raphe nucleus. This study sought to determine whether TBZ would also interfere with NTS stimulation-produced analgesia.

Male Sprague-Dawley rats (200-350 g) were anesthetized with pentobarbital (55 mg/kg) and a bipolar stimulating electrode was stereotaxically positioned in the NTS. Pain sensitivity was measured using the tail-flick test. After determination of SPA threshold intensity, TBZ (10 mg/kg, i.p.) or 0.9% saline was administered. SPA threshold intensity was determined again 30 min later. In other rats, electrodes were aimed at the dorsal raphe nucleus; SPA thresholds were determined before and after TBZ as described above.

Unlike SPA from dorsal raphe sites, that from the NTS was not consistently affected by TBZ. Serotonin, found in high concentrations in the dorsal raphe nucleus, is known to be involved in a descending pain inhibition system. Our results confirm the importance of this transmitter in the production of SPA from the dorsal raphe nucleus. Monoamines do not appear to mediate SPA from the NTS. (Supported by NIH grant NS 07628)

272.4 STIMULATION OF NUCLEUS TRACTUS SOLITARIUS CAUSES ANALGESIA BY ACTIVATING PERIAQUEDUCTAL GRAY MATTER IN THE RAT. J.-H. Sohn, A. Lohof, M. M. Morgan, and J. C. Liebeskind. Department of Psychology, UCLA, Los Angeles, CA 90024-1563.

Electrical stimulation of the nucleus tractus solitarius (NTS) produces opioid-mediated analgesia (Lewis et al., in press). The NTS has extensive reciprocal connections with higher brain structures. Notable among these are connections with the periaqueductal gray matter (PAG), itself known to be involved in pain inhibition. This study sought to determine whether NTS stimulation-produced analgesia (SPA) relies on connections with the PAG.

Male Sprague-Dawley rats were anesthetized with pentobarbital (55 mg/kg) and mounted in a stereotaxic apparatus. Pain sensitivity was measured using the tail-flick test. In the first experiment, a stimulating electrode was stereotaxically positioned in the NTS and holes were drilled in the lateral aspect of the skull so that a guide cannula could be lowered into the hole. Through this cannula a wire was inserted projecting 10 mm laterally allowing coronal brain sections to be made. Thresholds for SPA were determined before and after coronal sections. Sections were made rostral to the PAG and between the NTS and PAG. In the second experiment, similar procedures were followed except selective electrolytic lesions (2 mA DC, 15 sec) of the PAG were made rather than transections. Stimulation and lesion sites were histologically verified.

Transections between the NTS and PAG increased the threshold for SPA in the NTS. Thresholds were doubled in most rats, and in some animals SPA could no longer be obtained below intensities causing motor effect (i.e. tail movement). In some rats, transection also caused an increase in baseline tail-flick latency, but this effect disappeared after one or two minutes. Cuts made rostral to the PAG had no effect on SPA threshold. Selective PAG lesions also increased SPA threshold. In this experiment, baseline tail-flick latencies were always increased for approximately 2 to 5 minutes after the lesion.

Taken together, these results suggest that stimulation of the NTS produces analgesia via a descending system arising in the PAG. In related work, Morgan et al., (this volume) have shown that NTS analgesia is due to stimulation of cell bodies and thus not attributable to stimulation of descending PAG fibers. (Supported by NIH grant NS 07628).

272.5 SUPPRESSION OF THE HINDLIMB FLEXOR REFLEX THROUGH MEDIAL THALAMIC STIMULATION IN THE LIGHTLY ANESTHETIZED RAT. J. Duysens and J. Gybels*. Lab. of Experimental Neurology, University of Leuven, B-3000 Leuven, Belgium.

Although the descending control of flexor reflex pathways has been documented extensively, the nociceptive flexor reflex has been rarely used in studies of stimulation produced analgesia (SPA). The lightly anesthetized rat (Nembutal 10-20 mg/kg/hr) is a suitable preparation for testing the flexor reflex, as elicited by electrical stimulation of the pad. This model was used to further explore the analgesic effects obtained on the tail-flick and hot plate test in response to stimulation of the medial thalamic nuclei. Both the timing of the pad stimulation (interstimulus interval of 10 s) and the sampling of the integrated EMG responses of the pretibial flexors from the stimulated leg, were controlled by a small computer.

Provided the anesthesia was sufficiently light, a reliable complete suppression of the nociceptive flexor reflex was obtained with the same central stimuli which were previously shown to be effective in producing SPA in awake rats (1000 ms train at 100 Hz, 0.1 ms pulses at 50 μA). Such stimuli were sometimes effective over distances of more than 2 mm. Optimal sites were located near the midline, 1.8 mm behind the bregma and at depth of 4.5-5 mm and 7-7.5 mm below the cortical surface. Depending on the stimulus parameters a total train duration of minimum 300 to 500 ms was required with a pulse frequency exceeding 20 Hz. The suppression occurred during the period of stimulation and was sometimes followed by a rebound contraction after the completion of the stimulation.

Since the amount of suppression remained constant at different reflex amplitudes an additive inhibitory mechanism is postulated. Intravenous injection of naloxone (0.05 mg/kg) or methysergide (15 mg/kg) failed to reverse the suppression.

It is concluded that the hindlimb withdrawal reflex of the lightly anesthetized rat is suppressed by the same stimulus parameters and at the same sites yielding effective SPA in the awake rats. Moreover, the automated testing of the flexor reflex allows a rapid and detailed characterization of these sites.

272.6 EFFECTS OF INTRATHECAL (IT) THYROTROPIN-RELEASING HORMONE (TRH) ON IT SEROTONIN (5HT) ANALGESIA AS MEASURED BY THE TAIL FLICK (TF) AND HOT PLATE (HP) TESTS. E.S. Culhane*, C.L. Thurston, D.K. Douglass*, I.G. Campbell*, E. Carstens and L.R. Watkins. (SPON: S.N. Suberg) Dept. Animal Physiology, UC Davis, Davis, CA 95616.

TRH has been reported to have a modulatory effect on analgesia systems. IT TRH blocks IT morphine analgesia and ICV TRH potentiates both opiate and nonopiate stress induced analgesias. Recent anatomical evidence suggests a possible interaction between TRH and 5HT in the spinal cord. TRH and 5HT are colocalized in raphe-spinal neurons projecting to the ventral horn, while TRH containing cell bodies and terminals appear to be separate from, but do overlap, the descending 5HT projections to the dorsal horn. The present study investigated the possible modulatory effects of TRH on IT 5HT analgesia using the TF and HP tests.

Forty-six male Sprague Dawley rats (450-600g) were implanted with IT catheters terminating at the lumbosacral enlargement. After post-operative recovery and habituation to the restrainers used during testing, baseline TF and HP latencies were measured, and equivolume IT injections of either saline, 5HT (125 ug), TRH (10, 50 or 500 ng), or 5HT (125 ug) + TRH (10 or 50 ng) were made. Following injection, TF latencies were tested every 5 min for 40 min, and HP latencies were tested immediately following the 5, 15, 30 and 40 min TF tests.

5HT alone produced a rapid and significant analgesia for 15 min on the TF test which was significantly attenuated by 10 ng TRH. Conversely, 5HT did not produce analgesia on the HP test, but 10 ng TRH + 5HT caused significant analgesia for 15 min post-injection. 50 ng TRH did not affect 5HT analgesia on either test. TRH alone produced a significant dose dependent hyperalgesia 25-40 min post-injection on the TF test. No hyperalgesia occurred when both 5HT and TRH were injected.

These data indicate that TRH may affect 5HT analgesia in an inverted dose dependent manner. In addition, TRH appears to modulate a spinally mediated reflex (TF) differently than a supraspinally organized behavior (HP). A more complete dose response is currently being investigated. The slow-onset, dose dependent TRH hyperalgesia on the TF reflex may be due to its excitatory effect on motoneurons. Attenuation of TRH hyperalgesia by 5HT at times when 5HT alone is not analgesic suggests that 5HT may antagonize TRH's actions in the ventral horn.

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272.7 ANALGESIA INDUCED BY MICROINJECTION OF ARGININE VASOPRESSIN INTO THE LATERAL HABENULA OF CONSCIOUS RATS. K.D. Lake*, C.L. Thurston, E.S. Culhane*, E. Carstens, and L.R. Watkins. Dept. of Animal Physiology, University of California, Davis, Ca. 95616.

In a previous study, Lake et al. (FASAB, 1987) showed that microinjections of arginine vasopressin (AVP) into the lateral habenula (LHb) of anesthetized rats elicited an immediate and significant increase in mean blood pressure and heart rate for over 30 min. Since acute and genetically hypertensive rats can display elevated plasma AVP levels, high AVP content in certain brain nuclei, and a reduced sensitivity to painful stimuli, these data raise the possibility that the LHb may be involved in pain modulation secondary to its hypertensive effects. In order to study the possible role of the LHb in pain processing, we investigated the effect of AVP injection into the left LHb or control site just dorsal to LHb [dorsal hippocampus (Hi)] on the tail flick latency (TFL) in conscious rats.

Male Sprague Dawley rats, 450-520 grams, were surgically implanted with a 27-gauge guide cannula 10 days prior to testing. The rats were habituated to the restrainers used in testing. Baseline TFL values were recorded prior to injection with a 0.5 ul volume of either 20, 50, or 100 ng AVP. TFL were tested at 3, 6, 10, 15, 20, 25, 30, 35, and 40 min post-injection (PI). Correct placement of the injections was confirmed by histological examination of dye-injected brain sections. During our pilot studies, we observed these AVP-induced behaviors: circling, barrel rotations, scratching, partial flaccidity and hypnotic staring as well as potential seizure indices (piano playing and wet dog shakes). The rats were placed in an observation area at 5, 10, 20, 40 min. PI for behavioral observation.

Our preliminary results have indicated no dose dependency for analgesia or behavior. No analgesia or abnormal behavior occurred in rats injected with saline. Of the behaviors noted, the clearest differences between groups were for analgesia and "seizure". 5 of 12 LHb rats showed both "seizure" and 100% analgesia (TFL = 8.0 s). 1 of 5 Hi rats showed "seizure"; 0 of 5 showed analgesia.

These data suggest a potential interrelationship between analgesia, "seizure" and hypertension elicited from the LHb. While we have not yet tested whether AVP can elicit hypertension from the Hi, the data to date strongly indicate that neither analgesia nor "seizure" are readily elicited from this region. In contrast, AVP clearly can induce analgesia, "seizure" and hypertension when injected into the LHb. Whether AVP-induced "seizure" and/or hypertension from the LHb cause, or are simply correlated with, analgesia is yet to be determined.

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272.8 DEVELOPMENT OF TOLERANCE FOLLOWING INTRATHECAL (IT) INJECTIONS OF ARGININE VASOPRESSIN (VAS). C.L. Thurston, R.J. Jones*, E.S. Culhane*, E. Carstens, and L.R. Watkins, Dept. Animal Physiology, Univ. of California, Davis, CA 95616

IT VAS produces analgesia, scratching bouts, and motor suppression. Repeated injections of VAS have been shown to result in tolerance to the scratching and motor effects (Long, J.B., et al., Neurosci. Abs. 12:1496, 1986). The purpose of the present study was to determine if rats develop tolerance to VAS-induced analgesia and, if so, to compare the time course of tolerance development to the analgesic, scratching, and motor effects of IT VAS.

Nineteen male Sprague Dawley rats were implanted with lumbosacral IT catheters. Following habituation to the restrainers used during test sessions, each rat was assigned to a drug group (25 ng VAS or saline) and then subjected to the following protocol on 7 consecutive days. Baseline tail flick latencies (TFL) were measured prior to drug injection. Following IT injection, TFL were measured every 15 min for 90 min on Day 1, and at 5, 15, 60, and 90 min on subsequent days. Motor ability of the rats was assessed from 5-15 and at 30 min post-injection. Throughout the 90 min test time, rats were observed for any signs of abnormal behavior.

On Day 1, 7/11 rats receiving 25 ng VAS reached the 8 sec TFL cutoff throughout the 90 min test; saline produced no significant change in TFL. The 4 nonanalgesic VAS rats were not tested after Day 1. The remaining VAS rats exhibited tolerance to the analgesic effects of VAS on Days 2-5, with the mean TFL across time varying between 4 and 6 sec, as opposed to 8 sec on Day 1. On Days 2-4, the baseline TFL were significantly higher than Day 1 (5 sec compared to 3.5 sec on Day 1). On Days 6 and 7, VAS again produced analgesia with mean TF latencies between 6.2 and 8 sec. Tolerance also developed to the scratching and motor effects of IT VAS. Scratching bouts occurred in 3 rats receiving VAS on Days 1 and 2, but did not occur in any rats on Day 3. However, on subsequent days, one or two rats would show scratching bouts (not always the same rat across days). Motor dysfunction occurred in 6/7 rats receiving VAS on Day 1, with the severity of motor dysfunction decreasing on subsequent days. Two rats developed barrel rotations with repeated injections.

This study shows that tolerance develops to repeated IT injections of VAS, but this tolerance reverses after a few days. This suggests the possibility that non-opiate analgesics such as VAS may be used for long term pain management without the problem of tolerance that accompanies opiate analgesics. The tolerance to analgesia, motor suppression, and scratching bouts follow different time courses showing a dissociation of these effects.

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272.9 **DECEREBRATION BLOCKS THE ANALGESIA OBSERVED AFTER VERY BRIEF BUT NOT LONG SHOCKS.** M. W. Meagher and J. W. Grau. Dept. of Psychology, UNC, Chapel Hill, NC 27514 and Dept. of Psychology, Texas A & M University, College Station, Texas 77843.

We have suggested that the memory of an aversive event may activate the brainstem systems which modulate the flow of nociceptive information at the level of the spinal cord (Grau, J. W., Beh. Neurosci., 101: 272, 1987). Supporting this hypothessis, we have shown that factors which should speed the decay of the memory also speed the decay of analgesia. On the basis of this behavioral evidence we have suggested that forebrain systems may mediate the activation of the analgesic systems when an organism is exposed to mild aversive events. By contrast, others (e.g. Watkins, L. R. et al., Brain Rsch., 276:317, 1983) have suggested that the analgesic systems may be directly activated at the level of the brainstem and that forebrain systems are not essetial. Supporting this notion, they have shown that decerebration has little impact on the magnitude of shock induced analgesia. However, the duration of shock exposure employed in these studies (90 sec) is considerably longer than the shock duration (2.25 sec) we typically use. Thus, it is possible that forebrain systems do mediate the analgesia observed after very brief shocks, whereas long shocks may directly activate the brainstem analgesic systems. The present experiemt tests this hypothesis.

The subjects were 48 male Sprague-Dawley rats (100-120 days old). One-half of the subjects experienced sham surgery under Pentothal anaesthesia. The other half received mid-collicular decerebrations. Eight to ten hr later the subjects were placed in restraining tubes and allowed to acclimate for 15 min. Baseline pain reactivity was then assessed. One third of the subjects then recieved 3 very brief (0.75 sec) 1.0 mA shocks spaced 20 sec apart. Another third received 3 long (25 sec) 1.0 mA shocks spaced 20 sec apart. The remaining subjects served as unshocked controls. Five tail-flick tests were then administered at 2 min intervals.

No significant differences existed between the groups prior to shock exposure. Both the very brief shocks and the long shocks induced significant analgesia in the sham controls. However, the very brief shocks did not induce analgesia in decerebrate subjects. By contrast, exposure to long shocks did induce a strong analgesia. In fact, exposure to long shocks induced a greater analgesia in decerebrate subjects than it did in the sham controls.

This study shows that forebrain systems mediate the activation of the analgesic systems when subjects are exposed to very brief shocks. It also shows that long shocks are capable of directly activating the brainstem analgesic systems. The fact that decerebration potentiated this analgesia suggests that forebrain systems may actually inhibit the direct activation of the analgesic systems by long shocks.

272.10 **LESIONS OF THE FRONTAL CORTEX ATTENUATE THE ANALGESIA OBSERVED AFTER VERY BRIEF BUT NOT LONG SHOCKS.** J. W. Grau and M. W. Meagher. Dept. of Psychology, Texas A & M University, College Station, Texas, 77843 and Dept. of Psychology, University of North Carolina, Chapel Hill, NC 27514.

Considerable evidence exists that neural systems in the brainstem can modulate the flow of nociceptive information at the level of the spinal cord. Elsewhere we investigated whether neural systems in the forebrain play a role in activating these brainstem systems (see the abstract by M. W. Meagher and J. W. Grau). We showed that decerebration blocks the analgesia observed after very brief shocks, which suggests that forebrain systems mediate the activation of the analgesic systems in this situation. By contrast, decerebration potentiated the analgesia observed after long shocks. This suggests that long shocks can also directly activate the analgesic systems in the brainstem, and if anything, neural systems in the forebrain may inhibit the direct activation of the analgesic systems by long shock. The present study assesses whether lesions of the frontal cortex would have a similar impact on the analgesia observed after very brief or long shocks.

The subjects were 48 male Sprague-Dawley rats (100-120 days old). One-half of the subjects experienced sham surgery under Pentothal anaesthesia. The other half received lesions of the frontal cortex. The subjects were then tested 24 to 30 hr later. The subjects were placed in restraining tubes and allowed to acclimate for 15 min. Baseline pain reactivity was then assessed with the tail-flick test. One third of the subjects then received 3 very brief (0.75 sec) 1.0 mA shocks spaced 20 sec apart. Another third received 3 long (25 sec) 1.0 mA shocks spaced 20 sec apart. The remaining subjects served as unshocked controls. Five tail-flick tests were then administed at 2 min intervals.

No significant differences existed between the groups prior to shock exposure. Both the brief and the long shocks induced significant analgesia in the sham controls. In lesioned subjects exposure to brief shocks did not induce analgesia. By contrast, exposure to long shocks induced a strong analgesia. However, unlike decerebration, lesions of the frontal cortex did not potentiate this analgesia.

These findings suggest that neural systems within the frontal cortex play a critical role in mediating the analgesia observed after very brief shocks. This finding is of particular interest since others have shown that electrical stimulation of cells in this region can elicit analgesia on the tail-flick test (e.g. Hardy, S. G. P., Brain Rsch, 339:281, 1985). The fact that lesions of the frontal cortex did not affect the analgesia observed after long shocks suggests that neural systems in this region may not play a role in modulating the direct activation of the analgesic systems.

272.11 SACCHARIN INTAKE DIFFERENTIALLY CORRELATES WITH THE ANALGESIC STATES ELICITED BY TWO FOOTSHOCK PARAMETERS. J.T. Cannon, K.H. Gunn*, B.J. Cannon*[1], B.L. Thomas* and J.P. Utz*. Depts. of Psychology, University of Scranton, Scranton, PA 18510 and [1]SUNY Binghamton, Binghamton, NY 13901

Increasing the severity of relatively brief stressors can change an opioid form of stress-induced analgesia (SIA) into one that is nonopioid (Terman et al., 1984). For example, 3 min of 3.0mA pulsed footshock produces SIA that is diminished by naloxone, whereas, SIA elicited by 3 min of 3.5mA is naloxone insensitive (Missar and Cannon, 1986).

Opioids appear to be involved in the hedonic qualities of several motivational states, among them the response to sweets (e.g., Lieblich et al., 1983). Previously, we observed a negative relationship (r = -.77) between consumption of a 3mM saccharin solution and the analgesic response to morphine (2.5mg/kg) that appeared to depend upon individual differences across subjects, altering both preference for saccharin and the analgesic effectiveness of morphine (Gogas et al., 1985).

Here we examined the relationships between saccharin intake and analgesia elicited by footshock parameters that previously have been found to generate either naloxone sensitive or naloxone insensitive forms of SIA. We expected to find a significant negative correlation between saccharin intake and, at least, the naloxone sensitive form of SIA.

Thirty adult, male, albino rats (350-450g) were studied during the dark phase of a 12-12 hr light-dark cycle. The animals were exposed to pulsed footshock (1 sec pulse every 5 sec) for 3 min at 3.0 or 3.5mA in a counterbalanced fashion on two consecutive days. Tail-flick latencies were determined at 1 min intervals for 5 min preceding footshock and for 10 min following footshock. Vocalizations emitted between shock pulses were recorded for all animals. On the third day after stress, saccharin intake was determined by providing the animals with a 3mM saccharin solution as their only fluid source for 24 hr.

ANOVAs on tail-flick latencies and vocalizations revealed significant effects of repeated testing and significant interactions between test day and footshock intensity. Consequently, correlations between saccharin intake and either tail-flick latencies or vocalizations (none of the latter were significant) were performed separately for each stress day.

As predicted, saccharin intake and post-3.0mA tail-flick latencies exhibited a significant negative correlation in previously unstressed animals (r = -.84). In contrast, a significantly different correlation existed between saccharin and post-3.5mA tail-flick (r = .19). Surprisingly, saccharin intake and post-3.0mA tail flick latencies showed a significant positive correlation on the second stress day (r = .57). Once again, the correlation between saccharin and post-3.5mA tail-flick latencies was significantly different (r = -.44).

These findings, once again, suggest a linkage between the hedonic value of a sweet substance and opioid analgesic mechanisms. In some situations, a relationship also may exist between saccharin intake and nonopioid SIA, however, this relationship appears to be independent from the preceding.

272.12 HISTAMINE H_2-RECEPTOR MEDIATED FOOTSHOCK-INDUCED ANALGESIA (FSIA) IS DEPENDENT ON NEITHER PITUITARY NOR ADRENAL ACTIVATION. K. R. GOGAS AND LINDSAY B. HOUGH. DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, ALBANY MEDICAL COLLEGE, ALBANY, NY 12209.

Evidence suggests that the putative neurotransmitter, histamine, is involved in the mediation of non-opiate FSIA. We previously showed that H_2-receptor antagonists, such as cimetidine and ranitidine, but not the opiate antagonist, naloxone, significantly inhibit the analgesia elicited by exposure to 3 min of 3.5 mA FS (e.g., Gogas et al, Brain Res., 370:370, 1986). More recently, we found that the brain-penetrating H_2-receptor antagonist, zolantidine also significantly antagonizes this FSIA (Gogas and Hough, submitted). In order to determine whether stimulation of the pituitary-adrenal cortical axis is necessary for activation of this pain inhibitory system, we have determined the effect of adrenalectomy and hypophysectomy on H_2-receptor mediated FSIA.

Hypophysectomy and adrenalectomy were performed by the breeder (Taconic Farms) on male Sprague-Dawley rats (150-175 g). Sham operated animals served as controls. All animals were housed 2 to a cage at 27.5 °C with free access to food and water. Dextrose (5%) and NaCl (1%) served as water for hypophysectomized and adrenalectomized animals, respectively. Pain sensitivity was assessed using a modified version of the D'Amour tail-flick test, with a 7 sec upper limit of exposure. Three hrs into their dark cycle, animals received blinded injections of either naloxone HCL (10 mg/kg, i.p.), zolantidine dimaleate (5 mg/kg, s.c.) or saline vehicle. The intervals between injection and the first post-stress test were 10 and 30 min for naloxone and zolantidine, respectively. Following injection, animals were tested for baseline pain sensitivity, exposed to 3 min of 3.5mA FS, and tested for 15 min post-stress as previously described (Gogas et al, Brain Res., 370:370, 1986). Difference scores (obtained by subtracting each animal's mean baseline from its post-stress latencies) were analyzed by 3-way repeated measures ANOVA.

Neither adrenalectomy nor hypophysectomy had any effect on baseline or FSIA when compared to sham controls. Zolantidine significantly attenuated the FSIA in both the surgery and sham treated groups ($p < .0001$), indicating that the pharmacological nature of the response was unaffected by the surgical treatments. Likewise, naloxone had no effect on FSIA in any of the groups tested, showing that the naloxone-insensitivity of the FSIA was maintained following surgery. The present results show that neither hypophyseal nor adrenal mechanisms are necessary for the expression of the H_2-receptor mediated FSIA, and imply that the response is mediated by neural and not hormonal pathways. (Supported by DA-03816 and a grant from Sterling Winthrop Research Institute).

272.13 RESPONSES OF RVM NEURONS TO NOXIOUS STIMULI ARE BLOCKED BY FOOT-SHOCK STRESS. M.W. Friederich and J.M. Walker, Department of Psychology, Brown University, Providence, RI 02912.

It is now well established that several distinct endogenous pain inhibitory systems exist within the brain and spinal cord. These systems can be activated by noxious and physically or psychologically stressful stimuli. Much evidence suggests that brainstem structures in the rostral ventral medulla (RVM), especially the serotonin containing nucleus raphe magnus (NRM), are part of the descending pain control system that mediates environmentally induced analgesias. Most of the evidence for the involvement of general anatomical areas in stress analgesia has come from lesion studies. The effects of these stressors on neurons that respond to noxious input are unknown.

Previous work has demonstrated that some types of stress analgesia can be induced in anesthetized rats. We have used anesthetized rats to study the functional role of RVM neurons in stress analgesia through extracellular unit recordings. The change in rate of electrical discharge of neurons in the RVM, particularly in the NRM, was recorded in response to noxious mechanical pressure (2.8 kg/cm^2) to the tail before, and after, exposure to a stressor (5 or 7 mA of electrical current to the hindlimb). Recording sites were histologically verified to be within the RVM.

Our findings indicate that, for the majority of cells, exposure to the stressor for two minutes temporarily blocks the neuron's responses to the noxious stimulus. More specifically, neurons that evidence a decreased rate of discharge in response to noxious mechanical pressure (off-cells) tend to be disinhibited after two minutes of stress. Neurons that are excited by noxious mechanical pressure (on-cells) tend to be inhibited following exposure to the stressor. In some cells, this effect can be replicated by administration of morphine (i.v., 4mg/kg). Naloxone administration (i.v., 1 mg/kg or less) reverses this morphine effect. In other cells, in which stress is effective in blocking the response to pain, morphine (i.v., 4 mg/kg) remains without effect. These latter cells appear to be part of a non-opioid analgesia system. Whether naloxone or naltrexone can block the effect of stress on these neurons is currently under investigation to clearly determine the opioid or non-opioid involvement in this system.

272.14 DIFFERENTIAL EFFECTS OF INTRATHECAL ADMINISTRATION OF PHENTOLAMINE AND METHYSERGIDE ON ANALGESIA INDUCED BY CONTINUOUS VERSUS INTERMITTENT COLD WATER SWIM IN THE RAT. J.Rochford* and J.L.Henry Dept. of Psychiatry, McGill Univ., Montreal, Quebec, H3A 1A1

Continuous cold water swim elicits analgesia that is partly mediated by noradrenaline, but is independent of both serotonin and endogenous opioid peptides. In contrast, intermittent cold water swim elicits analgesia that is partly mediated by an opioid mechanism. Because the contribution of the monoamines in intermittent cold water swim analgesia is not known, the present study was conducted to determine whether the analgesia elicited by intermittent cold water swim is dependent upon noradrenaline and/or serotonin. Male Sprague-Dawley rats (225-250 g) were implanted under anaesthesia with chronic intrathecal catheters (Intramedic PE-10 tubing) via the atlanto-occipital junction so that the inner tip lay at the fifth lumbar vertebral level. Two weeks after surgery the rats were randomly divided into 6 groups (N=8 per group). Following determination of baseline pain sensitivity using the tail flick test (3 trials at 5 min intervals), two groups received intrathecal administration of artificial cerebrospinal fluid (CSF; 20 µL), two received phentolamine mesylate (30 µg in 10 µL CSF followed by 10 µL CSF to flush the catheter) and two received methysergide bimaleate (30 µg). Fifteen minutes later, one group within each drug condition was subjected to continuous cold water swim (3.5 min in 4° C water). The other group received intermittent cold water swim (10 s in, 10 s out for 6 min). Tail flick latency was determined 30 and 60 min after the swim. In CSF-treated animals, continuous cold water swim elevated tail flick latencies to 29.76% (SEM = 7.43) of the maximum possible effect (MPE). Intrathecal phentolamine produced a nearly complete blockade of continuous cold water swim analgesia (MPE = 5.53% ± 3.33, $p < .05$), but methysergide was without effect (25.37% ± 3.15, $p > .05$). In contrast to continuous cold water swim analgesia, intermittent cold water swim analgesia was more pronounced (MPE in CSF-treated animals = 67.06% ± 8.84), and was significantly attenuated by both phentolamine (34.36% ± 7.91) and methysergide (20.72% ± 4.31; both ps < .05). The difference between the phentolamine and methysergide groups was not significant ($p > .05$). These data suggest that noradrenaline is involved in both continuous cold water swim and intermittent cold water swim analgesia, whereas serotonin is involved only in the analgesia elicited by intermittent cold water swim.

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272.15 ENHANCEMENT OF SYMPATHETICALLY EVOKED ACTIVITY IN SENSITIZED WDR NEURONS IS DEPENDENT ON CONDITIONING STIMULUS MODALITY. W.J. Roberts, M.E. Foglesong* & R.C. Kramis*. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, Oregon 97209, USA.

Earlier, we hypothesized that a persistent sensitization of spinal wide-dynamic-range (WDR) neurons subsequent to noxious input is the essential dysfunction in sympathetically maintained pains such as causalgia and reflex sympathetic dystrophy (Pain 24:297-311, 1986). The present study was designed to test this hypothesis by determining whether noxious stimulation results in a persistent enhancement of the responsiveness of WDR neurons to sympathetically evoked afferent activity in anesthetized cats.

We recorded from single WDR neurons with cutaneous receptive fields on the hindlimb. The responsiveness of these neurons to afferent input was assessed with two types of test stimuli: non-noxious pressure applied to the receptive field; and electrical stimulation of the sympathetic trunk. Two types of noxious conditioning stimuli were used to sensitize WDR neurons: pinching small folds of skin with serrated forceps; or heating 1 sq cm of skin to 50°C for 10 sec.

In preliminary experiments, we have found that noxious pinch increased the responsiveness of WDR neurons to non-noxious pressure, but it did not increase their responsiveness to sympathetically evoked afferent activity. In contrast, noxious heat enhanced their responsiveness to sympathetically evoked afferent activity but not to non-noxious pressure. The increases in responsiveness persisted well beyond the period of discharge of nociceptors activated by the conditioning stimuli.

The finding that noxious pinch has different effects than noxious heat on the sensitization of WDR neurons to afferent input suggests that multiple mechanisms are involved.

272.16 MIDBRAIN SUPPRESSION OF SIMULTANEOUSLY RECORDED LUMBAR DORSAL HORN NEURON (DHN) AND BICEPS FEMORIS MOTOR UNIT (MU) RESPONSES DURING THE HINDLIMB FLEXION WITHDRAWAL REFLEX (WR). E. Carstens, Dept. of Animal Physiology, Univ. of California, Davis, CA 95616.

Stimulation in midbrain periaqueductal gray (PAG) or lateral reticular formation (LRF) suppresses DHN responses to noxious hindfoot heating in deeply anesthetized rats; this suppression is phasic (restricted to the midbrain stimulation period). In contrast, initial PAG or LRF stimulation frequently suppresses WR and MU responses for prolonged periods in lightly anesthetized rats. This study addressed whether there are different time courses for PAG or LRF suppression of DHN and MU/WR responses to noxious heat recorded simultaneously in the same rat.

In pentobarbital-anesthetized rats, the lumbar spinal cord was exposed to record single DHNs with a tungsten microelectrode, the left biceps femoris was exposed to record single muscle fiber (ie., MU) action potentials using a single-fiber EMG electrode, and the left hindfoot was attached to a force transducer to record isometric withdrawal force. WR force and DHN and MU responses to noxious heat (50-54 deg. C, 10 s duration, 2 min intervals) on the volar hindfoot surface were recorded simultaneously.

To date, 43 paired DHN and MU recordings were made in 15 rats. Mean latencies to onset and peak of response were shorter for DHNs (1.9 and 4.6 s, respectively) than MUs (3.5 and 5.9 s). Mean response magnitude was higher for DHNs than MUs (301 vs. 247 impulses/10 s). DHN were larger than corresponding MU responses in 19 cases, the reverse was seen in 12, and 12 were about equal. MU spikes never followed those of the DHN in a 1:1 correspondence.

In 7/14 rats, WR and MU responses were markedly suppressed for prolonged periods (5 to >40 min) following stereotaxic placement of PAG and LRF stimulating electrodes; DHN responses were stable before and after electrode placement in each case. Neither MU nor DHN responses were affected by electrode placement in the other 7 rats. Initial PAG or LRF stimulation (3 100 ms, 100 Hz trains/s, 25-400 uA) induced a prolonged suppression of WR and MU but only phasic suppression of DHN responses in 4 rats. Only phasic suppression of WRs, MUs and DHNs was seen in 8 rats. Phasic suppression of DHNs and MUs was recruited at lower current intensities by LRF compared to PAG stimulation. Equal PAG or LRF stimulation more effectively suppressed responses of MNs than DHNs in 11/15 and 4/10 cases, respectively. MN and DHN responses could also be dissociated by (1) habituation (12/42 cases) of MU but not DHN responses to the initial 2-4 heat stimuli, and (b) abolition of MU but not DHN responses following supplemental pentobarbital.

These results indicate that MUs contributing to the WR are more susceptible to prolonged suppressive effects than are DHNs (some of which might serve as interneurons in the WR pathway).

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273.1 PEPTIDE-CONTAINING NEURONS IN THE ENTERIC NERVOUS SYSTEM OF PRIMATES. K. Anderson* and C. Sternini (SPON: D. Aures-Fisher). Dept. of Medicine and Center for Ulcer Research and Education, UCLA School of Medicine, Los Angeles, CA 90024.

Numerous biologically active peptides have been reported to innervate the enteric nervous system (ENS) of mammals. The aim of this study was to examine the distribution of peptide containing neurons in the ENS of the Macaca monkey, using rabbit antisera directed to [Tyr] rat calcitonin gene-related peptide 23-37 (CGRP), neuropeptide Y (NPY; provided by Dr. McDonald), vasoactive intestinal peptide (VIP; provided by Dr. Walsh), galanin (GAL; from Peninsula) and a rat monoclonal antibody to substance P (SP; from Pel-Freeze). Specimens of the gut were fixed with a paraformaldehyde-picric acid solution and processed as whole mount preparations or cryostat sections for immunohistochemistry using either immunofluorescence or avidin-biotin methods. Peptide-containing nerve fibers were present in ENS along the entire length of the gastrointestinal tract with a differential distribution in the different layers. The muscle coat, particularly the circular layer, harbored numerous SP, VIP and GAL immunoreactive fibers, a smaller number of CGRP positive processes and only a few NPY fibers. CGRP axons in the smooth muscle were more numerous in the pyloric region than in other areas of the gut. A great number of peptide (particularly CGRP, SP and VIP) immunoreactive fibers was associated with the myenteric plexus. In the submucosa, peptide-containing processes were mainly found in the ganglia and associated with the vasculature. Blood vessels received a rich supply of CGRP, NPY and SP fibers, whereas the number of perivascular VIP and GAL processes was much lower. In the mucosa, SP innervation was the most abundant, especially in the intestine, where SP fibers surrounded the glands, often running close to the lumen. VIP mucosal fibers were also numerous, with a similar distribution as those immunoreactive for SP. CGRP and GAL processes were usually restricted to the base of the mucosa, being more numerous in the intestine than in the stomach. In some cryostat preparations, CGRP fibers were also observed running parallel to the duodenal villi. Only a few, thin NPY fibers were visualized in the mucosal layer. Ganglion cells positive for VIP, GAL and SP were observed in the myenteric plexus of the stomach and intestine as well as in the submucosal plexus of the intestine. CGRP ganglion cells were restricted to the intestinal plexuses. No positive NPY somata were observed. Co-localization studies have demonstrated that the majority of CGRP fibers in the enteric ganglia also contained SP immunoreactivity.

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273.2 N-ACETYLASPARTYLGLUTAMATE IMMUNOREACTIVITY IN NEURONS OF THE MONKEY'S VISUAL PATHWAY. S.B. Tieman, C.R. Hamilton, B.A. Vermeire, M.A.A. Namboodiri and J.H. Neale. Dept. of Biol. Sci., State Univ. of New York, Albany, NY 12222, Div. of Biol., Calif. Inst. of Technol., Pasadena, CA 91125, and Dept. of Biol., Georgetown Univ., Washington, D.C. 20057.

The neurotransmitters used by the major projection neurons of the mammalian visual system remain unknown, although there is some evidence implicating receptors for the acidic amino acids, aspartate and glutamate. N-acetylaspartylglutamate (NAAG) is an endogenous brain peptide thought to act at an acidic amino acid receptor. We have previously identified NAAG-like immunoreactivity in retinal ganglion cells and lateral geniculate neurons in both rat and cat, and in visual cortical neurons in the cat. We now report the immunohistochemical localization of NAAG in the visual pathway of the monkey. Frozen sections through the retina, lateral geniculate nucleus (LGN), superior colliculus and visual cortex of one adult female Macaca mulatta (approx. 10 yrs old), who had undergone midsagittal section of the optic chiasm at the age of 4 yrs, were reacted for the presence of NAAG. In temporal, but not nasal retina (where the ganglion cells had degenerated because they were axotomized by the optic chiasm section), we observed staining of retinal ganglion cells, their dendrites in the inner nuclear layer, and their axons in the optic nerve fiber layer. In both temporal and nasal retina we also observed stained amacrine cells, including some displaced amacrines. We also saw staining in the target regions of the retinal ganglion cells, the superior colliculus and the LGN, where both neuropil and cell bodies were stained. In LGN, staining was confined to layers 2, 3, and 5, that is, to the layers innervated by the intact ipsilateral pathway. Immunoreactivity was also seen in the pyramidal cells of layer V, upper layer II, and lower layer III of occipital cortex (including areas 17 and 18). This staining was not observed when normal rabbit serum was substituted for NAAG antiserum, or when the antiserum was blocked by preabsorption with NAAG coupled to bovine serum albumin (BSA). In retina and cortex, NAAG-BSA was more effective than conjugates of BSA with either N-acetylaspartate or aspartylglutamate, whereas in LGN, AG-BSA blocked staining of the cells as effectively as did NAAG-BSA. Thus, in retina and cortex, it is likely that the antisera are recognizing NAAG, whereas, in LGN, they may be recognizing some other, related peptide. These results are very similar to those in cat, and demonstrate the presence of NAAG and/or related compounds in neurons of the monkey's visual pathway. A determination of the role that these compounds play in mammalian visual neurotransmission must await further study. Supported by NSF grant #BNS 8217479 to SBT, NIMH grant #MH 34770 to CRH, and NIDA grant #DA 02297 to JHN.

273.3 SUBSTANCE P-LIKE IMMUNOREACTIVITY IN SUPERFICIAL LAYERS OF CHICK OPTIC TECTUM: DEPENDENCE UPON RETINAL INPUT. W.J. Crossland, G.R. Ten Eyck,* D.J. Goebel* and R.H. Granda* (SPON: R. Pourcho) Dept. of Anatomy and Cell Biology, Wayne State Univ. Schl. Med., Detroit, MI 48201.

Substance P-like immunoreactivity (SPLI) has been reported in retinal ganglion cells and retinal terminals in mammals and amphibians as well as in several layers of the avian optic tectum. We undertook this study to determine if substance P might be present in the retinofugal projection of the chick by observing the distribution of SPLI in normal chicks and those which had undergone unilateral eye removal at hatching.

Newly hatched chicks were unilaterally enucleated under deep halothane anesthesia. After eight weeks survival two enucleated and two normal chicks were perfused transcardially with phosphate-buffered paraformaldehyde. Two other enucleated chicks were perfused with 0.1% glutaraldehyde added to the fix. Frozen sections of the brains and retinae were reacted with a monoclonal antibody to Substance P (Pel Freez) then reacted with PAP (Polysciences). Sections fixed with glutaraldehyde were embedded in epon for electron microscopy.

The brain sections contained many regions of intense SPLI in fibers although relatively little of it was associated with the visual centers, optic nerve, chiasm or optic tract. However, a prominent band of SPLI was clearly demonstrated in sublaminae a-c of the stratum griseum et fibrosum superficiale of the optic tectum as well as a dense network of SPLI fibers in the deeper tectal layers. The superficial band was most dense dorsomedially and least dense lateroventrally. In retinal sections some SPLI cell bodies were found in the retinal ganglion cell layer. Whether these are ganglion cells or displaced amacrine cells (many cells in the inner plexiform layer also reacted) is yet to be determined. Electron microscopic observation of the superficial SPLI-containing layer in the tectum revealed densely labeled profiles of synaptic terminals contacting small dendrites or spines.

After contralateral eye removal the SPLI in the superficial tectal laminae completely disappeared although staining in the deeper layers was not affected.

The dramatic alteration of SPLI in the superfical tectum suggests that the SPLI is either contained in retinal terminals or that the production of the peptide is closely regulated by the retinal afferents.

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273.4 A CRF SYSTEM IN THE RAT RETINA: DISTRIBUTION AND EFFECT ON RETINAL NEURONS. Hermes H. Yeh and John A. Olschowka, Dept. Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

The demonstration of the presence of peptides in the mammalian retina has presented a unique challenge to research in CNS peptidergic systems. Whereas their localization in discrete retinal cell types favors a neurotransmitter role in the retina, their neuronal effects remain largely unexplored. Here, we present an immunohistochemical demonstration of a system of corticotropin releasing factor(CRF)-containing cells in the rat retina and, based on their anatomical disposition, report results of an ongoing electrophysiological study to elucidate the effect of CRF on cholinergic retinal neurons.

Retinal wholemounts, prepared from adult female Long Evans rats anesthesized with chlorpent and perfused intra-aortically with 4% paraformaldehyde, were incubated in rabbit-anti-CRF in the presence of Triton X-100 and visualized using the unlabeled peroxidase-anti-peroxidase procedure. One wholemount from each animal was subsequently embedded in epon to obtain 2- to 10-um transverse sections. Serial sections of the entire retina were obtained from 2 epon-embedded specimen.

Wholemount preparations revealed that the CRF-labeled cells could be found in two separate layers. The great majority of them were situated in the ganglion cell layer. These cells were predominantly displaced amacrine cells based on their soma diameter (8-12 um), their overall morphology, and on one preliminary double labeling experiment which indicated that the distribution pattern of ganglion cells, retrogradely labeled with fluorogold, did not overlap with that of the CRF-immunopositive cells in the ganglion cell layer. A small portion of the CRF-labeled cells were amacrine cells found in the vitreal aspect of the inner nuclear layer. Seen to better advantage in transverse sections, processes of CRF-labeled cells from both layers elaborated densely within sublamina 4 and 5 of the inner plexiform layer. Some amacrine cells in the inner nuclear layer also emitted a thin process which ramified within sublamina 1 and, on rare occasions, processes emanating from some cells in the ganglion cell layer were found to ascend across the inner plexiform layer toward sublamina 1.

We are using a cell culture system to assess electrophysiologically the effects of CRF on cholinergic retinal neurons. This experimental system permits continuous monitoring of neuronal release of acetylcholine. Our results indicate that CRF (0.1-1.0 um), dissolved in recording medium and ejected by pressure near cholinergic retinal neurons, had a potent effect on evoking acetylcholine release. Typically, when compared to that of pressure-ejected glutamate, the excitatory response to CRF had a longer onset and response duration. Most of the cholinergic neurons examined in this culture system were responsive to micropressure-ejected CRF.

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273.5 ULTRASTRUCTURE OF SOMATOSTATIN IMMUNOREACTIVE CELLS IN THE DEVELOPING VISUAL CORTEX OF THE RAT. B.W. Bakkum*, R.S. Cohen and L.A. Benevento. Dept. of Anatomy, Univ. of Illinois at Chicago, Chicago, IL 60612.

Somatostatin (SRIF) has been implicated as a trophic factor. Light microscopic studies have shown that somatostatin-immunoreactive cells (SRIF-cells) are present in the visual cortex of the rat as early as embryonic day 20 (Laemle et al., 1982). The number of postnatal SRIF-cells progressively increases in layers V and VI until postnatal day 7 (PND=7) at which time SRIF-cells in the superficial layers begin to appear. Up to PND=13, SRIF-cells in layer V and VI remain constant in number or may even decline. In order to add to the knowledge of the cytological development of the cortex, we monitored the development of this dynamic population of SRIF-cells in areas 17, 18 and 18a of the early postnatal rat. Rats (PND=4, 7, 10 and 13) were anesthetized and perfused with 5% acrolein, 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.4 and the brains were removed immediately and placed in a similar solution, omitting the acrolein, overnight at 4°C. Vibratome sections (40 µm) were processed with Immunonuclear antibody #20H2T and the Avidin/Biotin immunoperoxidase procedure. Tissue pieces from the visual cortex containing SRIF-cells were then postfixed in osmium tetroxide and embedded in Epon according to standard procedures. At PND=4 and 7, when SRIF-cells are increasingly present in layers V and VI, SRIF-immunoreactivity was ubiquitously distributed throughout the cytoplasm. It surrounded, but was not contained within the cisternae of rough endoplasmic reticulum. At this stage, developing axosomatic synaptic profiles appeared immature and it was not possible to classify them into specific synaptic types. The pre- and postsynaptic densities appeared symmetrical with little development of the postsynaptic density. Synaptic vesicles were sometimes present in the presynaptic endings. At PND=10 and 13, (during the time when light microscopy shows SRIF-cell number leveling off or decreasing in layers V and VI and appearing in layer II/III), the synaptic profiles on SRIF-cells in layers V and VI could be differentiated into symmetric and asymmetric types, with the former being the predominant type. The distribution of the reaction product was similar to that seen in the younger cells. From this qualitative data, no correlation could be made as to the relationship of the appearance of SRIF and the initiation of synaptogenesis. Therefore, SRIF-cells in layers V and VI of the visual cortex during the dynamic developmental phase (4-13 days) showed (1) typical morphologic patterns of synaptic development and (2) similar patterns of distribution of SRIF reaction product to those seen in the adult.

273.6 LHRH NEURONAL SYSTEM IN THE ACCESSORY OLFACTORY BULB OF THE PRAIRIE VOLE, MICROTUS OCHROGASTER. R.L. Reger*, A.A. Gerall, C.J. Wysocki, C.S. Carter, Dept. of Psychol., Tulane U., New Orleans, LA 70118, Monell Chem. Senses Ctr., Philadelphia, PA 19104, and Dept. of Zool., U. of Maryland, College Park, MD 20742.

Olfactory stimulation derived from male urine is necessary for estrous behavior and ovulation in the prairie vole (Carter, et al., Biol. Reprod. 23:1038, 1980). These and other reproductive processes are mediated by the vomeronasal-accessory olfactory system (Wysocki, Neurosci & Biobeh. Rev. 3:301, 1979). The present research studied the contribution of the vomeronasal organ (VNO) to the LHRH neurons in the accessory olfactory bulb (AOB) and whether male urine affects them.

Ten virgin females whose VNO had been unilaterally extirpated were exposed to male odor through a wire mesh cage divider for 36 hr. Male and dividers were then removed and 200 µl of either male urine or water was applied to the females' noses. One hr later they were perfused with 1%-4% paraformaldehyde. Their brains were sectioned, and LHRH was visualized by the Sternberger PAP method (a-LHRH H16; S.Vigh, Hungary). Complete VNO elimination was verified by H&E stain. Fiber densities in the AOB were determined using computer-aided image analysis.

Extirpation of the VNO results in degeneration of the glomerular layer of the ipsilateral AOB. LHRH-containing immunoreactive fibers seen looping among the glomeruli and forming contacts within or between them on the intact side are absent on the extirpated side. Male odor present for 36 hr increases LHRH immnoreactive fiber density in the deep layers of the AOB more than 1 hr exposure used in our previous studies. The lesioned side of females receiving male odor for only 36 hr had more LHRH fibers, especially in the deep layers of the AOB, than the intact side. The additional 1 hr of direct urine application non-significantly increased LHRH immunoreactive density on the intact side. Several LHRH-immunoreactive cell bodies were seen in the AOB; of all animals regardless of treatment or side.

Extirpating the VNO diminishes or redirects LHRH from the glomerular region and increases the density of LHRH fibers coursing along the AOB dorsal surface. The increase in LHRH fibers density which must be due to sources other than the VNO might include the Nervus Terminalis or centrifugal fibers. Olfactory cues apparently modify these as well as the vomeronasal system.

273.7 IMMUNOCYTOCHEMISTRY OF NEUROPEPTIDES IN THE CEPHALIC NEUROENDOCRINE SYSTEM OF THE LUBBER GRASSHOPPER, ROMALEA MICROPTERA. C.A. Zahnow*, K.R. Rao, C.J. Mohrherr* and J.P. Riehm*. Dept. of Biology, Univ. of West Florida, Pensacola, FL 32514

Pigment-dispersing hormones (PDHs) are a family of arthropod neuropeptides. Recently a pigment-dispersing factor (PDF), NSEIINSLLGLPKLLNDA-amide, which shows 78% sequence homology with ß-PDH from the crab Uca, has been isolated from lyophilized heads of the lubber grasshopper Romalea microptera (Rao et al., J. Biol. Chem., 262: 2672-2675, 1987).

In the present study we examined the localization of PDH/PDF in the cephalic neuroendocrine system of Romalea, utilizing an antiserum raised against ß-PDH (Dircksen et al., Cell Tissue Res., in press, 1987). Additional immunocytochemical work was carried out utilizing antisera for FMRF-amide (O'Donohue et al., Peptides, 5: 563-568, 1984) and adipokinetic hormone (N-terminal specific antiserum 433; Schooneveld et al., Cell Tissue Res., 243: 9-14, 1986). Immunocytochemistry was done utilizing the PAP method on sections of tissues (supraesophageal ganglia, optic lobes, and corpora cardiaca) embedded in Paraplast.

PDH-positive perikarya were found only in the optic lobes, whereas cell bodies reacting to FMRF-amide antiserum were located in the optic lobes, various parts of the brain, and the glandular lobes of the corpora cardiaca. Immunostaining of successive sections revealed co-localization of FMRF-amide and PDH reactivity in two clusters of somata, located dorsally and ventrally, between the medulla and lamina of the optic lobe. The PDH positive cells situated between the medulla and lobula were distinct from the FMRF-amide reactive cells in this region; the latter were more abundant and more widely distributed. An extensive network of immunoreactive fibers was found in the optic lobes and supraesophageal ganglion. The PDH and FMRF-amide reactivities in these fibers and plexuses were co-localized mainly in the lamina and, to a lesser extent, in the medulla. Marked differences were noted between the PDH-positive and FMRF-amide immunoreactive elements in the supraesophageal ganglion. The corpora cardiaca showed no reactivity to PDH antiserum, although strong staining was evident for AKH and FMRF-amide. The PDH immunoreactivity noted here seems to be attributable to the octadecapeptide PDF of Romalea, as preincubation of antiserum with the latter peptide abolished the staining. The function of PDF in insects and the chemistry of AKH-like and FMRF-amide-like substances in Romalea remain unknown.

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273.8 DETECTION OF VASOPRESSIN MESSENGER RNA IN CELLS WITHIN THE BED NUCLEUS OF THE STRIA TERMINALIS BY IN SITU HYBRIDIZATION HISTOCHEMISTRY. D.M. Dorsa, M.A. Miller*, and R.T. Zoeller, GRECC, VA Medical Center, Seattle, WA 98108 and Laboratory of Cell Biology, NIMH, Bethesda MD 20892

Vasopressin (VP)-immunoreactive neurons have been reported to be present in several extrahypothalamic sites including the medial amygdala and the bed nucleus of the stria terminalis (BNST). Cells in the BNST have been suggested to be the primary source of vasopressin fibers in the lateral septum, habenula, and periventricular grey. Using in situ hybridization techniques, we have confirmed the presence of VP synthesizing cells within the BNST by identifying individual neurons which contain VP mRNA.

Adult male Sprague-Dawley rats were decapitated, their brains frozen, and 12µm sections obtained. In situ hybridization was performed on post-fixed sections using a synthetic 48-base oligonucleotide probe complementary to the VP mRNA encoding the last 16 amino acids of the glycopeptide region. ^{35}S-dATP and terminal deoxynucleotidyl transferase were used to 3'-end label the probe (SA=6000 Ci/mmol). Hybridization was carried out for 20h at 37°C. Tissues were washed in 2X SSC/50% formamide at 40°C. The sections were dehydrated, dipped in NTB3 nuclear emulsion, and exposed for 2 to 3 weeks. After developing and counterstaining, the sections were examined microscopically for evidence of hybridization.

Scattered cells within the BNST were found to be labeled by the DNA probe. Between 10 and 20 cells were visible per section. The cells, located almost exclusively in the medial aspects of the nucleus, were parvocellular in appearance (mean diameter was approximately 10µm). These cells differed clearly in size from magnocellular VP neurons which were labeled in the paraventricular n. Quantitative in situ hybridization methodologies will prove useful in studying the factors which modulate the activity of these extrahypothalamic vasopressin producing neurons.

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273.9 ONTOGENIC STUDY OF THE GENE EXPRESION OF PREPROENKEPHALIN IN THE RAT FOREBRAIN. Y. Morita, H. Kiyama*, K. Noguchi*, A. Wang*, and M. Tohyama*, Dept. of Anatomy and Neuroanatomy, Osaka Univ. Med. Sch., Osaka 530, Japan

Enkephalins (methionine- and leucine- enkephalins: met-ENK and leu-ENK) are endogenous pentapeptides which bind to the same sites as opiates. Biochemical and immunocytochemical studies have demonstrated the extensive but differential distribution of these endogenous opiate-ligands in the central nervous system as well as other organ systems, indicating that these opioid peptides play various roles in different tissues. Recent recombinant DNA technology have revealed that the proenkephalin contains 6 copies of met-ENKs and one copy of leu-ENK. In the present study, the cellular localization of the precursor mRNA of proenkephalin, preproenkephalin (PPE), was first examined in the rat forebrain by means of in situ hybridization histochemistry, and secondly our attempt was directed to the ontogenic changes of the PPE gene expression in the brain tissues. The complementary DNA (cDNA) of the PPE mRNA (generous gift from Dr. K. Yoshikawa et al., JCB., 259:14301, 1984) were labeled with ^{32}P or ^{35}S according to nick translation protocol.

Neurons containing the PPE mRNAs were observed in various cerebral structures; piriform cortex, ventral tenia tecta, nuclus accumbens, pyramidal and polymorph layers of the olfactory tubercle, caudate-putamen nucleus, lateral septum, lateral and posterior bed nuclei of the stria terminalis, diagonal band of Broca, medial and lateral preoptic areas, amygdala complex (central nucleus), anterior hypothalamic nucleus, perifornical area, lateral hypothalamus, premamillary nucleus, medial mamillary nucleus, ventral lateral geniculate nucleus. Among these structures, part(s) of the amygdala complex, central nucleus, exhibit(s) the high level of hybridization signals throughout ontogenic stages. This may provide additional data to understand the function of enkephalins in the central nervous system. While, other cerebral structures containing PPE gene expressing neurons show the gradual increase of the hybridization signals during ontogeny.

273.10 CELLULAR LOCALIZATION OF RAT GAMMA-PREPROTACHYKININ A mRNA IN THE RAT DORSAL ROOT GANGLION AND TRIGEMINAL GANGLION BY IN SITU HYBRIDIZATION HISTOCHEMISTRY. K. Noguchi*, H. Kiyama*, Y. Morita, A. Wang*, and M. Tohyama*. (SPON: E. Semba). Dept. of Anatomy and Neuroanatomy, Osaka Univ. Med. Sch., Osaka 530, Japan

Substance P (SP) is one of the most characterized neuropeptides belonging to the tachykinin family, and regarded as a transmitter candidate involved in the pain sensation in the central and peripheral nervous tissues. It has been demonstrated that substance P and related peptide, substance K (Neurokinin K), are coded on a single gene by sequence analysis. This tachykinin gene produces three types of precursor mRNAs, alpha-, beta-, and gamma-preprotachykinin A (PPTA). Alpha-PPTA mRNA codes for only SP, while although beta- and gamma-PPTA mRNAs have different sizes they yield both SP and SK as final products. The present study uses the complementary DNA (cDNA) of the rat gamma-PPTA mRNA (generous gift from Prof. S. Nakanishi; Kawaguchi et al., BBRC, 139:3, 1986), and demonstrates the cellular localization of PPTA mRNAs in the trigeminal and dorsal root ganglia by means of in situ hybridization histochemistry. The gamma-PPTA cDNAs (-600bp, containing the coding region of SP and SK) were labeled with ^{35}S according to nick translation protocol.

After the autoradiographic exposure from one to two weeks, silver grains were observed on and in the close vicinity of neurons in the trigeminal and dorsal root ganglia. In the two ganglia, labeled neurons are small to medium in size. The population of the gamma-PPTA gene expressing neurons in the trigeminal and dorsal root ganglia are about 6.0% and 11.0% respectively. The size of the gamma-PPTA gene expressing neurons appears to be comparable to the previous data drawn from immunocytochemical studies.

273.11 ONTOGENY OF SUBSTANCE P AND ELEDOISIN BINDING SITES IN RAT BRAIN: A COMPARATIVE AUTORADIOGRAPHIC STUDY. T.V.Dam, E.Escher* and R.Quirion. Douglas Hospital Research Centre and Dept. of Psychiatry, McGill University, Verdun, Québec. H4H 1R3 and Departement de Pharmacologie, Faculté de Medecine, Université de Sherbrooke. Sherbrooke. Québec. J1H 5N4.

The existence of various classes of tachykinin receptors in peripheral tissues and brain has been proposed. We previously reported that the distribution of Substance P (SP)-NK-1 receptor binding sites undergoes major redistribution during postnatal ontogeny. (Quirion and Dam. J. Neurosci. 6:2187-2199, 1986). For example, high densities of [^{125}I]BH-SP binding sites are present in most brain stem nuclei at post-natal days 1 and 4 (P1 and P4) while it is not the case in adults. In the striatum, [^{125}I]BH-SP binding sites are distributed in "patches" early after birth but not in adults. We now report on the comparative ontogenic development of two putative tachykinin receptor classes namely NK-1 and NK-3 using [^{125}I]BH-SP and [^{125}I]BH-Eledoisin (ED) as respective ligands. The development of those two sites has been studied both pre- and post-natally in E3, E1, P1, P6, P14, P28 and P35 days old rats of either sexes. Brain sections were prepared and incubated with 100 pM [^{125}I]BH-SP or 50 pM [^{125}I]BH-ED as described before (Quirion and Dam, ibid). Our data indicate that the distribution of [^{125}I]BH-ED binding sites is discrete and different from that of [^{125}I]BH-SP. The distribution of [^{125}I]BH-ED binding sites also undergoes major modifications during brain development. High densities of sites are present in certain brain stem nuclei at E3 to P6 while it is not the case in older animals. Moreover, dense cortical [^{125}I]BH-ED labelling in layer IV and V does not become apparent until P14. Finally, [^{125}I]BH-ED binding sites are fairly homogeneously distributed in striatum thoughout brain development. These data clearly indicate the differential ontogeny of NK-1 and NK-3 receptora binding sites in rat brain.

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273.12 SUBSTANCE P TERMINALS IN THE RAT CUNEATE NUCLEUS. C.Y. Wen, S. DeBiasi*, S.L. Van Eyck, P. Petrusz* and A. Rustioni. Dept. of Anatomy and Physiology, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

Substance P-positive fibers and terminals have been demonstrated in the cuneate nucleus of rats and cats by light microscopic immunocytochemistry (ICC); Double-labeling experiments have suggested that substance P (SP) may be present in non-primary (post-synaptic) afferents to the dorsal column nuclei (Conti and Rustioni, 1986).

In this work substance P-positive terminals in the cuneate nucleus of rats were investigated by electron microscopy (EM). Both pre- and post-embedding EM ICC were used for this purpose. Rats were perfused with 4% paraformaldehyde and 0.5% to 1% glutaraldehyde. The caudal brainstem was subsequently post-fixed overnight in 4% paraformaldehyde (for pre-embedding procedure) or for five hours in 1% paraformaldehyde (for post-embedding procedure). Forty micrometer-thick sections were cut with a Vibratome and post-fixed in osmium tetroxide. For pre-embedding procedure, sections were pre-treated with alcohol. Antibodies for substance P were prepared in our laboratory (post-embedding) or obtained from Immunonuclear (pre-embedding). For pre-embedding staining peroxidase ICC was used, while in the post-embedding material substance P was revealed by 20 nm colloidal gold particles coated with goat anti-rabbit IgG.

Substance P-positive terminals are mostly of small size, dome-shaped, and contain dense-core vesicles interspersed among round, clear vesicles. They form asymmetric synapses with dendrites of various sizes and are not involved in axo-axonic synapses. In the post-embedding material, gold particles are found predominantly over dense-core vesicles. Given their morphology, substance P-positive terminals are likely to be endings of non-primary afferents to the dorsal column nuclei (Rustioni and Ellis, 1978). This is supported by the sharp reduction in number of substance P-positive terminals in the cuneate nucleus after spinal cord transection but not after dorsal rhizotomy.

Experiments currently in progress aim to verify the origin of these terminals by a double-labeling technique employing the anterograde transport of horseradish peroxidase, revealed by TMB histochemistry and post-embedding ICC for substance P.
Supported by USPHS grants NS 12440 and F05-T203695

273.13 GALANIN-CONTAINING SOMATA IN THE PRIMATE NUCLEUS BASALIS/DIAGONAL BAND COMPLEX. L.C. Walker†, V.E. Koliatsos†, C.A. Kitt†, R.T. Richardson§ and D.L. Price†. †Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; §Neurophysiology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The nucleus basalis/diagonal band complex in primates is characterized by large, basophilic neurons that innervate amygdala, hippocampus, neocortex, and other regions in the central nervous system. Most neurons forming the major subdivisions of this basal forebrain complex are cholinergic [Mesulam et al., J. Comp. Neurol. 214:170-197, 1983]. Furthermore, recent studies in rats have shown that many neurons in the medial septum/diagonal band contain both the neuropeptide, galanin, and choline acetyltransferase (ChAT), whereas cholinergic cells of the nucleus basalis magnocellularis lack galanin [Melander et al., Brain Res. 360:130-138, 1985]. In the owl monkey, a nocturnal New World primate, galanin and acetylcholinesterase coexist in neurons in all parts of the basal forebrain complex [Melander and Staines, Neurosci. Lett. 68:17-22, 1986]. However, the extent to which galanin and specific cholinergic markers, such as ChAT, coexist within neurons of the primate basal forebrain complex is not yet known. To address this issue, colchicine was injected into the lateral ventricle of an anesthetized rhesus monkey (Macaca mulatta) to enable visualization of galanin-containing somata. After 24 hours, the animal was perfused with 4% paraformaldehyde for immunocytochemical analysis. In 40 µm-thick frozen sections, galanin-immunoreactive neurons (mean size approximately 14 µm x 27 µm) were present throughout the rostro-caudal extent of the basal forebrain complex. In adjacent sections, ChAT-immunoreactive neurons of a similar size were present in the same locations as galanin-immunoreactive cells. Many neurons in the basal forebrain complex contained both markers, as demonstrated by double immunostaining with diaminobenzidine and benzidine dihydrochloride. Intense galanin immunoreactivity of cells in the nucleus of the diagonal band could be used to segregate these neurons from large cells of the nucleus accumbens and olfactory tubercle, which stained for ChAT but showed little or no galanin immunoreactivity in this preparation. These results show that, unlike the case in rodents, galanin coexists with ChAT in neurons throughout the nucleus basalis/diagonal band complex of the rhesus monkey. Basal forebrain neurons containing other peptides, such as somatostatin, neuropeptide Y, and leu-enkephalin, were generally small and sparse compared to ChAT- and galanin-containing neurons. The extensive coexistence of galanin and ChAT in neurons of the nucleus basalis/diagonal band complex in primates suggests that galanin could act as a cotransmitter with acetylcholine in areas innervated by these cells.

273.14 DISTRIBUTIONS AND RELATIONSHIPS TO EACH OTHER OF CLUSTERS OF NEUROTENSIN- AND SUBSTANCE P-IMMUNOREACTIVE NEURONS IN THE CAUDATE-PUTAMEN AND VENTRAL STRIATUM OF THE SPRAGUE-DAWLEY RAT IN THE EARLY POSTNATAL PERIOD. K.W. Eggerman*, R.F. Sprung*, D.E. Wesche*, E. Payne* and D.S. Zahm (SPON: R. Walsh). Department of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Neurotensin (NT)-immunoreactive (IR) neurons can be demonstrated in vibratome sections through the striatum in adult S-D rats in the absence of pretreatments, but they are present in greater numbers following exposure of the sections to H_2O_2, and are most numerous 48 hours following ICV injection of colchicine in clusters in n. accumbens and ventromedial caudate-putamen (c-p) (Zahm, 1987; Zahm and Heimer, 1987). They are not observed, however, in the dorsolateral quadrant of the c-p, where the striosomal (Graybiel and Ragsdale, 1983) organization of the striatum is distinct. Evidence from cat suggests that dense NT-IR terminal stain (Goedert et al., 1984) and, perhaps, neuron clusters (Sugimoto and Mizuno, 1987) correspond to striosomes in that species. We sought to determine, using a reported striosomal marker (Gerfen, 1985), substance P (SP)-IR, if striatal NT-IR neurons might be more apparent in striosomes in the immature rat. Anesthetized pups collected on postnatal days 1, 3, 5, 12, and 20 were perfused through the left ventricle first with a saline rinse and then with phosphate buffered aldehydes. Brains were sectioned at 50µm. Adjacent series of sections were treated with sodium borohydride (1%, 15 min.) and appropriate normal sera (2%) prior to incubation in anti-NT (1:3000, Immunonuclear), anti-SP (1:000, Sera), carrier buffer alone or one of the two antibodies preabsorbed with the appropriate antigen. Immunostaining was produced using biotinylated second antibodies (1:200), the ABC kit (Vector), and DAB. Controls resulted in absence of staining. On P1 NT-IR neurons were present in the subiculum, amygdaloid complex, endopiriform region, lateral hypothalamus, the septal area and, in the striatum, only along the caudal dorsolateral margin of the c-p. The latter were very numerous, however, extending ventrally toward ventral striatum by P5. At P12 NT-IR neurons were distributed in conspicuous clusters throughout lateral parts of the c-p and in ventral striatum. Dense patches of SP-IR terminal stain which contained clusters of SP-IR neurons were located more medially in the c-p, however, and where the distribution of NT-IR and SP-IR cell clusters did overlap the positions of NT-IR and SP-IR clusters themselves almost never corresponded. To the contrary, they appeared to be distributed in an inverse or complementary fashion. By P20 the arrangement observed was that of the adult, with NT-IR striatal neurons being few in the absence of section pretreatment. Support: NS-23805 and the American Parkinson Disease Association.

273.15 **THYROTROPIN-RELEASING HORMONE (TRH) IN SUBREGIONS OF THE HIPPOCAMPAL FORMATION: IMMUNOCYTOCHEMICAL LOCALIZATION AND QUANTITATION BY RADIOIMMUNOASSAY (RIA).** M.J. Kubek, J. Roepke, M. Zaphiriou, S.H. Murphy, T.G. Hill, A. Sattin, and W.C. Low, Depts. of Anatomy, Psychiatry, Physiology and Biophysics, and Program in Medical Neurobiology, Indiana University School of Medicine, Indianapolis, IN 46223.

Previous studies from our laboratories have demonstrated that TRH afferents project to the hippocampal formation by way of the fornix pathway (Neurosci. Abs. 11:680). Radioimmunoassay of TRH indicated that the ventral hippocampal formation is more densely innervated by TRH afferents than the dorsal hippocampus. In addition, fornix lesion studies sugggested that intrinsic sources of TRH existed within the hippocampal formation (Neurosci. Abs. 12:300). To determine the source of intrinsic hippocampal TRH, immunocytochemical methods were used to stain TRH-containing neural elements. TRH quantitation in microdissected subregions of the hippocampus was also determined using RIA.

In the immunocytochemical study, rats were perfused with phosphate buffer and lysine-periodate fixative. Hippocampal sections were cut 10 um thick using a cryostat at -20°C and mounted on glass slides. Tissue sections were incubated in primary antisera (1:3200) containing polyclonal antibodies raised in rabbits against BSA-conjugated TRH. Antibody localization was visualized using the peroxidase-anti-peroxidase method. TRH-positive staining was observed in pyramidal cells of regions CA1, CA3, and hilus. Granule cells of the dentate gyrus were also stained along with neurons in the subiculum. TRH-positive staining also displayed a unique laminar pattern: stratum oriens was moderately stained throughout the CA fields while strata radiatum and moleculare were sparsely stained. Within the dentate gyrus, stratum moleculare of the lower blade was more densely stained than the upper blade. Tissue incubated in preimmune sera at the same dilution and from the same rabbit exibited no staining.

In the RIA study, hippocampal subregions were microdissected on an ice-cold wax plate using low power magnification. Tissues were extracted by sonication using 90% methanol. TRH radioimmunoassay revealed that the CA3 region of the hippocampal formation contained the highest amounts of TRH (152 ± 5.8 pg, n = 8), followed by intermediate levels in region CA1 (116 ± 8.8, n = 9) and the dentate gyrus (76.5 ± 7.7, n = 7). The subiculum (38.4 ± 5.6, n = 9) and the hilus (26.6 ± 3.9, n = 7) contained the lowest levels. These TRH levels appear to correlate well with our immunocytochemical findings.

The results from this study suggest that (1) the intrinsic sources of hippocampal TRH appear to be pyramidal and granule cells, and (2) a discrete laminar distribution of TRH exists within subregions of the hippocampal formation. Finally, although the terminal zones of the extrinsic TRH afferents to the hippocampus have yet to be identified, we hypothesize that they will include projections to stratum oriens of cornu ammonis and stratum moleculare of the dentate gyrus.

273.16 THE LOCALIZATION OF SUBSTANCE P, CHOLINE ACETYLTRANSFERASE, LEU-ENKEPHALIN, AND ACETYLCHOLINESTERASE IN THE NORMAL AND DEAFFERENTATED INTERPEDUNCULAR NUCLEUS OF THE RAT. M.D. Kawaja*, B.A.Flumerfelt and A.W. Hrycyshyn*. Dept.of Anatomy, University of Western Ontario, London, Canada N6A 5C1.

The major afferent pathway to the interpeducular nucleus (IPN) arises from the medial habenula (mHb) and projects via the fasciculus retroflexus (FR). The purpose of this study was to examine the distribution of immunohistochemically demonstrable substance P (SP), choline acetyltransferase (ChAT), and leucine-enkephalin (l-Enk) reactivity, and acetylcholinesterase (AChE) in the normal IPN and following lesions of the FR. Neurons of the mHb exhibited moderate to weak AChE activity, SP reactivity dorsally, and ChAT reactivity ventrally. Within the IPN SP reactivity was localized in the lateral subnuclei (IP-L) and in the dorsal cap of the rostral subnucleus (IP-R); l-Enk is found in the central subnucleus (IP-C) and the IP-R; ChAT reactive axons were present in the rostral portion of the IP-R, and further caudally in the IP-C and intermediate subnuclei (IP-I); AChE staining was heaviest in the entire IP-R and in the IP-L. One week following unilateral electrolytic lesions of the FR, a significant decrease in SP reactivity was demonstrated ipsilaterally in the IP-L and in the dorsal cap of the IP-R; ChAT activity declined within the IP-C, IP-I, and in the rostral portion of the IP-R. Bilateral FR lesions substantially reduced SP reactivity in the IPN, while ChAT activity was moderately decreased. A perceptible decrease in AChE staining was observed bilaterally in the IP-L, while activity in the IP-R was unchanged. l-Enk reactivity was not altered following lesions to the FR. IPN afferents arising from the dorsal mHb projected predominantly to the IP-L and possessed both SP and AChE activity, while those axons that arise from the ventral mHb possessed high levels of ChAT activity and projected to the IP-C, IP-I and the rostral IP-R. AChE activity in the IPN appeared to be mainly intrinsic with only a moderate response in IP-L to FR lesions. Also, the distribution of AChE and ChAT activities overlapped the rostral portion of IP-R, while in ChAT-positive IP-C and IP-I AChE staining was weakest. Since l-Enk reactivity was unaffected following FR lesions, a habenular source for the enkephalinergic terminal fields in the IPN is unlikely.
(Supported by the Medical Research Council of Canada)

273.17 ORIGIN OF ATRIAL NATRIURETIC PEPTIDE-CONTAINING FIBERS IN THE RAT INTERPEDUNCULAR NUCLEUS: A COMBINED IMMUNOCYTOCHEMICAL AND FLUORESCENT RETROGRADE LABELLING STUDY IN THE RAT. G.S.Hamill (SPON: W.P.Bartlett).Department of Anatomy, Penn State University College of Medicine, Hershey, PA 17033.

Recent studies have reported a widespread distribution of atrial natriuretic polypeptide (ANP) within the CNS by radioimmunoassay and immunocytochemical techniques. ANP is heterogeneously distributed within the rat interpeduncular nucleus (IPN) principally within axon terminals localized in the rostral (R), dorsal medial (DM), dorsal lateral (DL) and lateral subnuclei (L). In this study, the origin of ANP-positive fibers identified in the IPN was determined using a technique combining retrograde fluorescent labelling and immunocytochemistry on the same tissue section.

Sprague-Dawley adult rats were anesthetized with Nembutal (40mg/kg), and 125-250 nl of fast blue dye (0.05%) stereotaxically injected into the IPN. Following 8 days survival and an intraventricular injection of colchicine (200ug/25ul H20), the rats were perfused through the heart with 4% paraformaldehyde. 14-18um cryostat sections were cut coronally through the entire brain prior to processing for immunocytochemistry. The ANP antiserum (1:250 dilution) used in this study was raised in rabbits against rat atriopeptin III (Zamir et al., Brain Res.365:105-111,1986). Preabsorption of the ANP antiserum with 1uM atriopeptin III resulted in a complete absence of immunostaining.

Preliminary data revealed that ANP-positive cell bodies projecting to the IPN were localized in the medial habenula (MH) and dorsal tegmental region (DTR). Most ANP-positive cell bodies within the (MH) were labelled with fast blue, and were confined to a narrow, vertically-oriented band adjacent to the lateralmost margin of the nucleus. In the (DTR) region, cells positively stained for ANP only, fast blue only, and ANP + fast blue, were localized on the midline just ventral to the fourth ventricle, in a narrow zone encircling the dorsal tegmental nucleus, and within the dorsal lateral tegmental nucleus.

The results of this study indicated that the IPN receives ANP-positive afferents from both the (MH) and dorsal tegmental region. The (DTR) also contained ANP-positive cells that projected to other brain regions, and contained cells that projected to the IPN that were not ANP-positive. In light of previous studies demonstrating reciprocal projections between the (R), (DM), (DL), and (L) subnuclei of the IPN and the (DTR) and raphe, the present data suggests that ANP may have some role in modulating the physiological activity within this feedback loop.

This study was supported by a grant from the American Heart Association, South Central PA chapter.

273.18 ULTRASTRUCTURAL LOCALIZATION OF CHOLECYSTOKININ-8 LIKE IMMUNOREACTIVITY IN THE CAT NUCLEUS SOLITARIUS. B.E. MALEY,Dept. of Anatomy and Neurobiology, Univ. Kentucky Med. Ctr., Lexington, KY. 40536

Cholecystokinin-8 like immunoreactivity (CCK) has been reported to be present in neuronal cell bodies and fibers in the cat nucleus solitarius at the light microscopic level. The nucleus solitarius is known to be involved in the central regulation of cardiovascular functions. CCK plays a role in this regulation, although the morphological basis for its action is not understood yet. The present study was undertaken to define the specific synaptic circuitry involving CCK in the nucleus solitarius.

All animals used in the present study were perfused with 4% paraformaldehyde and 0.5% glutaraldehyde in Sorenson's phosphate buffer, pH 7.4. The nucleus solitarius was isolated, cut on a vibrating microtome and immunostained with antiserum to CCK.

CCK was localized to specific synaptic terminals, dendrites, axons and neurons of the nucleus solitarius. The CCK reaction product was generally associated with synaptic vessels in terminals as well as the cytoplasmic side of other membrane bound structures. Immunolabelled synaptic terminals contained a mixture of clear and dense core vesicles in addition to mitochondria and other presynaptic structures. The majority of CCK labelled terminals contacted spines and smaller, distal dendrites, although CCK labelled terminals were also present on larger dendrites and occasionally on the neuronal cell body.

Results of this study indicate that CCK is present in presynaptic terminals in the cat nucleus solitarius, suggesting a possible role for it in the synpatic circuitry of this neural region. The morphological characteristics of CCK immunolabelled terminals is very similar to that of other peptidergic immunolabelled terminals in the cat nucleus solitarius. However, its pattern of distribution along the neuron is different than that of enkephalin and similar to that of substance P. This suggests that the pattern of termination on neurons is critical component of the synpatic circuitry in the nucleus solitarius.

Supported by NIH grant NS23861 to B.E.M.

273.19 IMMUNOCYTOCHEMICAL STUDIES OF PROENKEPHALIN-DERIVED PEPTIDES IN THE RAT CEREBELLUM. D. Zhao* and J. S. Hong (SPON: M. Ng Cheong Ton). Lab. of Neurobehav. Toxicol., NIEHS/NIH, Research Triangle Park, NC 27709.

Radioimmunoassay studies revealed that the cerebellum contains little amount of enkephalin immunoreactivity. Previous immunocytochemical studies either failed to identify the subpopulation(s) of neurons that contain the enkephalin-like immunoreactivity or only demonstrated a few positively stained Golgi cells. The existance of considerable amount of messenger RNA coding for proenkephalin prompted us to investigate this discrapancy by re-examining the distribution of the enkephalin-like immunoreactivity at cellular level.

Forty adult Fisher-344 rats were used. Purfused brains were sliced at either 10 or 20 um on the cryostat and further post-fixed in paraformaldehyde. Three different antisera raised against methionin-enkephalin (ME) conjugated with either thyroglobulin or BSA, one antibody directed against methionin-enkephalin-Arg-Phe (MEAP) were employed as the parimary antibodies in the immunostaining (these antibodies have been used by several laboratories and their specificities have been examined). All 3 antibodies against ME and the one against MEAP yielded identical staining pattern, i.e. outlayer stellate cells, basket cells, some Golgi cells and all Purkinje cells were positively stained while granule cells were not stained. The neurons in the deep cerebellar nuclei (DCN) and the fiber tracts from Purkinje cells to the DCN were also demonstrated to contain enkephalin-like immunoreactive substances. These indicate that the proenkephalin-derived peptides may exist in most, if not all, cerebellar inhibitory neurons and interneurons. Since all these neuronal components are known to use GABA as inhibitory neurotransmitter, it is extremely interesting that inhibitory proenkephalin-derived peptides coexist with GABA in these cells. Because the architecture of the cerebellum is relatively simple and the circuits between the cerebellar neurons are well defined, our finding may offer a unique model to study the fuctional significance of colocalized neurotransmitters and neuromodulators.

274.1 EFFECTS OF FENFLURAMINE ON LANGUAGE PRODUCTION IN CHRONIC SCHIZOPHRENIA. A.A. Rejzer*, R.O. Elliott*, B.D. Marshall*, and H.V.Soper. Camarillo State Hospital and UCLA-NPI Research Program, Camarillo, CA 93011.

Reported behavioral improvement among the autistic following fenfluramine treatment and high serotonin levels among certain chronic schizophrenic patients suggest that fenfluramine may be beneficial to schizophrenic patients. In particular, such control of serotonin level might improve expressive language.

The subjects, 8 (7 male) chronic schizophrenic (DSM-III diagnosed) patients, received minimal optimal neuroleptics and either a placebo or fenfluramine through the duration of the study. The first linguistic evaluation was conducted toward the end of a staggered baseline (placebo) period. Then the fenfluramine was introduced at 60 mg/day. The levels were titrated up 20 mg/day at 2-week intervals to a peak of 120 mg/day where they were maintained for 6 weeks, except for 1 subject who received the sequence 60, 80, 100, 80 mg/day. The second evaluation was performed at the end of the last dosage interval.

All evaluations were tape-recorded and the subject was asked 3 open-ended questions ("Tell me about"). The first 25 spontaneous utterances were rated by 2 experts on 2 scales - 14 factors of a pragmatic usage (PU) scale and 17 factors of a thought, language, and communication (TLC) scale. The PU scale included ratings of intonation variation, turn taking, topic maintenence, presuppositions, referencing, organization, fragments, pauses, repetitions, revisions, dysfluencies, vocal level, and rate. The TLC scale included ratings of poverty of speech, poverty of content of speech, pressure of speech, distractible speech, tangentiality, derailment, incoherence, illogicality, clanging, neologisms, word approximations, circumstantiality, loss of goal, perseveration, echolalia, blocking, and stilted speech.

The results suggest not only no beneficial effect from fenfluramine on language, but in fact an overall impairment in function. On the PU scale 5 subjects showed overall deterioration in linguistic functioning and 3 showed very little change. Overall performance deteriorated on most of the pragmatic variables assessed. The results were similar for the TLC scale. Although the deterioration was not as severe, 1 subject showed mild improvement, 1 showed no change, and the rest become more impaired.

The results of this study - lack of any beneficial effect from fenfluramine on linguistic functioning - are very similar to those obtained for neuropsychological functioning reported previously (Soper & Marshall, neurosci. Abstr. 12:480, 1986).

We would like to thank the staff of the Clinical Research Unit at Camarillo State Hospital for their help with the subjects, and Philip R. A. May, M.D., and Kamal Midha, Ph.D., for their help with the design.

274.2 EFFECTS OF A NEW SERENIC DRUG, DU 28853, IN EXPERIMENTAL MODELS FOR AGGRESSIVE BEHAVIOUR. B.Olivier and J.Mos*. Dept. Pharmacology, Duphar b.v., P.O.Box 2, 1380 AA Weesp, Holland.

DU28853 (1-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine hydrochloride) is a new drug from the class of serenics, which has been developed as specific anti-aggressive compounds. DU28853 was tested in various animal experimental aggression models in different species.

Isolated male mice confronted with a group housed partner rapidly attack. DU28853 very effectively inhibited this behaviour (ED_{50}=0.4 mg/kg p.o.) and even at doses 25 times the ED_{50} the behavioural effects were specific, i.e. animals were not sedated.

In a resident-intruder paradigm, aggression by male resident rats was dose-dependently (ED_{50}=1 mg/kg po) decreased by DU28853, whereas at the same doses social interest and exploration were not affected, neither were animals sedated. After chronic treatment with DU28853 (10 mg/kg once per day for 14 days orally) no tolerance was observed for its anti-aggressive action in a resident-intruder paradigm. In a large colony, in which a dominant (α) male, a subordinate male and a female were permanently housed, introduction of a strange male rat evoked aggression largely from the α-male. DU28853 reduced this aggression not only in the α-male but also in the subordinate (ED_{50}=2.5 mg/kg po).

Lactating female rats very rapidly attack intruders placed into their home cage during the first two weeks postpartum. DU28853 inhibited aggression (ED_{50} ~1 mg/kg po) against male intruders without interference with pupcare. Social interest remained intact while exploration increased.

In a paradigm of brain-stimulation induced aggression in rats, DU28853 at doses from 2 to 8 mg/kg po, enhanced the threshold currents to induce attacks whereas threshold currents to evoke locomotion via the same electrode were even decreased. This points to the very specific influence of this drug on aggression.

DU28853 inhibited a juvenile form of agonistic behaviour, play fighting in rats, but had no influence on locomotor activity.

Aggression resulting from mixing piglets from different litters was markedly reduced by DU28853 without sedation.

Defensive behaviour and flight, behaviours very important for species survival were unaltered by DU28853; this also held for shock-induced fighting, another commonly used model for defence.

DU28853 exerts a specific anti-aggressive activity in all species and models tested so far. This serenic property is characterized by decreases in offensive aggression which are not directly caused by behavioural interference from sedation or senso-motoric impairment. The latter may heavily interfere with the proper performance of behaviour. The specificity of the behavioural effects is further supported by the absence of effects on defence and flight.

The specific and unique profile of DU28853 warrants further testing of the drug in certain human disorders in which aggression is one of the symptoms.

274.3 ROLIPRAM-INDUCED INCREASE IN SENSORIMOTOR REACTIVITY MEASURED WITH ACOUSTIC STARTLE: SITES OF ACTION AND POSSIBLE MONOAMINERGIC MECHANISMS; J.H. Kehne, N. Boulis, and M. Davis, Dept. Psychiat., Yale Univ. Sch. Med., 34 Park St., New Haven CT 06508.

Previous work demonstrated that the phosphodiesterase inhibitor rolipram (4-(3-cyclopentyloxy-4-methoxy-phenyl-2-pyrrolidone)) increased sensorimotor reactivity manifested as elevated startle responses to auditory stimuli (Kehne et al., 1986, J. Neurosci., 6, 3250-3257). The present study further investigated sites of action and possible monoaminergic mechanisms underlying this behavioral effect of rolipram. For all systemic studies an ED_{50} dose of 0.5 mg/kg (IP) was used.

Two lines of evidence indicate that rolipram increases startle by enhancing neurotransmission on the motor (output) side of the startle reflex: (1) IP rolipram increased startle responses elicited by single-pulse, bilateral electrical stimulation of the nucleus reticularis pontis caudalis. Because stimulation of this relay nucleus directly activates the reticulo-spinal limb of the neural circuit mediating startle, the startle-enhancing effect of rolipram must be expressed, at least in part, at the level of the reticular formation or spinal cord. (2) Direct spinal (intrathecal) infusion of rolipram increased startle amplitude (Kehne et al., ibid.), though supraspinal infusions were not tested. In the present study, a 5 μg dose increased startle after intrathecal but not after supraspinal (lateral ventricle) infusion, implicating the spinal cord as a site of action.

Biochemical studies have demonstrated that compounds that elevate intracellular levels of cAMP can increase the evoked release of monoamine neurotransmitters. However, in the present study, the startle-enhancing effect of rolipram was not blocked by a variety of treatments which interfere with presynaptic monoaminergic function or which block postsynaptic monoamine receptors. These treatments included: (1) α-methyl-para-tyrosine (100 mg/kg; 3.5 hr before rolipram) + reserpine (10 mg/kg; 4 hr before); (2) para-chlorophenylalanine (400 mg/kg; 24 hr before) + reserpine (10 mg/kg; 4 hr before); (3) the noradrenergic neurotoxin DSP4 (50 mg/kg; 3 wks before); (4) pretreatment 30 min before with high doses of several monoamine antagonists: (i.e. β-adrenergic antagonist propranolol (20 mg/kg), α_1-adrenergic antagonist prazosin (5 or 10 mg/kg), dopaminergic antagonist haloperidol (1 mg/kg), serotonergic antagonists cyproheptadine (10 mg/kg) or cinanserin (10 mg/kg). These data indicate that a direct release of monoamines does not account for rolipram's enhancement of the startle response.

In summary, the increase in startle amplitude produced by the phosphodiesterase inhibitor rolipram is mediated, at least in part, by an action at the spinal level, and this effect is not attributable to increased release of monoamines.

274.4 RADIATION- AND DRUG-INDUCED EMESIS IN THE FERRET. G. L. King, Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Emesis is the most prominent prodromal symptom in man following low-level (300-1000 cGy) radiation. The ferret, Mustela putorius furo, has been recently introduced as an alternative species to dogs and non-human primates for studying this phenomenon (Gylys & Gidda, Gastroent. 90:1446, 1986; Andrews, Davis & Hawthorn, J. Physiol. 378:16P, 1986). Present work describes my findings on radiation-induced emesis in the ferret and preliminary results on those compounds that may act via the area postrema to induce emesis. For all studies, I used adult male ferrets (Fitch strain, castrated, descented, 1-1.5 kg, Marshall Farms), fed ad lib. For the radiation studies, a total of 48 animals were head-shielded and radiated (bilateral ^{60}Co, 100 cGy/min) at the following calculated midline absorbed doses (in cGy): 601 (n = 6), 401 (n = 6), 201 (n = 6), and n = 30 for steps between 49 and 127. Emetic latencies were standardized as T_0 = time that radiation ended. The emetic threshold is about 70 cGy and the ED_{50} = 95 cGy. This latter value becomes 79 cGy if retching (nonproductive emesis) is included as an emetic episode. The latency to first emesis decreased with increasing radiation dose (68.9 cGy = 40 min [n = 1]; 601 cGy = 8.5 min) whereas the duration of the prodromal response increased (68.9 cGy = 0.5 min, 601 cGy = 57 min). Increasing the dose also increased the number of both (a) emetic "bouts" within the prodromal episode (68.9 cGy = 1; 601 cGy = 2-7), and (b) expulsions (68.9 cGy = 2; 601 cGy = 7-15). For the chronic drug studies, animals were anesthetized and implanted with sterile jugular catheters. Compounds were injected under aseptic conditions 2-3 times weekly as a bolus, followed by a bolus of saline. Compounds were injected at various doses on a dose-weight basis, in volumes that never exceeded 1 ml. At least 48 hr passed between drug injections. Compounds tested thus far (on 3-6 animals) include: apomorphine, WR-2721 (a radioprotectant with emetic properties), peptide YY, and several opioid peptides (Leu- and Met-enkephalin, DADLE, DAGO, DPDPE, DSLET, and DTLE). WR-2721 produces an emetic episode that lasts for about 10 min; responses to all other emetics are brief (0.5-2.0 min). Of all compounds tested, peptide YY and the μ receptor agonist DAGO produce emesis at the lowest dose (3 μg/kg); DAGO-induced emesis is 100% at 5 μg/kg (n = 3). In contrast, the threshold dose for emesis induced by the δ-receptor agonists DADLE and DSLET is 100 μg/kg. Studies with opioid-receptor antagonists and other μ-, δ- or κ-receptor-selective agonists are in progress to determine the relative contribution of each receptor type to opioid-induced emesis.

274.5 INTRACRANIAL SELF-ADMINISTRATION OF NEUROHUMORS. J. E. Smith, K. H. McAllister*, C. A. Van Osdell*, G. F. Guerin, N. E. Goeders and S. I. Dworkin, Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Intracranial self-stimulation experiments indicate that specific neuronal circuits may mediate the neurobiological consequences of reinforcer presentation. Neurobiological investigations of intravenous drug self-administration further indicate reinforcing neuronal activity to be initiated through receptors in discrete brain regions which activate neuronal circuits that mediate these processes. Furthermore, these studies imply that continued reinforcing neuronal activity results from the release of neurohumors that interact with discrete receptors at specific sites. Experiments were initiated to identify such neurohumors and brain loci using intracranial self-administration procedures.

Adult male F-344 rats were implanted with intracranial guide cannulae into either the nucleus accumbens (NA), medial prefrontal cortex (MPC), preoptic nuclei (PO), ventral pallidum (VP) or ventral tegmental area (VTA). Following recovery from surgery the rats were allowed to self-administer 100 nanoliter infusions of either aspartate (Asp), dopamine (DA), glutamate (Glu), gamma aminobutyric acid (GABA), oxotremorine (OXT) or serotonin (5-HT) into one of these regions in 150-1500 picomole doses using electrolytic microinfusion transducer systems. Each animal was exposed to several doses of the neurohumor and vehicle twice per week during 3 to 8 hour sessions.

Animals self-administered DA and Glu significantly greater than vehicle at some brain sites. Presentation of DA into the NA and Glu into the PO and VP maintained responding in a number of animals at rates significantly above vehicle, while 5-HT into the NA or Asp into the VTA did not. OXT infusions into the VTA and MPC and GABA infusions into the VP may also maintain lever pressing but these data are less consistent at this time. Two lever discrimination and pharmacological blockade experiments are currently in progress for DA self-administration in the NA and Glu self-administration into the PO and VP. Intracranial self-administration may be useful in further characterizing and delineating the neuronal receptors and circuits that initiate and mediate reinforcement processes activated by drugs of abuse. (Supported in part by USPHS Grant DA-03832).

274.6 DOSE-DEPENDENT AND BASELINE-DEPENDENT CONDITIONING IN THE PLACE PREFERENCE PARADIGM. N.L. COSTELLO*, J.N. CARLSON AND S.D. GLICK. (SPON: J.S. DEITCH) Department of Pharmacology and Toxicology, Albany Medical College, Albany, NY 12208

The place conditioning paradigm has been used extensively as an animal model for the evaluation of drug reinforcement. Previous studies have demonstrated that a wide range of doses of d-amphetamine (d-A) which when consistently paired with an initially non-preferred environment will induce a significant increase in the total time spent in that environment (place preference). This laboratory recently reported that when compared to saline control rats, female Long-Evans rats exhibited a 'relative place aversion' to a dose of d-A (1.25 mg/kg) which was within the range of doses previously reported to induce a place preference in male rats (Costello et al. Neurosci. Abst. 1986). The present study was conducted to determine the dose dependency of this phenomenon.

Six doses (0 to 5 mg/kg) of d-A were given i.p. to female Long-Evans rats. These doses produced an inverted U-shaped function in place conditioned behavior. Contrary to the reports of similar studies, which have reported larger effects with a 5 mg/kg dose of d-A in male rats, the present study revealed that this dose failed to produce a significant place preference in females. In contrast to our previous findings, a 1.25 mg/kg dose now induced a place preference. It was noted that overall baseline preference for the conditioned side, as determined in a pre-conditioning phase, ranged from 20 to 360 sec. On this basis the control and drug groups were seperated into low [< 200 sec] or high [>200 sec] initial preference sub-groups. We evaluated the ratio of post-conditioning / pre -conditioning time spent on the drug paired side to determine if baseline preferences interacted with d-A preference induction. Place preferences were more readily induced when high initial baseline preferences were present at preconditioning. An initially low baseline preference with 1.25 mg/kg group may account for our previous findings.

After three post-conditioning test days we conducted a reinstatement test. All subjects were given a 1.5mg/kg dose of d-A, prior to placement in the testing chamber. All groups which exhibited a preference on the first three test days continued to do so when tested with the drug. Although the 5 mg/kg group did not exhibit a preference over the first three test days, when a dose of 1.5 mg/kg was administered on the fourth test day, a significant preference for the drug paired environment was observed. This suggests that this dose, elicits competing rewarding and aversive effects and that the former generalized to the effects of the 1.5 mg/kg dose. (Supported by NIDA grant DA03817 to S.D.G.)

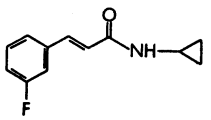
274.7 EFFECTS OF IONIZING RADIATION ON FIXED-RATIO ESCAPE PERFORMANCE IN RATS. P.C. Mele*, C.G. Franz* and J.R. Harrison* (SPON: S. Kandasamy). Behavioral Sciences Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

This laboratory recently reported that acute, sublethal doses of ionizing radiation produced reversible, dose-related disruptions in responding when maintained by fixed-ratio (FR) and fixed-interval schedules of milk presentation (Toxicologist 7: 250, 1987). The present study extended these findings by irradiating rats responding under an FR schedule of termination of electric foot-shock (FR escape).

Adult male rats pressed a lever to terminate scrambled foot-shock under an FR20 schedule; shock-off duration was 30 sec. Test sessions occurred Mon-Fri and were 60 min in duration. Separate groups of rats (n=6/group) were exposed to 0, 4.5 or 7.5 Gy of 60Co gamma radiation. Over 6 wk of testing after exposure, there were no consistent changes in performance of the 0 and 4.5 Gy exposure groups. In the 7.5 Gy exposure group, response rates were reduced over the first 30 days after exposure; individual rats had response rates falling below their control range during 16, 15, 12, 11, 8 and 4 of the 22 test sessions that occurred during this time. For each rat, response rates were almost completely reduced during some of these sessions and only slightly or moderately reduced during others. Subsequently, recovery of preirradiation control rates occurred in all rats. Thus, radiogenic disruption of FR escape performance was reversible and dose-related though variable both within and between animals. Next, rats of the 0 Gy exposure group received 150 Gy/day for 5 consecutive days. Over 6 wk of testing response rates were moderately reduced in only 1/6 rats (wk 4-5), indicating that dose-fractionation was less effective in disrupting performance than a single, acute dose. To evaluate the relative sensitivity of the FR escape baseline, chlorpromazine (C, 0.1-3.0 mg/kg) and d-amphetamine (A, 0.3-5.6 mg/kg) were used as reference compounds. C and A typically decreased response rates in a dose-dependent manner to 15 and 30% of control at the highest doses tested, respectively, although the lowest dose of A increased rates slightly (by 25%). Thus, FR escape responding was disrupted by these prototypical drugs at doses that have been shown to disrupt a variety of other schedule-controlled behaviors.

274.8 CINFLUMIDE, A CHEMICALLY NOVEL CENTRALLY ACTING SKELETAL MUSCLE RELAXANT. F.E. Soroko*, B.T. Kenney*, E. Grivsky+, R.A. Maxwell and B.R. Cooper. Department of Pharmacology, +Department of Chemistry (Retired), Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Cinflumide was compared with the clinically-used central muscle relaxants, chlorzoxazone, carisoprodol and methocarbamol in rodents using weakness of the abdominal muscles, ataxia and loss of righting reflex as indices of skeletal muscle relaxation. Cinflumide was found to be more potent or of equal potency to the above clinically-used muscle relaxants depending on species and routes of administration. The duration of action of cinflumide compared favorably to the three standards.



In the conscious cat cinflumide produced signs of skeletal muscle relaxation. It was found to be more potent than methocarbamol and approximately equal in potency to chlorzoxazone and carisoprodol. No evidence was obtained in the rodent or the cat that cinflumide produced sedation as a side effect at doses equal to the ED_{50} for muscle relaxation. Electrophysiological studies show that cinflumide acts on the spinal cord to depress selectively polysynaptic reflexes. Clinically, cinflumide is in Phase 2 studies.

274.9 LOCOMOTOR ACTIVITY PATTERNS INDUCED BY DESIGNER DRUGS. H.D Christensen, D.A. Christensen*, W. Cao*, R.A. Brumback* and J.M. Carney*. Depts. of Pharmacol. and Pathol., Col. Med., OUHSC, Oklahoma City, OK 73190

Locomotor activity effects of 3,4-methylene dioxymethamphetamine (MDMA) was compared to saline controls in five strains of inbred mice where recombinant sets exist. In the SWR mouse there was a progressively greater stimulation as the dose of MDMA was increased. In contrast in the C57L mouse, MDMA at 5 and 10 mg/kg i.p. stimulated locomotor activity to twice control values with a slight decrease at 30 mg/kg, compared to the 5 and 10 mg/kg dose levels. The response of AKR mice to MDMA was a four-fold depression in activity at 5 and 10 mg/kg while 30 mg/kg induced stimulation. The BALB/cBy mouse, which is not stimulated by amphetamine, had an activity depression of four-fold after 5 and 10 mg/kg and two-fold after 30 mg/kg MDMA. One of the four mice receiving 30 mg/kg daily showed stimulation on day 3 and 4. The C57BL/6By mouse showed no change at 5 mg/kg and a mild stimulation at 30 mg/kg. Thus, five mg/kg MDMA would be an appropriate dose to study genetics since locomotor activity for AKR and BALB/cBy is a depression, C57L stimulation and SWR and C57BL/6By are not significantly different from saline treated animals.

Locomotor activity was measured after daily administration of four designer drugs, MDMA, MDA, MDEA and methamphetamine 5 mg/kg in A/J and C57BL/6J mice. MDMA showed a depression on day 1 for A/J mice, but no change from saline values on days 2-4. MDA and methamphetamine did no differ from saline on day 1 but progressively stimulated activity on day 2-4 reaching a 4 to 5 fold increase from day 1. MDEA did not vary from saline. In C57BL/6J mice there were no differences on day 1, small but significant depression for MDMA, MDA and MDEA on day 4, while methamphamine caused stimulation. When these drugs were given daily for 15 days, MDMA induced a decrease in A/J but not in the C57BL/6J or C57BL/10J mouse. In the two F1 generations, B6AF1 and B10AF1, MDMA caused a decrease like the A/J sire, although their basal rates are more like their dams. One day post administration there was a relative rebound and by seven days post administration control values were found. Fifteen days of MDA and MDEA treatment resulted in an activity decrease in C57BL/10J but not in A/J or C57BL/6J mouse.

Depending upon the inbred strain MDMA, MDA and methamphetamine elicit different responses suggesting separable CNS mechanisms. One approach to the identification and characterization of these separable mechanisms is to utilize recombinant inbred mouse strain sets (AKXL, CXB/By and SWXL).

(Supported by USPHS grants DRS 2S07-RR05411 and DA04028)

274.10 THE EFFECT OF INTRACEREBROVENTRICULAR INJECTIONS OF VEHICLE SOLUTIONS ON LOCOMOTOR ACTIVITY IN THE HAMSTER. P. Schnur and C.M. Archuletta*. Dept. of Psychology, University of Southern Colorado, Pueblo, CO 81001.

A series of experiments investigated the effects of 5 µl intracerebroventricular injections of commonly used physiological vehicle solutions on running wheel activity in six female golden Syrian hamsters, Mesocricetus auratus. Stainless steel cannulas were implanted in the left lateral ventricle (1.1 mm anterior to bregma, 1.7 mm lateral to the midline, 2.7 mm below the dura) under sodium pentobarbital anaesthesia. Animals were given at least one week to recover from surgery before testing commenced. The solutions studied (and their compositions in grams/liter) were as follows: bacteriostatic saline (9 g sodium chloride (NaCl), 9 g $C_6H_5CH_2OH$ (benzyl alcohol)), saline (9 g NaCl), artificial cerebrospinal fluid 1 (7.46 g NaCl, 0.19 g potassium chloride (KCl), 0.14 g anhydrous calcium chloride ($CaCl_2$), 0.19 g magnesium chloride ($MgCl_2 \cdot 6H_2O$), 1.76 g sodium bicarbonate ($NaHCO_3$), 0.18 g sodium biphosphate, (Na_2HPO_4), 0.61g glucose ($C_6H_{12}O_6$)), artificial cerebrospinal fluid 2 (1.8 g NaCl, 0.093 g KCl, 0.088 g calcium chloride ($CaCl_2 \cdot 2H_2O$), 0.042 g potassium dihydrogen phosphate, (KH_2PO_4), 0.04 g magnesium sulfate ($MgSO_4$), 0.55 g $NaHCO_3$), Ringer's solution (7 g NaCl, 0.14 g KCl, 0.13g $CaCl_2$, 0.20 g $NaHCO_3$) and deionized water. All solutions were freshly prepared and filtered prior to injection. Following the injection, animals were placed in the running wheels for a three hour test session and the number of wheel revolutions was recorded at 20-min intervals. Compared with no injection or sham injection controls, which did not differ from one another, all solutions except Ringer's solution produced a temporally-specific decrement in running wheel activity: Little or no effect was evident for one hour after the injection, but activity decreased dramatically thereafter for the next two hours. The mechanism(s) underlying this effect is under investigation.

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274.11 REPEATED ADMINISTRATION OF MURINE-RECOMBINANT INTERFERON-GAMMA (murIFN-gamma) PRODUCES DECREASED LOCOMOTOR ACTIVITY IN MICE. S.B. Weinberger, G. Schulteis, A.G. Fernando*, C. Bakhit, and J.L. Martinez, Jr. Psychology Department, University of California, Berkeley, CA 94720 and Genentech, Inc., South San Francisco, CA 94080.

In addition to their important immune augmenting effects, the interferons may modulate, either directly or indirectly, neuronal and central nervous system function. In some human patients, interferons may cause several presumably centrally-mediated, but reversible, effects, including lethargy, anorexia, psychomotor retardation, and cognitive changes that persist throughout the treatment period. Although several investigators studied the effects of interferon-alpha in animals, the behavioral effects of peripherally administered interferon-gamma have not been characterized, nor are the biochemical changes that may underlie these effects known. We report here the effects of treatment with murIFN-gamma on spontaneous locomotor activity in mice.

All studies were done in male Swiss-Webster mice. Locomotor activity was measured in an open field chamber, the floor of which was divided into 16, 3-inch square sections. The number of lines crossed during a 60 min observation period was tabulated in one-min intervals.

The acute effects of murIFN-gamma injection were evaluated both immediately after and 4 hrs after a single 30 µg i.p. injection. In both studies total crossovers over the 60 min observation period were not significantly different in animals treated with murIFN-gamma or saline.

The locomotor effects of repeated daily treatment with murIFN-gamma were evaluated over a 60 min period commencing 4 hrs after the fifth daily i.p. injection of 30 µg of murIFN-gamma. As compared to the saline-treated mice, a decrease in locomotor activity was seen in the murIFN-gamma animals (total crossovers for mins 25-60: murIFN-gamma, 90 +/- 29, n = 7; saline, 230 +/- 28, n = 8; p = 0.035 [Mann-Whitney U-Test]). Additionally, the murIFN-gamma-treated animals gained significantly more weight over the 5-day treatment period than did the saline-treated controls.

The observed decrease in locomotor activity suggests a centrally mediated effect of murIFN-gamma. Biochemical experiments following murIFN-gamma treatment in mice (Bakhit et al., in preparation) suggest changes in striatal dopaminergic and serotonergic activities, which may contribute to the observed murIFN-gamma effect on locomotor activity.

(Supported by NRSA #1-F32-DA05313-01 from NIDA; NRSA #5-R32-MH15860-08 from NIMH; NIDA # DA04195; and S07-RR07006.)

274.12 THE EFFECTS OF ADENOSINE AGONISTS ON DISCRETE TRIAL AVOIDANCE BEHAVIOR IN RATS. P.M. Fritz* and D. Luttinger. Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, NY 12144

Adenosine agonists have been suggested to produce effects in animals that are similar to those produced by antipsychotic drugs (e.g. Heffner et al., The Pharmacologist 27:293, 1985 and 28:89, 1986 and Luttinger and Patton, Society for Neuroscience 12:911, 1986). The present studies were conducted to further explore the effects of adenosine agonists in a procedure that detects antipsychotic activity. Thus, the ability of adenosine agonists to selectively inhibit discrete trial avoidance responding was assessed as well as the effects of theophylline on adenosine agonist induced effects.

Male Sprague-Dawley rats were trained on an avoidance schedule to press a lever to avoid (or escape) electric footshock. The schedule consisted of a 30-second intertrial interval. During the intertrial interval, the number of lever presses were recorded but had no programmed consequences. Following the 30-second intertrial period a 10-second interval during which a continuous tone was presented ensued. When the tone was on, a lever press would result in termination of the tone and initation of another 30-second intertrial interval. A response during this component of the schedule is considered an avoidance response. If the rat did not press the lever during the 10 seconds when the tone was on, then the tone remained on and electric current (0.60 mA) was applied through the grid floor resulting in the rat being shocked. This component continued until the rat pressed the lever or 5 seconds elapsed. If the animal pressed the lever to terminate the shock, the response was considered an escape. Each session consisted of 50 such trials. Adenosine agonist and antipsychotic compounds were administered orally one hour (theophylline -45 min) before the session in a volume of 1 ml/kg.

The two antipsychotic drugs tested, chlorpromazine and clozapine, produced dose-related inhibiton of avoidance responding without affecting escape responding. Similarly, administration of the adenosine agonists 5'-N-ethylcarboxyamidoadenosine (NECA), 5'-N-methylcarboxyamidoadenosine (MECA) N^6-cyclohexyl adenosine (CHA), 2-chloroadenosine (CLA), N^6-cyclopentyladenosine (CPA) and the R- and S- isomers of phenyl isopropyladenosine (RPIA and SPIA) inhibited avoidance responding without affecting escape responding. Except for CLA (active at 55 mg/kg) and SPIA (inactive at 3 mg/kg), the other agonists were active at 3 mg/kg. Theophylline antagonized RPIA-induced inhibition of avoidance responding without affecting clozapine or chlorpromazine induced inhibition of avoidance responding.

These results extend the observations that adenosine agonists produce effects similar to antipsychotic drugs in a variety of procedures. Furthermore, the effects of the adenosine agonists, but not the antipsychotic drugs are antagonized by theophylline.

274.13 BEHAVIORAL AND BIOCHEMICAL EVIDENCE FOR NORADRENERGIC INVOLVEMENT IN THE STIMULANT EFFECTS OF AMFONELIC ACID. G.L. Robinson, B.K. Koe, and P.A. Seymour. Central Research Division, Pfizer, Inc., Groton, CT 06340

Tests that evaluate the ability of drugs to antagonize the behavioral effects of d-amphetamine, have become important preclinical tools for the evaluation of potential antipsychotic agents. D-amphetamine, however, has been shown to release norepinephrine (NE) as well as DA from central catecholaminergic neurons, and to enhance the metabolism of both NE (Kalisher et. al., J. Pharmac. Exp. Ther., 193:64, 1975) and DA (German et. al., J. Neural Trans. , 44:39, 1979). These nonspecific effects of d-amphetamine can pose problems when this agent is used for the measurement of in vivo DA antagonism, especially when the agents in question posess alpha1-antagonist properties. The psychostimulant amfonelic acid, however, has been reported to be selective for DA (McMillen, B.A. and Shore, P.A., J. Pharm. Pharmac., 30:464,1978), and may present an advantage over the use of d-amphetamine. The present studies examined the effects of the alpha1-adrenergic antagonist, prazosin (PRA), on d-amphetamine vs. amfonelic acid-induced locomotor stimulation in rats. D-amphetamine and amfonelic acid both induced dose-related stimulant effects from .32 - 1.78 mg/kg, s.c., and a mixed stimulant/stereotypy effect at 3.2 mg/kg, s.c., with remarkable similarity in all respects. It was found that PRA significantly attenuated d-amphetamine-induced (ID50 < 1 mg/kg) and amfonelic acid-induced (ID50 < 1 mg/kg, s.c.) locomotor activity at doses that had no effect on spontaneous locomotor activity. These findings are in agreement with those of Menon and Haddox (Neuropharm., 23:555, 1984) who reported that PRA antagonizes amfonelic acid-induced stimulation in mice. Finally, in contrast to the aforementioned selective dopaminergic effects of amfonelic acid, it was found that amfonelic acid inhibited the uptake of both DA and NE from rat brain synaptosomes (IC50=.05 and .09 uM respectively) more potently than did d-amphetamine (IC50=1.5 and .4 uM). These data do not support the hypothesis that amfonelic acid is more selective for dopaminergic systems than d-amphetamine.

274.14 CEREBRAL ACTIVITY IN THE DECORPORATE RAT: DRUG EFFECTS. C.H. Vanderwolf, G. Buzsaki, D.P. Cain, R.K. Cooley* and B.J. Robertson*. Dept. of Psychology, Univ. of Western Ontario, London, Canada, N6A 5C2.

Mikeska and Klemm (Lab Anim. Sci., 1975, 25, 175-179) suggested that decapitation of conscious animals causes pain and distress. In a reinvestigation of this, we implanted chronic recording electrodes transcortically in the hippocampus and neocortex in 29 rats. Polygraphic recordings were taken before and during decapitation. Hippocampal activity was filtered (usually 6-12 Hz), rectified and integrated as a measure of rhythmical slow activity (RSA). In waking rats atropine-resistant forms of hippocampal RSA and neocortical low voltage fast activity (LVFA) occur during voluntary movement. These patterns appear to depend on central serotonergic pathways (Vanderwolf, C.H., and Baker, G.B. Brain Research,1986, 374, 342-356). Atropine-sensitive forms of RSA and LVFA appear to depend on central cholinergic pathways and may occur during waking immobility and during anesthesia (Vanderwolf, C.H., and Robinson, T.E. Behav. Brain Sci., 1981, 4, 459-514). Thus, atropine-resistant (serotonergic) activation could be said to be restricted to the conscious state but atropine-sensitive (cholinergic) activation is not so restricted.

After decapitation at C1 in undrugged rats, neocortical activity (sometimes clear LVFA) persisted for 6-9 s. Hippocampal activity (usually some clear RSA) persisted for about 15 s. Following decapitation of ether anesthetized rats, similar patterns occurred but hippocampal activity persisted for about 24 s. When rats pretreated with atropine (50 mg/kg, i.p.) or scopolamine (5 mg/kg, s.c.) were decapitated, some RSA was present for 1-3 s but at a level of only 35% of what was seen during locomotion prior to decapitation. Hippocampal activity persisted for about 11 s. Clear LVFA was not observed. These results suggest that rats lose consciousness almost immediately when decapitated.

Further, previous anatomical studies have shown that dorsal root fibers from C1-C3 terminate no higher than C2. Trigeminal pain fibers terminate as low as C4 in rats (Torvik, A. J. Comp. Neurol. 1956, 106, 51-142). Therefore, following section at C1, little or no pain input can reach the brain.

Conclusion. Decapitation is probably virtually painless.

This research was supported by NSERC grants to D.P. Cain and C.H. Vanderwolf.

274.15 CENTER TIME: A TEST FOR ANXIOLYTIC ACTIVITY IN RATS. N.F.Nichols* and P.J.K.D.Schreur (SPON: L.T.Rutledge). CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

In a novel enclosure, rats and mice spend more time near the walls than in the exposed central space. Anxiolytic compounds increase the "center time," the amount of time spent away from the walls.

Method. Male Sprague-Dawley rats (10/group), 170-190g, were injected s.c. 20 min before the test. Activity was recorded for 5 min in the light in 16-beam Omnitech Digiscan Activity Monitors (16" x 16") with 16 regularly-spaced 1" holes in the plastic floors. Center time is the amount of time the rat spends more than 1/2" from the walls of the cage. Groups were compared by Student's t-test.

Results. Center time was significantly increased by diazepam and the proposed 5-HT1a agonists, buspirone and 8-OH-DPAT (8-hydroxy-dipropyl-aminotetralin). Chlordiazepoxide (10 mg/kg) tended to increase center time, but the effect did not reach statistical significance. Sodium pentobarbital increased center time at 10 mg/kg; haloperidol (0.1 -1 mg/kg) did not increase it at all.

In conclusion, center time is a predictor of anxiolytic efficacy of drugs.

274.16 PHENCYCLIDINE-INDUCED ATAXIA: COMPARISON OF MOVING BELT AND GAIT ANALYSIS. G.C. Haggerty and J.M. Johnson*, Battelle Columbus Division, Columbus, OH 43201 and College of Pharmacy, Ohio State University, Columbus, OH 43210.

Phencyclidine is a psychotomimetic agent which has experienced continual and increased popularity as a drug of abuse since the mid 1960's. Its effects in animals vary according to the species and dosage of drug used. In rodents, phencyclidine appears to exert a biphasic effect on behavior. Investigators have found that administration of phencyclidine to rats in the dose range of 2 - 7.5 mg/kg primarily produces excitation, while higher doses cause gross motor ataxia and central nervous system depression (Murray et al., Life Sci., 24:2217-2226, 1979; Haggerty et al., Toxicol. Appl. Pharmacol., 75:444-453, 1984). The objective of the present work was to determine the feasibility of measuring ataxia in rats following doses of phencyclidine lower than those which induce severe functional impairment and depression. Two methods were used: 1) Performance of a moving belt task, a well documented method for the measurement of ethanol-induced gait impairment, and 2) The assessment of footprint patterns during forward locomotion.

Phencyclidine at a dose as low as 2 mg/kg impaired performance of the moving belt task, suggesting the presence of drug-induced ataxia. At 4 mg/kg, impairment was present, but performance was also adversely affected by hyperexcitability and moderate stereotypy. Phencyclidine did not induce abnormal gait patterns, as measured by footprint analysis, although at the higher doses tested, an increase in step length was observed. This effect was attributed primarily to an increase in the walking speed of the animals due to the excitatory effects of phencyclidine. An ethanol-treated group, included in the study for comparison purposes, showed a clear increase in step width and a concomittant decrease in step angle, indicating a wider gait.

In summary, the results of this study showed that phencyclidine produced ataxia in the rat at doses that were clearly excitatory. Phencyclidine-induced ataxia was detected using a moving belt task, but not by gait analysis suggesting that the moving belt was a more sensitive technique for assessing drug-induced ataxia.

276 SYMPOSIUM. NEUROPEPTIDES/NEURAL SYSTEMS IN FEVER, INFLAMMATION AND IMMUNE RESPONSES. J.M. Lipton, Univ. of Tex. Hlth. Sci. Ctr. at Dallas (Chairperson); D.G. Payan, Univ. of California, Sch. of Med.; San Francisco, J.E. Blalock, Univ. of Alabama Med. Sch., Birmingham; D.L. Felten, Univ. of Rochester Sch. of Med.; T.L. Roszman, Univ. of Kentucky Sch. of Med.

Bidirectional interactions between the nervous system and the immune system were suggested initially by research on brain lesions and immunity and on immunologic modulation of neural function. Such interactions are now widely accepted, although the details remain to be established. Terms such as "neuroimmunomodulation", "immunotransmitters", "neuroendocrine-immune axis" reflect the recent development of ideas about parallels, shared chemical mediators and homeostatic relations between the nervous and immune systems.

Neuropeptides are believed to form a link between the two systems. For example, α-MSH, a naturally occurring CNS peptide, alters host responses such as fever and inflammation. Substance P, somatostatin and VIP are believed to be important to modulation of local immunological responses by the peripheral nervous system, via interaction between immunocompetent cells and the neuropeptides. Receptors for certain peptides, previously associated primarily with neuroendocrine function, occur on macrophages, lymphocytes and other tissues of the immune system. Lymphoid tissues such as the spleen, thymus and lymph glands are innervated by autonomic fibers. There is recent evidence that destruction of specific hypothalamic regions markedly alters immune function and that central administration of agents that impair neurotransmitter function can depress antibody responses. The aim of the symposium is to broaden understanding of relations between neuropeptides, neural structures and host responses through a review of these and other concepts. Investigators from several disciplines will outline this rapidly developing field of research.

277 SYMPOSIUM. DYNAMIC CHANGES OF SYNAPTIC STRUCTURE UNDER NORMAL AND EXPERIMENTAL CONDITIONS. D. Purves, Washington University (Chairperson); S. Smith, Yale University; C.H. Bailey, Columbia University; W.T. Greenough, University of Illinois.

The purpose of this symposium is to review recent work which indicates that synaptic connections in the mature nervous system are normally subject to substantial structural change. Steve Smith will begin the session by discussing the observation that the application of neurotransmitters (and other agents that alter levels of second messengers) can have marked effects on the structure of cultured mammalian neurons. These changes are particularly apparent among the growth cones and dendrites of such cells. Dale Purves will then describe the gradual changes in the configuration of synapses that have been observed in the peripheral nervous system of living mice when the pre- or post-synaptic elements of identified neurons are monitored over long periods (weeks to months). These observations, like those of Smith, depend on video-enhanced light microscopy and digital image processing; these techniques will also be discussed. Craig Bailey will then review recent work on the structural correlates of simple forms of learning in Aplysia. The emphasis of his talk will be on a specific set of identified synapses that he and his colleagues have used as a model system to explore a possible anatomical basis for the relationship between short- and long-term memory in Aplysia. Finally, Bill Greenough will discuss changes in synaptic numbers and dendritic branching patterns in the rat cerebral cortex elicited by exposure of adult animals to a complex environment, or to maze or motor training. He will also consider the possible relationship of these effects to long-term memory. These several lines of evidence suggest that structural changes in the arrangement of synaptic connections play an important part in the normal function of the mature nervous system.

OPIATES, ENDORPHINS AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS IV

278.1 INTERACTIONS OF PROGLUMIDE WITH MORPHINE ANALGESIA. G.W. Pasternak and R.J. Bodnar. Dept. of Neurol. Memorial Sloan-Kettering Cancer Ctr., New York, NY 10021.

Proglumide (PRO) antagonizes cholecystokinin (CCK) receptors and blocks CCK effects. The demonstration that CCK acts as a physiological antagonist of opiate analgesia led to the subsequent finding that PRO potentiates analgesia induced by morphine and other opioid manipulations, effects which are blocked by naltrexone, but which do not display monotonic dose-response relationships. PRO also potentiated analgesia in morphine-tolerant rats. The following four experiments explored PRO-opiate interactions further. First, PRO (10 mg/kg, IP) potentiated morphine (2.5-20 mg/kg, SC) analgesia on the tail-flick test in mice both in terms of peak effects and duration of action without affecting basal latencies. Further, PRO (5 and 10 mg/kg, but not 1 mg/kg) potentiated morphine (5 mg/kg) analgesia. PRO dose-dependently reinstated morphine analgesia in mice 48 h after morphine pellet implantation, and increased morphine analgesia in mice 24 h after morphine pellet implantation. Naloxone (0.01-1.0 mg/kg, SC) eliminated analgesia induced by both morphine (5 mg/kg) and morphine paired with PRO (10 mg/kg). Naloxonazine (35 mg/kg, SC) eliminated morphine (5 mg/kg) analgesia and the PRO-induced potentiation. As the morphine dose was increased (20 & 30 mg/kg), naloxonazine antagonized both morphine analgesia and the PRO-induced potentiations; the former effect was more pronounced. Finally, PRO (5 ug) potentiated d-ser$_2$-thr$_6$-leucine enkephalin (DSTLE, 5 ug) analgesia following administration of both into the periaqueductal gray. Again, central PRO failed to alter baseline latencies. These data indicate that the ideal circumstances under which PRO-induced potentiations occur is when the opiate system is active, and that PRO is an effective enhancer of opiate actions without possessing intrinsic analgesic activity itself. (Supported by ACS Grant PDT 169).

278.2 CHANGING PATTERNS OF ANALGESIA INDUCED BY LATERAL VENTRICLE OR SPINAL INJECTIONS OF MORPHINE OR KETOCYCLAZOCINE IN DEVELOPING RATS. G. A. Barr, D. Miya and W. Paredes. Biopsychology Doctoral Program, Dept. Psychology, Hunter College, CUNY, NY, NY 10021, USA and Dept. Psychiatry, Albert Einstein College of Medicine, Bronx, NY 10461, USA.

Morphine and ketocyclazocine, prototypic but non-specific μ and k opioid receptor agonists, produce changing and different patterns of analgesia during development. Morphine's effects first appear at 3 days of age when a noxious stimulus is applied to the forepaw of a pup but not until 4-10 days later (depending of the stimulus and its intensity) when the same stimulus is applied to the tail. Ketocyclazocine's analgesic actions do not show this strong caudad pattern of development and emerge at about 5-7 days for both the forepaw and tail. Further, analgesia produced by morphine, but not ketocyclazocine, to a thermal noxious stimulus was attenuated by intraspinal depletion of serotonin by 5,7 DHT. It was proposed that morphine acts at a μ opioid receptor in brain that in turn activates some descending system (e.g. serotonin) in spinal cord to mediate its analgesic actions and that this descending system develops in a rostral to caudal direction postnatally. In contrast, ketocyclazocine may act at local spinal k receptors that mature at the same age at different segmental levels. To test this model and to further understand the anatomical organization of morphine (μ) and ketocyclazocine (k) induced analgesia, we injected each drug into the lateral ventricle or subdurally into the spinal cord. Lateral ventricle injections were done free hand by penetrating the cartilaginous skull with a 30 ga. needle. Accuracy of the injection was verified by dye. For the intraspinal injections, a catheter constructed of dialysis tubing was implanted under the dura on the dorso-lateral surface of the spinal cord. Morphine or ketocyclazocine were injected in doses that ranged from 1 to 10 μg. Pups were tested at various ages beginning at three days. Both thermal and pressure noxious stimuli were used. In each case, the stimulus produced a quick (< 1 second) and consistent withdrawal response at each age tested. When injected into the lateral ventricle of 3 day olds, morphine, but not ketocyclazocine, produced a potent analgesia for the forepaws. No increase in latency was seen for the hindpaw or tail. In 10 day olds, morphine's effects were seen in all three appendages but only when the mechanical stimulus was used. No analgesia was noted in the hindpaw or tail when the thermal stimulus was tested. Intraspinal injections of ketocyclazocine or morphine produced a different pattern. Neither drug produced a consistent developmental pattern of analgesia until 14 days of age. At this age, ketocyclazocine began to produce analgesia in the tail flick test, but at a relatively high dose (30 μg). The data from both sets of experiments imply that analgesia produced by intracerebroventricular administration of morphine acts through descending spinal circuits that are mature at the level of the rostral spinal cord by three days of age and develop caudally over several weeks. Further, the circuitry for mechanical and thermal stimuli differ with the latter maturing more slowly. In contrast, spinal segmental opioid receptors mediating opiate induced analgesia develop relatively late since neither drug produces much analgesia until into the second week of life.

278.3 EFFECTS OF STRESS AND β-FUNALTREXAMINE (β-FNA) PRETREATMENT ON MORPHINE (MS) ANALGESIA AND OPIOID BINDING IN RATS. J.U. Adams*[1], J.S. Andrews*[1], J.M. Hiller[2], E.J. Simon[2] and S.G. Holtzman*[1] (SPON: F.J. Gordon). [1]Dept. of Pharmacology, Emory University, Atlanta, GA 30322 and [2]Dept. of Psychiatry, New York University, New York, NY 10016.

Stress has been hypothesized to induce the release of endogenous opioids, which in turn may mediate such phenomena as stress-induced analgesia and stress-induced potentiation of MS analgesia. β-FNA is an irreversible opioid antagonist that presumably acts by alkylating the mu-opioid receptor. Receptors can be protected from alkylation in vitro if occupied by reversible receptor ligands. This study was essentially an in vivo protection experiment designed to provide further evidence for the stress-induced release of endogenous opioids and their subsequent action at opioid receptors. Adult male rats that were either subjected to restraint stress or unstressed were injected ICV with β-FNA (2.5 µg) or saline. Twenty-four hours later, subjects were tested unstressed for MS analgesia using the tail-flick assay or were sacrificed and numbers of opioid binding sites in brain were determined. [^{3}H]D-Ala^2-NMePhe^4-Gly(ol)enkephalin (DAGO) served as a specific ligand for mu-opioid receptors, and [^{3}H]bremazocine as a general ligand for all opioid receptors.

Pretreatments did not affect baseline tail-flick latencies 24 hr later but markedly altered the analgesic response to MS (3.0-17.5 mg/kg SC). Rats injected with saline while stressed were significantly less sensitive to MS than were their unstressed counterparts. β-FNA pretreatment antagonized MS analgesia in an insurmountable manner, as characterized by a downward shift to the right of the MS dose-effect curve when compared to the curve for saline-pretreated controls. Animals pretreated with β-FNA while stressed were significantly more sensitive to the analgesic effect of MS than were animals that received β-FNA while unstressed. This is consistent with the hypothesis that stress induces release of endogenous opioids which would protect opioid receptors from alkylation by β-FNA. β-FNA caused small and similar decreases in ^{3}H-DAGO binding in brain of both stressed and unstressed animals. Stressed rats injected with saline tended to have an increased number of [^{3}H]DAGO and [^{3}H]bremazocine binding sites compared to the other groups. This outcome may be revelant to the tolerance to MS analgesia caused by stress. Stress-induced tolerance probably served to limit the magnitude of the difference in the analgesic effect of MS between the stressed and unstressed animals treated with β-FNA. (Supported in part by Grants DA0017 and DA00541 and by RSA DA00008.)

278.4 THE ANALGESIA OF PREGNANCY INVOLVES A SPINAL OPIOID MECHANISM. A.R. Gintzler, W. C. Chan* and H.W. Sander*, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

It has been demonstrated that during pregnancy and labor in rats and humans there is an opioid mediated elevation in the threshold for responsiveness to aversive stimuli which reaches a maximum at term. Acute administration of the opiate antagonist, naltrexone, into the lumbar intrathecal space of pregnant rats (day 20 of gestation) significantly reduces the threshold for reflexive jumping in response to electric footshock. The intrathecal administration of the inactive stereoisomer of a closely related narcotic antagonist, (+)naloxone, is devoid of any effect on pain threshold. No effect on pain threshold is observed following intrathecal saline administration to pregnant rats, intrathecal naltrexone administration to non-pregnant rats or following systemic administration of an intrathecally effective dose of naltrexone to pregnant rats. These data indicate that the analgesia observed during gestation is mediated, at least in part, via spinal opioid receptors which are activated by some aspect of the pregnant condition. Recent experiments suggest that this phenomenon involves both uterine and hormonal components. The former is suggested by experiments in which hypogastric neurectomy reduced by a factor of five the analgesia of pregnancy. A hormonal component is inferred by the observation that during pseudopregnancy jump thresholds are significantly higher than that observed in the period prior to its induction or following its offset.

278.5 EFFECTS OF NALOXONE AND U69593, A KAPPA OPIOID, ON HIBERNATION INDUCTION IN SUMMER ACTIVE GROUND SQUIRRELS BY "HIBERNATION INDUCTION TRIGGER" (HIT). T.-P. Su[1], D.B. Bruce*[2] and P.R. Oeltgen*[3]. [1]Neuropharmacol. Lab, NIDA Addiction Research Center, Baltimore, MD 21224, [2]Wheaton College, Wheaton, IL 60187 and [3]VA Hospital and Univ. of Kentucky, Lexington, KY 40511.

HIT is an endogenous plasma factor isolated from winter hibernating animals which, when injected, can induce hibernation in summer active ground squirrels (Dawe,A.R. and Spurrier,W.A., Science, 163:298, 1969; Oeltgen,P.R. et al., Prep. Biochem., 8:171, 1978). It was also observed that HIT, when given to monkeys, could induce inhibitory activities. Some of the inhibitory activities could be reversed by naloxone, raising a possibility that the action of HIT, at least in part, may involve opioid receptors. The present study examined this possibility by studying the effects of naloxone and a selective kappa opioid agonist U69593 (Lahti,R.A. et al., Europ. J. Pharmacol., 109:281, 1985) on HIT-induced hibernation in summer active ground squirrels (Citellus tridecemlineatus).

Drugs were delivered through osmotic minipumps which were implanted subscapularly into ground squirrels. Delivery rates were 1 mg/kg body weight/h for naloxone and 0.457 mg/kg/day for U69593. HIT was dissolved in saline and given to animals intrasaphenously. Animals were put into separate cages in the hibernaculum at 7°C. Hibernation frequency was determined by measurement of core temperature, respiratory rate and bouts of activity.

Injection of HIT caused summer active ground squirrels to hibernate. However, animals receiving naloxone hibernated four times less frequently than controls. After removal of pumps which were deliverying naloxone, reinjection of HIT into naloxone-treated animals caused them to hibernate in a frequency equivalent to controls receiving HIT. U69593 was tested to see if kappa receptors may be involved in the HIT-induced hibernation since naloxone is known to block mu, kappa and delta opioid receptors. U69593 did not cause hibernation in summer active animals, indicating that kappa opioid receptors may not be involved in the HIT-induced hibernation. However, unexpectedly, U69593 antagonized HIT-induced hibernation in summer active ground squirrels.

HIT depressed electrically stimulated twitches in a guinea-pig ileum myenteric plexus-longitudinal muscle preparation. However, the depression was not reversed by naloxone even at high doses (≥3000 nM).

Taken together, our results indicate that HIT may not by itself have opioid properties, but may induce hibernation as a potent precursor or releaser of endogenous opioid ligands.

278.6 MODULATION OF MESOLIMBIC DOPAMINERGIC PROJECTIONS BY BETA-ENDORPHIN IN THE RAT. S. Iyengar, H.S. Kim and P.L. Wood, Neuroscience Research, Research Dept., Pharmaceutical Division, CIBA-GEIGY Corp., Summit, N.J. 07901.

The basal forebrain limbic regions are known to receive a dense innervation of beta-endorphin from the arcuate N. and of dopamine from the ventral tegmental area. In addition, receptor autoradiography with [^{3}H]beta-endorphin has demonstrated a high density of putative epsilon or beta-endorphin binding sites in the N. accumbens. To study the interactions of these systems, we examined the potential modulation of the dopaminergic nerve endings in the N. accumbens and olfactory tubercle by beta-endorphin.

Intraventricular beta-endorphin stimulated dopamine metabolism in the N. accumbens and olfactory tubercle as evidenced by increased levels of DOPAC. These actions were dose dependent and reversed by the opiate antagonists naloxone and WIN 44441.

Since beta-endorphin has high affinities for mu, delta and epsilon receptors, we performed tolerance experiments to determine if a specific epsilon receptor was involved in the actions of beta-endorphin on DOPAC levels in the N. accumbens. For these experiments, 2 groups of animals were prepared: 1) tolerant to ascending doses of ip morphine and intraventricular DADLE; and 2) tolerant to an ascending dose of intraventricular beta-endorphin. Using this design, in the beta endorphin tolerant animals, morphine was still active while the actions of beta-endorphin were reduced. In the morphine/DADLE tolerant animals, beta-endorphin was still active while the action of morphine and DADLE were reduced.

These data are therefore suggestive of an epsilon mediated action of beta-endorphin in the rat N. accumbens.

278.7 **MU ANTAGONIST PROPERTIES OF KAPPA AGONISTS IN CENTRAL CONTROL OF BLADDER MOTILITY IN RATS.** Frank Porreca, Linda Nunan and Russell J. Sheldon. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724

Previous studies have shown that intracerebroventricular (i.c.v.) mu and delta opioid agonists inhibit volume initiated, spontaneous micturition contractions in the rat, while kappa agonists do not. (Dray and Metsch, Eur. J. Pharmacol. 104: 47, 1984). Recent findings from a number of laboratories have shown that kappa agonists block some, but not all, of the pharmacological actions of agents that have been classically termed mu agonists (Wood, Drug Dev. Res. 4: 429, 1984; Holaday et al., Fed. Proc. 44: 2860, 1985). In the present study, possible mu antagonist properties of 3 putative kappa agonists, U50,488H (U50), tifluadom (TIF) and ethylketocyclazocine (EK), as well as dynorphin-(1-17)(DYN) were tested against 9 mu agonists of various structure. Female, Sprague-Dawley rats were anesthetized with ketamine HCl (100 mg/kg, i.p.) and supplemented with urethane (1.2 g/kg, i.p.) as needed. The bladder was catheterized via the urethra and filled with warm saline until spontaneous contractions occurred as a result of central reflex activity. All compounds were were given i.c.v., with antagonists given 15 min prior to agonists. Administration of mu agonists [D-Ala^2, NMPhe^4, Gly-ol]enkephalin (DAGO, 0.01 nmol), [NMPhe^3, D-Pro^4]enkephalin (PL017, 0.03 nmol), morphine (0.08 nmol), normorphine (0.3 nmol), sufentanil (0.002 nmol), etorphine (0.004 nmol), phenazocine (17 nmol), meperidine (176 nmol) or the delta agonist, [D-Pen^2, D-Pen^5]enkephalin (DPDPE, 15 nmol) all inhibited contractions for periods of 20-30 min. The i.c.v. kappa agonists U50 (22 nmol), EK (3 nmol), TIF (3 nmol) and DYN (0.23 nmol) were unable to alter bladder activity. Unlike U50, higher doses of EK, TIF and DYN produced consistent suppression of bladder contraction. Pretreatment with sub-agonist doses of the kappa agonists consistently blocked the agonist effects of morphine and normorphine, but failed to antagonize DAGO, PL017, phenazocine, meperidine and DPDPE. Mu agonist effects of etorphine and sufentanil were antagonized by U50 and DYN but unaffected by EK and TIF. In addition, administration of U50 during a morphine-induced bladder shutdown resulted in either an immediate recovery of bladder activity or a shortened duration of action. Furthermore, pretreatment with U50 produced a parallel, rightward shift of the morphine dose-response curve with similar efficacy, suggestive of a competitive interaction. These findings may reflect either (a) the presence of mu-receptor subtypes (isoreceptors) within the central nervous system that are differentially antagonized by kappa agonists, or (b) differences in intrinsic activity of mu agonists at a common receptor, such that less efficacious compounds are more susceptible to antagonism by kappa agonists. Supported by NS 23710 and DK 36289.

278.8 ACTION OF OPIOIDS ON PERIPHERAL SENSORY NERVES: STUDIES USING THE RAT INFRAORBITAL NERVE MODEL. H.G. Hassan, H. Renck and C.W.T. Pilcher (SPON: S.R. Choudhury) Depts. of Surgery and Pharmacology, Faculty of Medicine, P.O. Box 24923 - Safat, Kuwait.

Opiates exert their profound analgesic effects by activating specific receptors in the CNS at spinal and supraspinal loci. Recent studies have shown that opiates can produce intense and prolonged analgesia following intrathecal and epidural administration but of the opioid drugs used clinically, only pethidine has been shown effective as a sole agent for surgery. This opioid is relatively selective for kappa-receptors, which are richly distributed in the spinal cord. However, at high doses pethidine also has a local anaesthetic action in peripheral nerves. Thus its spinal efficacy may result from conduction block, activation of opioid receptors or a combination of the two. Using the rat infraorbital model (Haggag, H.G. et al Acta Anaesthiol. Scan. 29: 375 - 379, 1985) we have examined further the ability of pethidine and selected opioids to produce peripheral nerve block. Pethidine rapidly produced abolition of nociceptive responses, the duration and degree of which was dependent on opioid doses over the range 1.0 - 5.0 mg/kg. Doses below 1.0 mg/kg did not produce a significant effect whilst at 5.0 mg/kg inhibition of responding was complete. Neither intravenous nor local administration of naloxone at 0.25 - 1.0 mg/kg had any effect on the pethidine-induced analgesia. All of the other opioid agonists examined were without effect in this model. It was concluded that the pethidine-analgesia was not mediated by an opioid receptor mechanism but by inhibition of conduction in the infraorbital nerve by a local anaesthetic action.

This work was supported by Grants MDS 181 and MR 015 from Kuwait University Research Council.

278.9 OPIATE DRUGS ALTER OPIOID PEPTIDE GENE EXPRESSION. J. Ryan*, R. Hanig*, J. Schwartz[1], J. Douglas[2] and G.R. Uhl. (SPON: R. Ackerman). Dept. of Neurology and Howard Hughes Medical Institute, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114. [1]LNGR, NINCDS, Bethesda, MD 20892. [2]IABS, Portland, OR 97201.

Opiate drug-induced alterations in function of brain circuits utilizing opioid peptides have been sought to explain the sequelae of long-term administration of opiate agonists, including tolerance and dependence. Nevertheless, there is still not agreement about the mechanisms responsible for these time-dependent concomitants of opiate drug administration. Since expression of several neuropeptide genes can vary in response to drug-induced alterations in neural function, we have examined the influences of opiate drugs on regional expression of the principle brain opiate peptide genes.

Animals were treated for 5 days with a regimen of increasing doses of morphine free base pellets implanted subcutaneously, brains rapidly removed, total striatal RNA prepared and subjected to quantitated Northern analyses. Blots were hybridized with cRNA probes directed against enkephalin, dynorphin, and somatostatin mRNAs, and with oligonucleotide cDNAs complementary to endogenous actin and tubulin mRNAs as well as B-globin mRNA added as a recovery marker.

Levels of both preproenkephalin and preprodynorphin mRNAs in the rat striatum are reduced by 30-40% following treatment with a 5 day schedule of ascending doses of morphine administered as subcutaneously-implanted pellets. These mRNA levels remain below normal during the animals' expression of withdrawal symptoms. Normal levels of methionine-enkephalin immunoreactivity are found at the end of treatment. Conversely, treatment with the opiate antagonists naloxone or naltrexone increased expression of preproenkephalin and preprodynorphin mRNAs to more than 200% of control levels. These changes in gene expression display the appropriate directions and temporal features to fit with a model of opiate tolerance and dependence.

278.10 **STUDIES ON THE PERIPHERAL ANTISECRETORY EFFECT OF OPIOIDS IN THE MOUSE SMALL INTESTINE.** R.J. Sheldon*, M.E. Malarchik*, F. Porreca and T.F. Burks. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

In recent years, the intestinal epithelia of several species have been suggested to be a possible site of opiate antidiarrheal actions. In the present study, we have investigated the actions of receptor-selective opioids on transepithelial potential difference (PD) and short-circuit current (Isc) in the small intestine of the mouse. Whole jejunal segments from male ICR mice (30-40 g) were cut open into flat sheets and mounted in Ussing chambers: mucosal and serosal surfaces were bathed in standard Krebs-Ringers media (pH 7.4, aerated with 95% O_2/5% CO_2) containing 10 mM mannitol or 10 mM glucose, respectively. Initially, PD and Isc gradually decreased with time, reaching a plateau after 30 min. Basal values (mean $\pm$ s.e.m.; N=30) at 30 min were: PD (-2.4 $\pm$ 0.2 mvolt; serosal positive), Isc (76.7 $\pm$ 4.6 uamp/cm^2) and tissue resistance (Rt)(30.6 $\pm$ 1.3 ohms·cm^2). Most tissues exhibited slow oscillations in PD (0.1-0.3 mvolt amplitude) and Isc (15-20 uamp/cm^2 amplitude) after 10 min in vitro. Tetrodotoxin (TTX) (0.1 uM; serosal media) caused a rapid reduction in basal PD and Isc, and eliminated the spontaneous oscillations of PD and Isc, suggesting tonic neural control of basal intestinal transport. In TTX-pretreated tissues, carbachol (10 uM) and theophylline (1 mM) produced prominent secretory responses (ΔIsc > 100 uamp/cm^2 increase), indicative of intact epithelia. Serosal addition of atropine (1-10 uM), however, had no effect on basal PD or Isc. The delta-selective opioid agonist [D-Pen^2, D-Pen^5]enkephalin (DPDPE) (0.003-1 uM) produced a sustained concentration-related decrease in PD and Isc when added to the serosal media. At higher concentrations, [D-Ala^2, NMePhe^4, Gly-ol]enkephalin (DAGO) (0.1-3 uM), a mu receptor agonist, and U50,488H (>3 uM), a kappa receptor agonist, caused similar reductions in PD and Isc when added to the serosal media. All of the opioid agonists abolished spontaneous oscillations in PD and Isc. Antisecretory effects of DPDPE (0.1 uM) and DAGO (1 uM) were reversed by naloxone (1 uM) and ICI 174,864 (1 uM), a delta receptor antagonist; effects of U50,488H were not reversed at these antagonist concentrations. Pretreatment of tissues with TTX (0.1 uM; serosal media) abolished the effect of DPDPE (10 uM). In contrast, DPDPE (1 uM) produced a prominent antisecretory effect in the presence of atropine (10 uM); 10 uM carbachol was completely blocked by this dose of atropine. These data suggest that delta opioid receptors are involved in the regulation of fluid and electrolyte transport processes in the small intestine of the mouse. Furthermore, peripheral regulation of transport by opioids appears to be mediated through non-cholinergic neuronal mechanisms. (Supported by NS 23710, DA 02163 and DK 36289.)

279.1 CHOLINERGIC MECHANISMS IN MOTONEURONS OF LOBSTER CARDIAC GANGLION. Joseph E. Freschi and David R. Livengood. Neurology Dept., Emory Univ., Atlanta, GA 30322 and Physiology Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

We studied the post-synaptic effects of acetylcholine (ACh) and nicotinic and muscarinic agonists on large motoneurons in the cardiac ganglion of Homarus americanus. Neurons were penetrated with one or two microelectrodes and studied under current- and voltage-clamp. To improve space-clamp, axons were ligated within several hundred micrometers of the soma (Tazaki & Cooke, J. Neurophysiol., 56:1739, 1986). At resting membrane potentials of -50 ± 5 mV, ACh caused a dose-dependent membrane depolarization at concentrations between 0.01 and 1 mM. Nicotinic responses were variable among ganglia. Concentrations between 0.1 and 1 mM were required to cause maximum depolarization, and these responses were invariably less than half the amplitude of the responses elicited by similar doses of ACh. Muscarine, however, in the same dose range, caused depolarizations 60-90% as large as those caused by ACh. Under voltage-clamp, muscarinic agonists caused inward currents of 1-3 nA. The response became larger at more positive and smaller at more negative holding potentials; no response to muscarinic agents could be obtained at potentials negative to -100 mV. We were unable to demonstrate a reversal potential for the agonist-induced current, even after shifting E_K to more postive potentials by increasing $[K]_o$ to 50-150 mM (normal 15 mM). The response amplitude was increased when $[K]_o$ was decreased to 1.5-5 mM. We examined the effect of cholinergic agonists on voltage- and time-dependent currents and could not identify a specific current uniquely affected by the drugs. Often no change in membrane conductance could be seen; the I-V curves before and after agonist were parallel. When a change was seen, it appeared to involve a reduction in leak current. The net effects of this change were a reduction in amplitude of total inward current following hyperpolarizing steps, and an enhancement of inward calcium current and reduction of total outward current following depolarizing steps. The agonist-induced inward current was blocked by 50 mM tetraethylammonium and 10 mM barium, but not by 10 mM 4-aminopyridine, 5 mM cesium, or 5 mM manganese.

We conclude that in motoneurons of lobster cardiac ganglion (1) ACh acts on both nicotinic and muscarinic receptors, but the muscarinic effect is predominant; (2) the muscarinic agonist-induced inward current is caused in part by a reduction of a resting potassium conductance; and (3) either the affected potassium conductance may not be voltage- and time-dependent or it may be generated in unclamped regions of the dendrites not excluded by axonal ligation. (Supported by NIH grant NS22628).

279.2 VASOACTIVE INTESTINAL POLYPEPTIDE MOBILIZES INTRACELLULARLY BOUND CALCIUM BY GENERATING INOSITOL TRISPHOSPHATE TO CAUSE EXOCYTOTIC SECRETION OF CATECHOLAMINES FROM RAT ADRENAL GLAND. R.K. Malhotra*, T.D. Wakade* and A.R. Wakade (SPON: K. Koizumi), Department of Pharmacology, SUNY Health Science Center at Brooklyn, N.Y. 11203

Secretion of catecholamines from the rat adrenal gland has been believed to be regulated by the activity of splanchnic neurons. Splanchnic neurons release acetylcholine, a principal transmitter, which activates cholinergic receptors of the chromaffin cells to secrete catecholamines. Our most recent study shows that in addition to acetylcholine some other non-cholinergic substance(s) might be released from splanchnic neurons to induce catecholamine secretion (Malhotra & Wakade, J. Physiol. 383, 639, 1987). At present the biochemical nature of such substance(s) has not been identified. Among various peptides tested (enkephalin, enkephalinamide, etorphine, etc.) vasoactive intestinal polypeptide (VIP) proved to be as potent as acetylcholine in evoking the secretion of catecholamines by directly stimulating the chromaffin cells (Malhotra & Wakade, J. Physiol., in press) Present study was aimed at determining the role of Ca in VIP-induced secretion of catecholamines. We now show that omission of Ca from the perfusion medium had almost no effect on VIP-induced secretion; however, addition of 1 mM EGTA to Ca-free medium abolished the secretion. Stimulation with VIP did not result in a net increase in Ca^{45} uptake, and the uptake was not modified by phorbol 12,13-dibutyrate, a protein kinase C activator. All these results were very similar to those obtained with muscarine. In contrast, nicotine-evoked secretion was associated with an increase in Ca^{45} uptake and this effect was further enhanced by the phorbol ester. VIP (0.3 to 10 μm) and muscarine (30-100 μm) produced time- and concentration-dependent increase in the production of ^{3}H-inositol phosphates. A VIP antagonist, Ac-Tyr-hGRF (10 μM), reduced VIP-induced ^{3}H-inositol phosphates production by about 70%. The production of ^{3}H-inositol phosphates by VIP and muscarine occured in Ca-free and EGTA medium. Although nicotine produced a brisk secretory response, there was no increase in ^{3}H-inositol phosphates. These findings suggest that inositol 1,4,5-trisphosphate generated upon activation of VIP or muscarine receptors is linked to exocytosis through mobilization of intracellularly bound Ca ions.

279.3 PROTEIN KINASE-C SUPPRESSES OUTWARD K^+ CURRENTS IN ISOLATED RAT VENTRICULAR MYOCYTES. M. Apkon, R. Miyake*, R. Gross*, J.M. Nerbonne. Departments of Pharmacology and Internal Medicine, Washington Univ. Sch. of Med., St. Louis, MO 63110

Previously, we reported the specific suppression of depolarization-activated outward K^+ currents in rat ventricular myocytes by α_1-adrenergic agonists. As phorbol esters and diacylglycerol analogues mimicked the effects of α_1-agonists, we postulated that protein kinase-C (PKC) activation underlies K^+ current suppression. In order to evaluate the involvement of PKC, we have examined the effects of exogenously supplied PKC on outward K^+ currents directly. PKC was purified from rat brain (Kitano et. al. (1986) Meth. Enzym. 124:349-353) and stored at -80°C in 10% glycerol with (in mM): 20 Tris; 0.5 EGTA; 0.5 EDTA; 10 β-mercaptoethanol. For physiological experiments, the PKC solution was dialyzed for 4 hours at 4°C against Tris (2mM) buffered KCl (135 mM) with 0.5 mM EGTA; after enzyme activity was assayed, the kinase was diluted 1:1 in pipette recording solution (see below) to a final PKC concentration of 15 μg/ml.

With Co^{2+} (5 mM) and TTX (20 μM) in the bath to block voltage-gated inward Ca^{2+} and Na^+ currents, whole-cell K^+ currents were measured during depolarizations to potentials positive to -30 mV from a holding potential of -60 mV; recording pipettes contained (in mM): 135 KCl; 10 EGTA; 10 HEPES; 5 Glucose; 3 Mg-ATP; 0.5 Tris-GTP. After control K^+ currents were measured, a whole-cell recording was established with a second pipette, which contained the diluted PKC solution, and K^+ currents were remeasured. The waveforms of K^+ currents after introduction of the PKC-containing pipette were then compared with the control K^+ currents measured in the same cell. In all experiments, the accuracy of the voltage clamp was monitored with the PKC-containing pipette and only data obtained from cells in which this pipette faithfully recorded a 20 mV voltage-step applied to the first pipette were accepted; this criteria minimized errors caused by damage from the second pipette. In all cells examined (n=3), peak outward K^+ currents, evoked by depolarizations to +50 mV, were reduced following introduction of the PKC-containing pipette. The absolute magnitude of the current suppression, as well as the timecourse of this effect, however, varied markedly among cells; mean K^+ current suppression was 22%. In contrast, no effects on K^+ currents were seen when the second pipette contained heat-inactivated PKC (n=2). We conclude that the direct intracellular application of (brain) PKC, at least in a qualitative sense, mimics the effect of α_1-agonists on outward K^+ currents in rat ventricular myocytes. These results, therefore, lend additional support to our postulate that the effects of α_1-adrenergic receptor activation in ventricular myocytes are mediated via a mechanism involving PKC-dependent protein phosphorylation. Support: NIH: T-32 #GM07200, #GM07800, #HL35864, and #HL34161; and AHA: Grant-in-Aid and Est. Inv. Award.

279.4 SELECTIVE EFFECTS OF PHORBOL ESTERS ON CA AND K CURRENTS IN HIPPOCAMPAL NEURONS. D. Doerner, T.A. Pitler and B.E. Alger, Dept. Physiol., Univ. Maryland Sch. Med., Balto., MD 21201.

Several neurotransmitters induce polyphosphoinositide breakdown and activation of protein kinase C (PKC). PKC regulates a number of ionic conductances in peripheral and invertebrate nervous systems, but there is less evidence regarding its effects in mammalian CNS. The hippocampus is rich in PKC and is therefore a good model for studying its role in the brain.

We have used the whole-cell voltage-clamp technique to study the effects of PKC activation on K and Ca currents in hippocampal neurons acutely dissociated from guinea pig (Kay and Wong, 1986, J. Neurosci. Meth. 16:227-38) and tissue cultured from fetal rat (Segal, 1983, J. Neurophys. 50:1249-64). Using phorbol esters to activate PKC, we have asked whether specific current components are subject to PKC regulation.

Electrodes filled with $CsMeSO_3$, ATP and leupeptin were used to identify distinct Ca current components in pyramidal cells. Ca current was identified as a TTX- and low-Na-insensitive inward current also carried by Ba and blocked by Cd. We observed a low-voltage-activated, transient current and a high-voltage-activated current with a large, rapidly inactivating phase and a smaller, slowly inactivating phase. The low-voltage-activated current was seen reliably in tissue culture. Both phases of high-voltage-activated current were seen in acutely dissociated and tissue-cultured neurons, and may correspond to the N and L currents identified in other preparations. Phorbol esters markedly reduced the N-like component, but reduced the L-like component only slightly. The low-voltage-activated transient current, which resembles the T current identified by others, was unaffected.

Using $KMeSo_4$-filled electrodes, we observed large outward currents with both rapid transient and late components. The late current had a large, voltage-dependent component (IK), and a cadmium-sensitive, slowly developing late component (I_{AHP}). When depolarizing voltage-clamp pre-pulses were used to inactivate the rapid transient current, I_K was seen to activate rapidly. Phorbol esters reduced both I_K and I_{AHP} without affecting transient current. Leakage and holding currents were unchanged.

All effects of phorbol esters could be reversed with washing.

We suggest that these effects are due to block of I_K rather than enhancement of calcium influx or block of other currents. I_{AHP} reduction may be due in part to reduction of I_{Ca}, although a direct effect of phorbol esters on IAHP cannot be excluded at present. (Supported by NIH grant NS22010)

279.5 THE LATE IPSP OF HIPPOCAMPAL NEURONS MAY BE CONTROLLED BY A GTP-BINDING PROTEIN THAT IS NOT COUPLED TO ADENYLYL CYCLASE. R.H. Thalmann, D. Grenet* and L. Birnbaumer*. (Spon: B. Lonsbury-Martin) Depts. of Cell Biology, Physiology and Biophysics and Program in Neuroscience. Baylor College of Medicine, Houston, TX 77030.

Orthodromic synaptic stimulation of hippocampal and other forebrain neurons is followed by an early IPSP (peak within 20msec following the stimulus) and then by a late IPSP (peak within 200 msec) that is associated with a potassium conductance and may be mediated by GABAb receptors. We report that the potassium conductance of the late IPSP is blocked by pertussis toxin, an inactivator of one or more GTP-binding proteins (G-proteins) other than Gs. Synaptic potentials were elicited in CA3 neurons of the rat hippocampal slice by an electrical pulse (50usec) to the mossy fiber pathway. When the stimulus was at threshold for elicitation of a postsynaptic action potential the early IPSP conductance was 140 nano Siemens (nS) and reversed at -75 mV, while the late IPSP conductance was 15nS and reversed at -97mV. When the hippocampus was injected with pertussis toxin (2.5ug, 1-3 days prior to recording), no change in the early IPSP could be detected, but the late IPSP conductance was reduced to 2.5nS, and reversed at -76mV. Mossy fiber EPSP's that exceeded control values did not reinstate that late IPSP in toxin treated cells. For example, in control cells, when the membrane potential was set at -95mV, a threshold stimulus was associated with a EPSP slope of 20mV/msec, while the most intense stimulus produced an EPSP with a rise time of 40mV/msec. The late IPSP conductance varied from 15 to a maximum of 30nS over this same range of EPSP slopes. By contrast, in toxin treated hippocampi, the EPSP slope varied similarly from 20-40mV/msec, but the late IPSP conductance increased from 2nS to only 6nS. The toxin treatment also reduced the response to the GABAb ligand baclofen (5um perfused 5-15 mins) but failed to affect the response to THIP (20um), a ligand for the GABAa receptor mediating the early IPSP. Control and toxin treated slices adjacent to those used for recording were frozen immediately after slicing for a subsequent assay of ADP ribosylation of G-proteins The toxin treatment had ADP-ribosylated a significant, but not total, portion of the total toxin substrate. Although pertussis toxin inactivates Gi, perfusion of forskolin (25um for 30-200mins) under conditions that increase cyclin AMP several fold in slices had no effect upon the late IPSP, suggesting that the late IPSP did not depend upon inhibition of cyclic AMP synthesis. By analogy with more direct observations on a similar potassium conductance that is gated by muscarine in cardiac cells (Yatani et al., Science 1987, Vol. 123-207) the late IPSP could depend upon a G-protein (Gk) that is directly linked to the potassium conductance of the late IPSP. Supported by NIH grant NS21713 and DK-19318.

279.6 PIRENZEPINE BLOCKS CALCIUM- AND PI-TURNOVER-RELATED MUSCARINIC ACTIONS IN RAT HIPPOCAMPUS. B. E. Alger and M. McCarren. Dept. Physiol., Univ. Maryland School of Medicine, Baltimore, MD 21201.

Muscarinic cholinergic agonists have a variety of effects on mammalian CNS neurons. Different receptor-effector mechanisms are probably involved, but there are few physiological data on this topic. Some muscarinic agonists may act via protein kinase C. Group A agonists (e.g., carbachol) dramatically increase PI turnover; group B agents (e.g., oxotremorine) are only weak partial agonists for this effect. Phorbol esters that activate protein kinase C and group A muscarinic agonists block the actions of adenosine and other G-protein-linked neurotransmitters; group B agonists are much less effective (Worley et al., PNAS, 1987, in press). Groups A and B are equipotent on other measures. Muscarinic receptors also differ in their sensitivity to block by pirenzepine (PRZ).

We are investigating the relationship between muscarinic effects associated with PI turnover and PRZ sensitivity. We now report that PRZ (1 uM) prevents or reverses carbachol's (50 uM) blockade of adenosine. At 1 uM, PRZ does not affect the plateau of carbachol-induced depolarization, whereas 10-20 uM PRZ strongly antagonizes the depolarization.

In control saline plus TTX, the bath-applied carbachol depolarization has an initial rapid rise. PRZ (1 uM) markedly slows dV/dt. "Low Ca" (nominally zero mM)/2 mM Mn saline slows dV/dt to the same extent as PRZ. The dV/dt of the oxotremorine depolarization is slow in control saline and is hardly affected by either low Ca/Mn or PRZ. Carbachol and oxotremorine produce similar plateau depolarizations.

We conclude that PRZ-sensitive muscarinic actions are associated with PI turnover. They are also associated with a transient Ca-dependent membrane depolarization. PRZ-insensitive receptors mediate maintained membrane depolarization. (Supported by NIH grant NS22010)

279.7 $GABA_A$ RECEPTORS OF HIPPOCAMPAL NEURONS: INTRA- AND EXTRACELLULAR REGULATORY FACTORS. Armin Stelzer* & Robert K.S. Wong (SPON: L.W. Haynes) Dept. Neurology, CPS Columbia University New York, N.Y. 10032.

There is evidence that GABA functions as a neurotransmitter in the CNS and that the GABAergic transmission is highly modifiable. We examined the postsynaptic action of GABA in acutely isolated cells prepared from the CA1 region of the guinea-pig hippocampus (Kay & Wong 1986). Whole-cell voltage-clamp experiments were carried out using low resistant pipettes (2-5 MOhm). K+-currents were suppressed by external TEA, Cs & 4AP, and internally by Tris. GABA (100 uM) was applied by short pressure pulses (10-25 ms) of low frequency (0.01 Hz) to prevent accumulative desensitization. We observed that the GABA-induced outward-current progressively decreased during intracellular recording down to about 10% of the control value after 10 minutes. This spontaneous run-down of the GABA-current was probably caused by alteration of the intracellular content by dialysis and exchange through the recording pipette. The inclusion of a suitable level of free Mg++ and ATP was critical for the maintainence of stable GABA responses.

The stabilized GABA current allowed us to examine the interaction of the GABA receptor with other transmitter agents. Glutamate (10 uM in the perfusate) increased the peak amplitude of the GABA-current to up to 160% over control. Other structurally related compounds such as NMDA and, surprisingly, the NMDA receptor antagonist APV produced a similar enhancement of the GABA-current with comparable efficacy. Short duration hyperpolarizing voltage-pulses applied to estimate the leak conductance showed that the GABA-induced conductance was also proportionally increased in the presence of the modulating agent . Thus the enhancement of GABA-current cannot be attributed to changes in the reversal potential.

The results show that the efficacy of the GABA receptor can be modified by physiologically-occurring agents at both extra- and intracellular membrane sites. The data are particularly interesting in that the modification of GABA-ergic transmission may contribute to long-term changes in the activities of the neuronal circuit following tetanic stimulation.

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279.8 BLOCKADE AND DESENSITIZATION OF A RAPID SEROTONIN RESPONSE IN NG108-15 CELLS AND MOUSE HIPPOCAMPAL NEURONS, Jerrel L. Yakel* & Meyer B. Jackson, Dept. of Biology, UCLA, Los Angeles, CA.

Serotonin(5-HT) produces a rapid depolarizing and desensitizing inward current in NG108-15 cells(a neuroblastoma X glioma hybrid cell line) [Christian et al, Brain Res. 147:261(1978)]. A similar fast desensitizing inward current is one of 3 different 5-HT responses observed in primary cultures of mouse striatum [Yakel et al, Soc. Neurosci. Abstr. 12:726, 1986] and hippocampus, being observed in less than 10% of these neurons.

5-HT produced a fast inward current in 86%(148 cells) of NG108-15 cells. This inward current is associated with a large conductance increase with a reversal potential of 6.5$\pm$4.1 mV(7 cells). Metoclopramide(1 μM), curare(1 μM), and spiperone(20 μM) reversibly blocked this response. 200 μM methysergide reduced this response by 42$\pm$7% (3 cells), while 20 μM methysergide had no effect. The 5-HT agonist 5-methoxy-N,N-dimethyltryptamine did not activate this response. Cobalt (1 mM) was found to reduce the amplitude by 67$\pm$9% (6 cells). This effect probably is not due to a decreased calcium influx, because a 0 mM calcium/2 mM EGTA bath solution did not alter the response. Therefore, the cobalt effect may be the result of a direct block of the 5-HT-gated channel. The pharmacology of this response in NG108-15 cells and cultured hippocampal neurons was found to be similar in the following respects: 1) metoclopramide and curare(both at 20 μM) are antagonists; 2) methysergide(20 μM) has no effect; 3) 5-methoxy-N,N-dimethyltryptamine(10 μM) has no effect. In addition, the time course and reversal potential of these two responses is quite similar. Thus, NG108-15 cells appear to be an excellent system for studying an apparent neuronal response.

The time course of desensitization during continuous 5-HT application(50 μM) in NG108-15 cells was found to be variable between cells, the mean time to 50% desensitization averaging 2.0$\pm$2.6 sec(64 cells). The rate of desensitization during individual experiments usually decreased with time, possibly due to intracellular dialysis by the patch electrode. The time course of desensitization at the beginning of an experiment was usually biphasic, with a fast and a slow component. Several minutes into an experiment, desensitization was monophasic, with only a slow component. Forskolin (30 μM), an adenylate cyclase activator, was found to increase the rate of desensitization; the time to 50% desensitization decreased by 28$\pm$10% (4 cells) after forskolin treatment. The rate of desensitization of the 5-HT response in these cell types may be regulated by phosphorylation.

279.9 PHARMACOLOGICALLY DISTINCT ACTIONS OF 5-HT ON SINGLE NEURONS IN RAT HIPPOCAMPUS. R. Andrade and R.A. Nicoll. Dept. of Pharmacol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104 and Depts. of Pharmacol. and Physiol., Univ. of Calif., San Francisco, San Francisco, CA 94143.

While multiple receptors for 5-HT have been postulated to exist on the basis of binding studies, their relationship to the electrophysiological actions of this amine at the cellular level remain unclear. We have reexamined the actions of 5-HT in the hippocampus using intracellular current-and voltage-clamp recordings in in vitro rat brain slices.

Administration of 5-HT to pyramidal cells of the CA1 region elicits a hyperpolarization which sags during prolonged administration. This hyperpolarization subsides upon removal of the 5-HT and is followed by a longer lasting depolarization which is associated with a decrease in the calcium-activated afterhyperpolarization (AHP). The hyperpolarization exhibits the pharmacological profile of a 5-HT_{1A} binding site and results from a selective increase in potassium permeability. Upon blockade of the hyperpolarization with spiperone or pertussis toxin, the remaining actions of 5-HT could be examined in isolation. The depolarizing action of 5-HT, exhibited a dose-response curve similar to that of the hyperpolarization but the response had a slower time course, and was mediated by the closure of an as yet unidentified potassium conductance. The decrease in the AHP was closely associated temporally with the depolarization. It did not involve reductions in the calcium spike and, as previously reported, it decreased the ability of the cells to show spike frequency adaptation. Both the depolarization and the decrease in the AHP were mediated by 5-HT receptors which do not appear to conform to any of the previously described 5-HT binding sites.

When cell excitability was examined using weak depolarizing current pulses, 5-HT causes an initial decrease in excitability which was followed by a long lasting increase in excitability upon removal of the 5-HT. With strong depolarizing pulses, however, only excitatory actions of 5-HT were evident despite the hyperpolarization. This is due to the blockade of spike frequency adaptation which occurs during and after the hyperpolarization. Thus the overlapping and opposing actions of 5-HT results in an unusual alternation in the input-output curve of the pyramidal cell.

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279.10 PERTUSSIS TOXIN BLOCKS AUTORECEPTOR-MEDIATED INHIBITION OF DOPAMINERGIC NEURONS IN SUBSTANTIA NIGRA AND SEROTONERGIC NEURONS IN DORSAL RAPHE NUCLEUS. R.B. Innis and G.K. Aghajanian. Dept. of Psychiatry, Yale University Sch. of Med., New Haven , CT 06508.

Guanine nucleotide binding proteins (G or N proteins) not only act as signal transducers resulting in stimulation (G_s) or inhibition (G_i) of the synthesis of the classical second messenger cAMP but may also have direct actions on ion channels which are independent of cAMP. Pertussis toxin inactivates the α subunit of at least two G proteins, G_i and G_o. Because the dopamine somatodendritic autoreceptor (of the D_2 subtype) and the serotonin somatodendritic autoreceptor (of the 5-HT_{1A} subtype) may be linked to the inhibition of adenylate cyclase, we have examined the effects of pertussis toxin on electrophysiological inhibtion mediated by these two receptors.

To examine the role of G proteins in receptor-mediated inhibtion of 5-HT neurons, we injected pertussis toxin (0.5-1.0 μg) into rat midbrain in a region immediately rostral to the dorsal raphe nucleus, and we recorded extracellularly from 5-HT neurons 1-15 days after injection. The baseline firing rate of 5-HT neurons in chloral hydrate anesthetized animals was not significantly affected by pertussis toxin. However, in comparison to saline-injected animals, toxin-treated animals showed markedly blunted sensitivity to agonists that act at 5-HT autoreceptors (ipsapirone, 5-HT, and LSD) and to baclofen, a $GABA_B$ agonist. This effect was demonstrated in vivo (with intravenous and iontophoretic application of drugs) and in vitro in the dorsal raphe brain slice preparation. The sensitivity to GABA itself was not significantly decreased with pertussis toxin treatment, consistent with evidence that GABA administered in this manner acts on dorsal raphe cells mainly through $GABA_A$ receptors.

To examine the role of G protein(s) in DA autoreceptor function, rat substantia nigra was injected with 1 μg pertussis toxin, and zona compacta DA neurons were recorded extracellularly 1-7 days after injection. In comparison to saline-injected animals, the toxin-treated animals showed almost no inhibition of DA neurons in response to DA applied iontophoretically or the dopamine agonist (-)-apomorphine given intravenously, although they maintained almost normal inhibitory responses to iontophoretically applied GABA.

This study is part of a series of experiments in which pertussis toxin has been shown to block inhibition mediated by the somatodendritic autoreceptors of the three major monoaminergic systems in rat brain: the α_2-adrenoceptor on norepinephrine-containing cells of the locus coeruleus (Aghajanian and Wang, Brain Res., 371:390, 1986); the D_2 receptor on DA-containing neurons of the substantia nigra (present study); and the 5-HT_{1A} receptor on 5-HT-containing neurons of the dorsal raphe nucleus (present study).

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279.11 NEUROMODULATORY ACTIONS OF NOREPINEPHRINE IN THE THALAMUS: POSSIBLE CONTRIBUTION TO THE ASCENDING CONTROL OF AROUSAL? David A. McCormick and David A. Prince. Dept. of Neurology, Stanford Univ. Sch. of Medicine, Stanford, CA 94305.

The mammalian thalamus is richly innervated by noradrenergic axons and terminals arising from the locus coeruleus. Activation of this ascending noradrenergic system in vivo has been previously shown to enhance the firing of lateral geniculate neurones to other inputs (Rogawski and Aghajanian, Nature, 287:731; Kayama et al., Neurosci. 7:655). Here we investigate the ionic mechanisms by which NE modulates the excitability of thalamic neurones.

Local application of NE to intracellularly recorded thalamic neurones maintained in vitro and located in the guinea pig lateral geniculate (dorsal division), medial geniculate, anteroventral, reticular, and parataenial nuclei resulted in a slow depolarization and decrease in input conductance of 2 to 15 nS. This was a direct effect since block of synaptic transmission by bathing the slices in 0.5 mM Ca^{++}, 5.0 mM Mn^{++} did not block the NE response. I-V plots performed before and during application of NE indicated that the equilibrium potential varied as a Nernstian function of $[K]_o$. Similarly, changing $[K]_o$ from 0.5 to 5.0 mM or visa versa affected the amplitude of the NE response as expected for a K-mediated event. These results indicate that the NE-induced slow depolarization is due to a decrease in K conductance.

Parataenial (Pt) neurones are electrophysiologically distinct from other thalamic neurones in that they display prominent accomodation and slow afterhyperpolarizations. Application of NE to Pt neurones not only caused the slow depolarization mentioned above, but also completely blocked the slow ahp and reduced accomodation without affecting fast ahps.

The NE-induced slow depolarization appears to result from the activation of α_1 receptors since it was activated by the α_1 agonist phenylephrine but not by the α_2 agonist clonidine or the β agonist isoprenaline. The subtype of adrenoceptor mediating the block of the slow ahp in Pt neurones is not yet known.

Thalamic neurones possess three possible excitability states: they generate single spike discharges at membrane potentials above approximately -55 mV, burst discharges at membrane potentials below -65 mV, and are silent when V_m is in between. The NE-induced slow depolarization completely inhibited burst discharges and promoted the occurrence of single spike activity by depolarizing V_m towards -55 mV, inhibiting the slow ahp, and by increasing the slow membrane time constant. These effects allow the ascending noradrenergic system to potently control the prevalence of single spike versus rhythmical burst firing in the thalamus and thereby influence the pattern and excitability of basal neuronal activity throughout the forebrain. Supported by NIH grants NS 12151, NS 06477 and NS 07331.

279.12 WHOLE CELL VOLTAGE CLAMP STUDIES OF ENZYMATICALLY DISSOCIATED DORSAL LATERAL GENICULATE NEURONS. S. Suzuki* and M.A. Rogawski, Neuronal Excitability Section, Medical Neurology Branch, NINCDS, NIH, Bethesda, MD 20892.

We have developed an isolated cell preparation from guinea pig thalamus that is suitable for gigohm seal patch clamp recording. The dorsal lateral geniculate nucleus (LGNd), a brain region that consists of a relatively homogeneous population of principal neurons with well characterized transmitter sensitivity and a small number of intrinsic interneurons, was chosen as a model system. Slices of guinea pig LGNd (1×1×0.6 mm) were exposed to trypsin, mechanically dissociated and plated on polystyrene dishes essentially as described by A.R. Kay and R.K.S. Wong (*J. Neurosci. Methods* 16: 227-238, 1986). Neurons isolated in this manner exhibited roughly spherical morphology with amputated, "stump-like" processes. Patch electrodes, filled with (in mM) 140 K gluconate, 1 $MgCl_2$, 10 HEPES, and 1.1 EGTA, were used for current clamp and voltage clamp recording. Current clamp recordings demonstrated that isolated LGNd neurons had resting potentials of -40 to -50 mV and fired tetrodotoxin (TTX)-sensitive action potentials (AP). In the presence of TTX (2 μM) and tetraethylammonium (20 mM), the cells exhibited longlasting Cd^{2+}-sensitive APs (100 msec) that were followed by slow afterhyperpolarizations (500 msec). Superfusion with GABA (100 μM) produced a rapid hyperpolarization, whereas norepinephrine (10 μM) or the α_1-agonist phenylephrine (25-50 μM) caused a slow depolarization in association with rapid spike firing as expected from previous *in vivo* studies (M.A. Rogawski and G.K. Aghajanian, *Nature* 287:731-734, 1980).

Under whole cell voltage clamp conditions, step depolarization elicited a TTX-sensitive fast inward current and two kinetically distinct outward currents, I_K and I_A. I_K, a slowly rising voltage-dependent outward current demonstrated a tail current that reversed about -70 mV, near the expected equilibrium potential for K^+. When LGNd neurons were depolarized from holding potentials negative to -50 mV, I_A, a rapidly activating and inactivating (transient) outward current, was observed. GABA stimulated a large picrotoxin-sensitive conductance increase with reversal potential about -50 to -60 mV. When KCl was substituted for K-gluconate in the pipette solution, the reversal potential of the GABA activated current shifted to about 0 mV, as expected of a Cl^- current. We conclude that acutely dissociated LGNd neurons exhibit excitability characteristics expected of neurons in the intact brain and are a suitable preparation for studying voltage-sensitive currents and transmitter responsiveness using the patch clamp technique.

280.1 FORMS OF GROWTH AND RETRACTION AT MOUSE NEUROMUSCULAR JUNCTIONS REVEALED BY A NEW NERVE TERMINAL STAIN AND CORRELATIVE ELECTRON MICROSCOPY. N. Robbins and J. Polak*. Dept. of Dev. Genetics & Anatomy, Case Western Reserve School of Medicine, Cleveland, Ohio 44106.

The extent to which adult motor nerve terminals engage in sprouting and remodelling is controversial. To address this issue, we combined a new stain for nerve terminals in whole mounts (Tetanus toxin C fragment coupled to Texas Red) with a stain for acetylcholine receptors (FITC-alpha-bungarotoxin) and used 100x oil objectives. Sprouts were then seen as fine protrusions from terminal branches, with no underlying acetylcholine receptors. In addition, tracings of video-recorded whole-nerve-terminal images were used to determine the extent of nerve terminal protrusion or retraction with respect to post-synaptic folds. Ultrastructure and immunocytochemistry were used to characterize sprouts and protrusions in parallel.

In mature (6-7 mo.) CBF-1 mice, fast twitch muscles had 0-1 sprout per endplate and slow muscles had 2-3. In slow muscle from 1 mo. old mice, sprouts were even more numerous and prevalent. In all muscles, sprouts often occurred in clusters and were always located at the upper (Schwann-cell associated) plane of the nerve terminal. Sprouts were also seen when fixation preceded staining. In young muscle, a 30 min. exposure to 0.1-to 5 ug/ml cytochalasin D decreased the percentage of endplates with sprouts from 81 to 32% (control). In whole mounts of mature soleus muscle, the majority of synapses showed one or more lamellar or angular protrusions of the nerve terminal beyond the receptor region as well as retractions. Such forms usually occupied no more than about 5% of the nerve terminal area.

In about 20% of 117 random sections, EM revealed nerve terminal protrusions at gaps between Schwann cell and muscle, extending about 0.5 um. Semi-serial sections indicated that at least some of these protrusions were lamellar, extending up to 2.3 um along the terminal branch. The protrusions were devoid of vesicles or mitochondria, contained fine filaments, and stained with anti-actin. Retraction of nerve terminals away from post-synaptic folds were also noted.

Thus, mature nerve terminals have filapodia-like sprouts which apparently do not last long enough to induce receptors (at least in young mice) and are readily withdrawn upon exposure to cytochalasin D. In addition, lamellipodia-like protrusions and retractions are additional forms of plasticity. The possible role of these forms in neuromuscular plasticity will be discussed.

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280.2 TRANSIENT FILOPODIAL-LIKE STRUCTURES OBSERVED AT THE LIVING MAMMALIAN NEUROMUSCULAR JUNCTION. Hill, R.* and Robbins, N. (SPON: R.J. Lederman). Department of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, Ohio 44106.

Static observations of the neuromuscular junction (NMJ) in slow twitch muscle has indicated that remodelling may occur at this much studied synapse. Recent observations, utilizing tetanus toxin C fragment conjugated to texas red (TT-TR) as a nerve terminal stain indicate that fine sprouts protrude from terminal branches (Robbins & Polak, this volume). Such sprouts possess no underlying acetylcholine receptors and thus may be newly formed. The purpose of the present research was to examine the possibility that these filopodia-like sprouts have only a transient existence, and as such, would represent a dynamic component of the NMJ.

To address this issue, the slow twitch pectineus muscles of anesthetized young (3-5 wks.) Swiss Webster mice were stained in situ with TT-TR and in final experiments with FITC-alphabungarotoxin as well to visualize underlying acetylcholine receptors. High fluorescence resolution was obtained with a 100x oil objective specially shielded from the preparation. Images were visualized by a SIT camera and recorded onto VCR tape. The incisions were then closed and the animals allowed to recover. The following day, the same procedure was repeated and the same NMJ relocated. Tracings of the recorded NMJ, from day one and day two were then overlaid. In some of the junctions, the number and location of sprouts had not changed while in other day 1 animals, sprouts were absent while new sprouts were generated in different parts of the nerve terminal. Two other stains - 4Di 2Asp and $DIOC_2(5)$ were evaluated for comparison to TT-TR, but neither stained the sprouts described above nor revealed some of the more detailed architecture of the nerve terminal.

This study is the first to identify filopodia-like structures in the living mammalian NMJ and to demonstrate that they are transient and dynamic entities. Studies are currently in progress to determine the fate of these structures as well their role in normal synaptic remodelling and in circumstances such as aging. (Supported by NIH grant AG 06641).

280.3 DYNAMIC ASPECTS OF CORNEAL INNERVATION VISUALIZED IN LIVING MICE. L.W. Harris* (SPON: D. Purves), Departments of Anatomy and Neurobiology and Neurological Surgery, Washington University School of Medicine, St. Louis, Missouri 63110.

Repeated visualization of identified neural elements in living mammals has recently been accomplished using non-toxic fluorescent dyes (Purves et al., J. Neurosci. 6:1051-1060, 1986; Purves et al., Soc. Neurosci. Abstr. 12:390, 1986; Lichtman et al., J. Neurosci. 7:1215-1222, 1987). Here I report the adaptation of these methods to test the anatomical stability of sensory nerve endings in the corneas of adult mice.

Topical application of a dilute solution of the fluorescent dye 4-(4-diethylaminostyryl)-N-methylpyridinium iodide (4-Di-2-ASP, Molecular Probes) labeled neural processes throughout the cornea. Images were acquired with a fluorescence microscope system coupled to a SIT camera and an image processor (op. cit.). Neural staining, which faded within one to two hours, could be repeated after an arbitrary interval.

Vital staining with 4-Di-2-ASP demonstrated the corneal innervation as completely as conventional stains (e. g., methylene blue, gold chloride). Nerves could be seen to enter the cornea radially from the sclera. Within the corneal stroma these nerves ramified to form a plexus, from which finer nerve fibers branched upward and divided dichotomously among the cells of the superficial layers of the corneal epithelium.

Observations of the same region of the cornea were made at intervals of up to two months. Stromal nerves maintained a constant position relative to the iris during this time. Thus, identified stromal nerves could be used to precisely localize superficial nerve endings. Over periods of a few hours, little or no change in the appearance of the same superficial sensory endings was noted. After 24 hours, however, changes in terminal arborizations were apparent (though an overall similarity to initial configuration was retained). After one week, terminal branching patterns bore little or no resemblance to those seen initially; indeed, the position of individual groups of sensory terminals relative to stromal nerves was often totally different. Thus, marked rearrangement of the surface terminals occurs over a period of several days.

These observations suggest that sensory endings within epithelial tissues in adult mammals may be quite dynamic under normal circumstances.

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280.4 REPEATED IMAGING OF NEUROMUSCULAR JUNCTIONS IN MOUSE SOLEUS MUSCLES IN VIVO. Donald J. Wigston, Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322.

Although it has been suggested that neuromuscular junctions (NMJs) in adult skeletal muscle are continually remodelled, the evidence for this is rather indirect. Epi-illumination fluorescence microscopy makes it possible to study a particular NMJ several times in the same animal, separated by arbitrary intervals, to see directly if any changes occur in its shape. Using this approach, Lichtman et al. (J. Neurosci. 7:1215, 1987) have found that NMJs in mouse sternomastoid muscles do not change their shape significantly over several months. However, much of the earlier evidence for remodelling of NMJs in mammals was obtained in soleus muscles. Since it is possible that intrinsic differences between muscles might influence synaptic reorganization, I have monitored identified NMJs in soleus muscles of adult mice by viewing them in living animals.

In preliminary experiments, fluorescent dyes that stain nerve terminals were used, but these stained soleus muscle fibers too heavily to be used routinely. However, the arrangement of postsynaptic acetylcholine receptors (AChRs) provides a reliable indication of the shape of the presynaptic nerve terminal: there is normally an exact correspondence between the shape of a nerve terminal and the arrangement of AChRs, and changes in the configuration of a nerve terminal produce corresponding alterations in the pattern of AChRs. To label AChRs, soleus muscles were exposed in anesthetized mice and bathed for 1 hr with rhodamine-conjugated α-Bungarotoxin (5 μg/ml). This clearly delineated superficial NMJs in the absence of any detectable effect on neuromuscular transmission. Labeled junctions were examined with epi-illumination using a 50X water-immersion lens (NA = 1) and an intensified video camera (SIT) operated at maximum gain so that low levels of incident light could be used. Images were digitally enhanced and recorded on videotape.

Staining and viewing were repeated 7-131 days later. Individual NMJs were easily identified from the original recordings as the overall morphology of each NMJ was remarkably similar each time. Minor rearrangements were detected in some NMJs viewed several months apart, however. Occasionally small parts of the original NMJ were not apparent at the second viewing (deletions); sometimes, extension of existing branches or their appearance de novo (additions) was observed. I have not so far observed such changes in NMJs studied at the shortest times after the initial viewing. The modest changes observed suggest that NMJs in adult mouse soleus muscles undergo only a minor degree of spontaneous remodelling.

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280.5 OBSERVATIONS ON THE ASSOCIATION OF SATELLITE CELLS AND SYNAPSES IN AN AUTONOMIC GANGLION. S.L. Pomeroy and D. Purves, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

Neurons in mammalian autonomic ganglia are associated with satellite cells (glia) whose function is not yet understood. Recently, gradual changes in the arrangement of synaptic terminals on the surfaces of neurons in mouse salivary duct ganglia have been described in normal adults (Purves et al., Soc. Neurosci. Abstr. 12: 390, 1986). Here we report some observations on the relationship of satellite cells and synapses in these ganglia which may be pertinent to ongoing synaptic rearrangement.

Both light microscopic observations of adult mouse salivary duct ganglia after methylene blue staining in vivo, and electron microscopical examination of fixed ganglion cells, indicate that the position of glial nuclei on the surfaces of these neurons tend to mark sites of increased numbers of preganglionic endings. Although this relationship is not strict--synaptic boutons are often observed on regions of the neuronal surface away from the satellite cell nuclei--the association is significant. Furthermore, the neuronal surface near glial nuclei often bears increased numbers of finger-like extensions, which are the usual sites of synaptic contact.

In the course of observing individual ganglion cells over time in living mice (Purves et al., J. Neurosci. 6: 1051-1060, 1986), it became evident that satellite cells can be seen in vivo with the same techniques (video microscopy and image processing) used to monitor nerve cells. In order to determine whether glia change their relationship to the nerve cells they invest, we made repeated observations of identified neurons and their associated satellite cells in unstained ganglia over periods of several weeks. In some of the cases that we followed, the position of a glial nucleus changed substantially within 1-3 weeks. In other cases, we observed the disappearance of an earlier identified nucleus, or appearance of a nucleus not previously seen.

Although these findings admit a number of explanations, our observations indicate that the position of satellite cell nuclei may be a useful marker of synaptic sites in living animals. They also raise the possibility that satellite cells play a specific role in the maintenance or rearrangement of synapses on these neurons.

Supported by NIH grants NS 18629 and 11699.

280.6 THE ROLE OF NMDA RECEPTORS IN LONG-TERM POTENTIATION IN THE PYRIFORM CORTEX: EFFECTS OF KETAMINE. D. K. Patneau and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Previous research from our laboratory has demonstrated a selective long-term potentiation (LTP) of the pyriform cortex (PC) potential evoked by olfactory bulb (OB) stimulation which appears to be functionally inhibitory (Soc. Neurosci. Abstr. 11:780, 1985). Period 1 of the evoked potential, representing both a mono- (A1) and di-synaptic (B1) EPSP in PC pyramidal cells, remains unchanged, while period 2, which is temporally associated with IPSPs, is dramatically increased by repeated high-frequency stimulation. The selective potentiation observed cannot be produced by stimulation restricted to the lateral olfactory tract (LOT), indicating that other pathways, activated either alone or in concert with the LOT, are involved in LTP produced by OB stimulation (Soc. Neurosci. Abstr. 12:508, 1986).

An extensive association fiber system in the PC, whose level of activation is reflected by B1 in the field potential, is a strong candidate for involvement in the production of LTP. Activation of N-methyl-D-aspartate (NMDA) receptors by glutamate has been implicated in the development of LTP in the hippocampus. Glutamate or an analogue is presumed to be the neurotransmitter of the association fibers in the PC. The present study therefore utilized an NMDA antagonist, ketamine, to investigate the role of NMDA receptors, presumably activated by association fibers, in the development of LTP in the PC.

Male Long-Evans rats were chronically implanted with electrodes in the OB and PC and an intravenous catheter. The effects of ketamine (5 mg/kg) on the PC evoked potential and on the induction and expression of LTP were examined. Half of the animals received ketamine immediately before thirty 100 Hz trains of 10 pulses each, and half received ketamine following the trains.

A number of measures were used to assess differential changes in the LOT volley, A1, B1, and period 2 of the PC evoked potential. Ketamine's primary effect was inhibition of B1, with no alteration of the LOT volley, indicating that input from the OB was unaffected. Ketamine significantly retarded the development of LTP when given prior to the potentiating trains. When given following the trains, it temporarily antagonized the expression of LTP, but did not block its development.

These results indicate a role for NMDA receptors in pyriform cortex LTP, and are consistent with a model for its development involving coactivation of LOT and association fibers.

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280.7 LONG-LASTING POTENTIATION IN TRANSMISSION DEVELOPS IN CHRONIC PREPARATIONS FOLLOWING SYSTEMIC ADMINISTRATION OF KAINIC ACID. N. W. Milgram, C. Sawicki*, J. Rick* and G. O. Ivy. Life Science Division, Scarborough Campus, U. Toronto, Scarborough ONT, M1C1A4.

Systemic administration of kainic acid (KA) produces sustained activation of limbic system structures which can lead to repeated afterdischarge and eventually to convulsive seizures. This treatment also has long lasting consequences as indicated by cognitive deficits, hyperactivity (Milgram, N. W., Soc. Neurosci. Abstr., 12, 1375, 1986) and the development of spontaneous seizures. The present work illustrates some physiological correlates of these long lasting changes in rats implanted with chronic stimulating electrodes in the angular bundle and with recording electrodes in the the dentate gyrus. Testing involved three phases: an initial period of stabilization, a treatment phase which involved a subcutaneous injection of KA (10mg/kg) and a post-treatment phase during which field potentials were monitored for up to 4 months. One group of animals received KA only, while a second group was also administered sodium pentobarbitol.

The immediate response to KA was a progressive decrease in field potentials and by two hours post-injection, they were virtually gone. Over subsequent days field potentials exhibited substantial growth resulting in both a potentiation in amplitude and a decrease in threshold compared with the pretreatment interval. The animals which were not administered pentobarbitol showed a similar growth, except that it occurred over a much longer interval and was preceded by a longer period of suppression. Tests using paired-pulses indicated that these results were not due to a selective suppression of inhibition. Histological analysis revealed extensive cell death in the animals given KA alone but not in the animals which were also treated with pentobarbitol.

The phenomenon we describe provides a possible neural correlate of memory which differs in two important respects from the long-term potentiation(LTP) induced by electrical stimulation. First, the KA-induced potentiation shows a growth which may continue over an extended interval of time while LTP is maximal shortly after the treatment. Second KA produces a permanent potentiation while LTP decays by a few weeks.

280.8 LATENT EXCITATORY SYNAPTIC PATHWAYS REVEALED AFTER TETANIC STIMULATION IN THE HIPPOCAMPUS. R. Miles* and R.K.S. Wong Dept. of Neurology, Columbia University, New York NY 10032.

Plasticity of hippocampal synapses is usually studied by stimulating a fibre pathway and monitoring changes in responses evoked in a single neuron or in the post-synaptic population. This technique precludes a direct examination of the possibility that changes occur at synapses between post-synaptic cells. We recorded from pairs of CA3 cells in slices from guinea pig hippocampus to examine whether tetanic stimulation affects recurrent excitatory or inhibitory synaptic circuits.

Two pathways which synapse with CA3 cells were stimulated repetitively (20-50 Hz, 5-10 sec x 2). 1. mossy fibres in transverse slices. 2. longitudinal association fibres in longitudinal slices from the CA3 region. Recordings were made from cell pairs where no interaction was detected initially. In 5 of 48 cases in transverse slices and in 3 of 22 cases in longitudinal slices latent excitatory interactions were revealed several minutes after tetanisation and persisted for at least 20 minutes. The latency and probability that post-synaptic events failed suggested that new pathways were poly-synaptic.

Since pharmacological disinhibition increases functional excitatory connectivity in the CA3 region (J. Physiol. 388, 1987) we asked whether tetanic stimuli modified recurrent inhibitory interactions. Recordings were made from pairs of burst firing cells, one of which could evoke inhibitory post-synaptic potentials in the other. The strength of di-synaptic inhibitory coupling was assessed by averaging post-synaptic responses to pre-synaptic bursts. It was reduced by more than 20% from its control level in 15 of 20 cell pairs when tested at 12-20 minutes after tetanic stimulation.

These modifications at intrinsic synapses changed activity in the post-synaptic population. In 21 of 36 cell pairs from both transverse and longitudinal slices simultaneous synaptic events of amplitude greater than 5 mV emerged after tetanic stimulation. They could be triggered by a single cell and probably reflect simultaneous activity in small groups of CA3 cells. The formation of groups of associated cells after tetanic stimuli and the ability of some cells to initiate simultaneous firing in a group via recurrent excitatory pathways may represent a mechanism for information storage and recall.

280.9 SUBCELLULAR DISTRIBUTION OF PROTEIN KINASE C (PKC) IN RAT BRAIN REGIONS SUGGESTS THAT HIPPOCAMPUS HAS THE HIGHEST CAPACITY FOR KINASE TRANSLOCATION, AND THAT THIS CAPACITY MAY BE DIURNALLY REGULATED. F-S. Sheu*, K. Murakami*, and A. Routtenberg. (SPON: M. deJonge). Cresap Neurosci. Lab., Northwestern Univ., Evanston, IL 60201.

PKC is active when present at the plasma membranes and its activation may occur by translocation from cytosol to membrane [Nature (1985) 317 546, J. Biol. Chem. (1985) 260 15718]. The subcellular PKC distribution in a given brain region may thus indicate the level of PKC activity in that region as well as its capacity for futher activation in response to extracellular signals. We thus assayed cytosol/membrane distribution of PKC activity in eight regions of adult rat brain.

Preliminary experiments indicated that: (1) maximum PKC activity could be extracted by 1% and 2% of Triton X-100 from membrane and crude homogenate fractions respectively; (2) a minimal amount of divalent cation chelator (0.1 mM EDTA) in homogenization buffer was sufficient to prevent proteolysis of PKC and minimized PKC extraction from membranes. Thus we used these conditions during tissue preparation in our studies of PKC distribution.

We observed a significant difference in the total PKC activity (cytosol plus membrane fraction, C+M) across the eight regions examined ($F=5.09$, $df=7,63$, $p<0.001$). The order of total PKC activity among the eight brain regions was: hippocampus>frontal cortex>neostriatum> septal area>cerebellar cortex>thalamus>entorhinal cortex >hypothalamus. In addition, there were significant differences in cytosol and membrane PKC activity among these eight brain regions ($F=5.68$ $df=7,63$ $p<0.001$ for cytosol; $F=2.21$ $df=7,63$ $p<0.05$ for membrane). The hippocampus had the highest level of cytosol PKC activity of the brain regions. Moreover, the mean proportion of cytosolic PKC activity (C/C+M) in hippocampus (0.53 ± 0.04) was consistently higher than that of other brain regions, such as neostriatum (0.39 ± 0.07), thalamus (0.40 ± 0.04) and cerebellum cortex (0.40 ± 0.06). Hippocampus may thus have the highest capacity for PKC translocation in response to transmembrane signals. In a separate experiment, we found that rats sacrificed at midnight when behavioral activity was high, had a significantly lower C/C+M ratio in hippocampus (0.447 ± 0.022) than that of rats sacrificed at noon when behavioral activity was low (0.520 ± 0.016) ($t=2.71$, $df=6$, $p<0.025$). The subcellular PKC distribution in rat hippocampus might thus display a diurnal rhythm possibly related to the animals' arousal state. (Supported by MH25281-13, and AFOSR-0042).

280.10 COOPERATIVE ACTION OF Zn(II) AND Ca(II) IN THE REGULATION OF PROTEIN KINASE C ACTIVITY FROM RAT BRAIN K. Murakami*, M.K. Whiteley*, and A. Routtenberg. Cresap Neurosci. Lab. Northwestern Univ. Evanston, IL 60201

Recently, PKC has been shown to have a cysteine repeating sequence which could be a potential metal binding site(s). We have reported that purified protein kinase C (PKC) can be fully activated by cis-fatty acid in the absence of Ca2+ and phospholipid. This suggests that PKC may be activated by another signaltransduction pathway, possibly cis-fatty acid liberation by Ca2+-dependent phospholipase A2 (PLA2) in addition to PLC/PI breakdown mechanism (FEBS Lett. (1985) 192:189, J.Biol. Chem. (1986) 261:15424). Since cis-fatty acid does not require Ca2+ for PKC activation, it is possible to examine the effects of metal ions on protein kinase C independently of Ca2+. Here we show a specific interaction of Zn2+ with protein kinase C and a positive and negative cooperativity with Ca2+.

At low concentrations (~5uM) of Ca2+, Zn2+ (~300uM) shows significant augmentation of PKC induced both by cis-fatty acid and by PS/diolein. In contrast, 300uM Zn2+ inhibits PKC activity at high concentrations (over 50uM) of Ca2+. In the absence of Ca2+, Zn2+ neither inhibits PKC activity induced by cis-fatty acid nor activates PKC with phospholipid and diacylglycerol. The dual and opposite effect of Zn2+ indicates that PKC has high and low affinity Ca2+ binding sites and at least one Zn2+ binding site and that Zn2+ does not interact with PKC unless Ca2+ occupies its high affinity site(s).

Our laboratory has proposed that PKC play an important role in synaptic plasticity in rat hippocampus: 1) an increase in membrane associated PKC activity following long-term potentiation (LTP)(Science (1986) 231:587) 2) an increase in phosphorylation of protein F1 (Behav. Neural. Biol. (1985) 43:3, Brain Res. (1986) 399:205), a PKC substrate (Brain. Res (1985) 334:147, Exp. Neurol.(1985) 89:213, J. Neurosci. (1986) 6:3618) 3) prolonging effects of LTP by PKC activators (Brain Res (1986)378:374, 379:358). Zn2+ concentration has been known to be high in hippocampus, especially in mossy fiber system (~490uM in wet tissue). Interestingly, chronic Zn2+ deficiency alters neural activity in the same system. Thus, it is possible that the cooperative action of Zn2+ and Ca2+ plays a role in synaptic physiology in mossy fiber system. (MH25281-13 and AFOSR 87-0042 to A.R.)

280.11 THE IDENTIFICATION OF PROTEIN F1 IN MAMMALIAN NERVOUS TISSUE USING AFFINITY-PURIFIED ANTIBODY AGAINST CALF PROTEIN F1. S.Y. CHAN, C. HASKELL* AND A. ROUTTENBERG. Cresap Neuroscience Laboratory, Northwestern University, Evanston, Ill. 60201.

The *in vitro* phosphorylation of protein F1 (Mr=47K, pI=4.5) has been found to be directly correlated with synaptic plasticity of long term potentiation (*Behav. Neural Biol.*,1985,43,3-11). Protein F1 has recently been co-identified with neuronal growth-associated proteins GAP43, pp46 and B-50, and as such may play a role in nerve growth and regeneration as well.

Protein F1 has been purified from rat synaptosomal membranes using column chromatography methods (*J. Neurosci.*, 1986, 6, 3618-3627). It was found to be phosphorylated by purified protein kinase C (PKC) but by no other kinases tested. Using the same purification method, protein F1-like molecules can be isolated from rat, calf and human but not from Aplysia. Aside from a slightly different molecular weight (rat=47K, human=53K, calf=55K), these molecules have very similar physical and biochemical properties to rat F1 including pI, PKC phosphorylation, V8 protease map and cross-reactivity by F1-specific antibodies. Such results indicated that protein F1 is generally present in mammalian nervous tissues.

Rabbit antisera were raised from purified calf F1 and subsequently purified by a calf F1-conjugated affinity column. This affinity-purified antibody specifically immunoprecipitated F1 from human, monkey, calf and rat but no cross-reactivity was observed using Aplysia nervous tissue. Initial results suggested that the antibody was capable of blocking F1 phosphorylation by PKC in a dose-dependent manner.

Preliminary immunolocalization studies using paraffin-embedded rat hippocampal and cortical sections showed staining by the F1-antibody in the hippocampus and frontal cortex. In the hippocampus, the heaviest staining was found in the stratum radiatum near the hippocampal fissure. Other areas of staining included the molecular layer of dentate gyrus, CA3 cell area, entorhinal cortex and subicular complex. Staining seemed to be confined to lamina containing neuronal projections of the hippocampal formation and no specific staining was observed in the pyramidal and granule cell bodies. (This work was support by MH25281-13 and AFOSR-0042)

280.12 ULTRASTRUCTURE OF DENDRITIC SPROUTS SEEN FOLLOWING CLOSE AXOTOMY OF GIANT CENTRAL NEURONS IN THE LAMPREY G.F. Hall, A. Poulos and M.J. Cohen. Dept of Biology, Yale University, New Haven, CT 06520

Axotomy of anterior bulbar cells (ABCs), a group of giant Muller interneurons in the hindbrain of the sea lamprey (Petromyzon marinus), results in dendritic sprouting if the site of axotomy is located close to the cell body (<500 um) (Hall & Cohen, Science 222 pp. 518-521 1983). In order to determine whether dendritic sprouts evoked by axotomy are intrinsically dendritic or axonal in nature, we have examined the ultrastructure of the dendrites, axon and dendritic sprouts of ABCs 2 months following close axotomy and compared it with the dendritic and axonal ultrastructure of intact ABCs.

The dendrites and axons of intact ABCs were distinguished by the following ultrastructural criteria: (1) the predominance of microtubules in the dendritic cytoskeleton and of neurofilaments in that of the axon, (2) the presence of polyribosomes and large numbers of mitochondria in the dendrites and their respective absence and scarcity in the axon, and (3) the exclusively postsynaptic status of the dendrites versus the presynaptic status of the axon.

We found that all neuritic sprouts evoked by axotomy resembled axons rather than dendrites whether they originated from the axonal stump or the dendrites. The dendrites of axotomized ABCs with dendritic sprouts possessed a mixture of dendritic and axonal characteristics, having the dendritic pattern of synaptic relations combined with axonlike cytoskeletons.

We conclude that axotomy of ABCs close to the soma causes axonal regeneration to occur from ectopic sites in the dendrites. Our results also suggest that the materials needed for axonal regeneration (such as axonal cytoskeletal elements) may be transported through the dendrites to dendritic sprouts following close axotomy, displacing the dendritic cytoskeleton while leaving dendritic synapses intact. Supported by NIH # NS-10174

281.1 CALCIUM CHANNELS IN TWO TYPES OF ANTERIOR PITUITARY CELLS IDENTIFIED BY A PLAQUE ASSAY. S.A. DeRiemer. Dept. of Biological Sciences, Columbia Univ., New York, NY 10027.

The presence of multiple types of calcium channels in many cells raises the question of what impact the existence of any one of these has on a cell's physiology. To address this question we have examined the calcium channels present in two cell types from the anterior pituitary gland, growth hormone secreting somatotrophs and prolactin secreting lactotrophs (DeRiemer & Sakmann, Exp. Brain Res. Ser. 14:139-154, 1986).

Primary cultures of rat anterior pituitary cells were prepared and maintained using standard techniques. Cells of a given type were identified using the reverse hemolytic plaque assay of Neill & Frawley (Endocrinol. 112:1135-37, 1983). Hormone secretion was measured by radioimmunoassay on fractions collected from a perifused cell column with materials supplied by the NIADDK. Patch clamp recordings of ion channels in identified cells were carried out according to standard methods.

Preliminary results indicated that these cells contained two classes of calcium current, low-voltage activated (Type I, T) and high-voltage activated (Type II, L), and that lactotrophs and somatotrophs had different relative amounts of the two currents. In further examination, it appears that both cell types have aproximately the same amount of the high-voltage activated current when records are normalized to cell capacitance, while the absolute level of low-voltage activated current is significantly higher in the lactotrophs, both with respect to the somatotrophs and to the other calcium current. The high-voltage activated current in these cells is sensitive to dihydropyridines, both BAY K8644 and antagonists such as nifedipine. The low-voltage activated current is selectively inhibited by alcohols as was shown in inferior olive cells by Llinas & Yarom (Soc. Neurosci. Abstr. 12(1):174, 1986).

Three functions are likely candidates for selective involvement of calcium channels: patterned electrical activity, basal or stimulated secretion, and modulation by neurotransmitters. The high-voltage activated current can be modulated by the adenylate cyclase activator, forskolin (1-10 μM), while no effect has been observed on the high-voltage activated current to date. Effects of dihydorpyridines on secretion suggest that basal release in lactotrophs is relatively insensitive to both BAY K8644 and nifedipine, but is inhibited by Cd^{++} (1 mM) suggesting that the low-voltage activated calcium channel may be involved in this process. These effects are being examined further as well as the involvement of these channels in electrical activity.

Experiments were begun at the Max-Planck Institut für biophys. Chemie, Göttingen with the support of Dr. Bert Sakmann and a Hoffmann-LaRoche fellowship from LSRF. Supported by NSF #8615840.

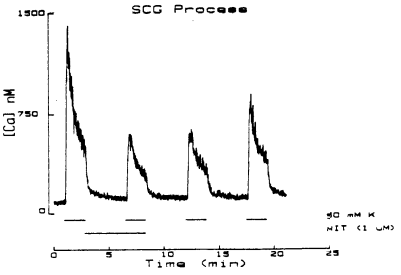
281.2 DISTRIBUTION OF MULTIPLE Ca^{2+} CHANNEL TYPES AND INTRACELLULAR Ca^{2+} STORES IN SINGLE CENTRAL AND PERIPHERAL NEURONS. S.A. Thayer*, L.D. Hirning*, K.M. Harris* and R.J. Miller Dept. of Pharmacological and Physiological Sci., Univ. of Chicago, Chicago IL 60637.

A rise in intracellular Ca^{2+}, $[Ca^{2+}]_i$, serves as a trigger for many neuronal processes, including the release of neurotransmitter. We investigated two means by which this rise in $[Ca^{2+}]_i$ can be produced. The influx of Ca^{2+} through voltage-sensitive Ca^{2+} channels and the release of Ca^{2+} from intracellular stores were measured with the Ca^{2+}-sensitive dye fura-2 in neurons grown in primary culture from the hippocampus (H) and the superior cervical ganglion (SCG) of the rat. The fura-2 fluorescence signal was measured with a microfluorimeter capable of alternating the excitation wavelength at a frequency of 60 Hz. Recordings were obtained from either a single or small bundle of neuronal processes and compared to recordings from single cell bodies.

In SCG and H neurons depolarization with 50 mM K^+ produced a rapid increase in $[Ca^{2+}]_i$ composed of both transient and sustained components. This response was seen in recordings from both the soma and processes of the cells. The entire response could be reversibly blocked by 30 μM La^{3+} and partially inhibited by 1 μM nitrendipine (NIT) as shown in the figure below. The transient component was blocked by predepolarization in Ca^{2+}-free solution and the remaining sustained component was substantially inhibited by 1 μM NIT. These data show that both dihydropyridine (DHP) sensitive and insensitive Ca^{2+} channels are present in processes as well as cell bodies of central and peripheral neurons.

Intracellular stores of Ca^{2+} can also be mobilized in these neurons. Both central and peripheral neurons responded to perfusion with 10 mM caffeine in Ca^{2+}-free bathing solution with small transient increases in $[Ca^{2+}]_i$. Multiple responses to caffeine could be produced only if these stores were allowed to replenish by either prolonged incubation or depolariztion in medium containing Ca^{2+}.

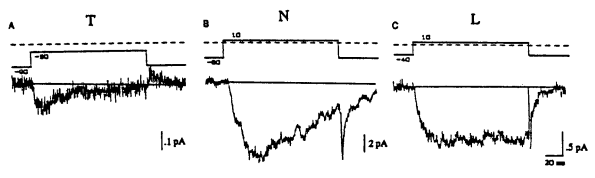
Neurotransmitter release from SCG and H neurons, when evoked by the same method of depolarization as used in these studies, is not sensitive to DHP Ca^{2+} channel blockers. Therefore, the DHP-insensitive portion of the rise in $[Ca^{2+}]_i$ seen in these recordings must be responsible for triggering neurotransmitter release. However our studies demostrate the presence of DHP-sensitive as well as insensitive Ca^{2+} channels in both the soma and processes of these neurons. This suggests that the DHP-insensitive channels may be clustered around release zones giving them a relative advantage for the initiation of neurosecreation (Miller, R.J., Science, 235:46, 1987). The contribution of Ca^{2+} released from intracellular stores to neurotransmitter release remains to be determined.



281.3 MULTIPLE TYPES OF CALCIUM CHANNELS IN HIPPOCAMPAL NEURONS: CHARACTERIZATION AND LOCALIZATION. *K.R. Bley*, D.V. Madison, and R.W. Tsien (SPON: R.D. Blakely), Dept. of Physiology, Yale Medical School, New Haven, CT 06510.*

We have studied Ca currents in cultured neurons of the CA3 region of the rat hippocampus using patch-clamp techniques. Both whole-cell and cell-attached recordings suggest the presence of three types of Ca channels in these cells, corresponding to those termed T, L, and N in other preparations (e.g. DRG neurons or dentate granule cells). In whole-cell recordings with 10 mM Ca outside and internal Cs-EGTA, weak depolarizing pulses from negative potentials elicit a transient Ca current (T) which decays with a time constant of approximately 50 msec and is nearly completely inactivated positive to -60 mV. In cell-attached patch recordings (110 mM Ba in the pipette), openings with a slope conductance of 7 pS give average currents with kinetics like whole-cell T current (panel A). L-type channels display a slope conductance of 25 pS, are evoked in isolation by strong pulses from relatively depolarized holding potentials, and inactivate very little in 150 msec (panel C). L-current responds to dihydropyridines: nifedipine potently reduces the whole-cell current while Bay K 8644 produces mixed agonist and antagonistic effects, with higher concentrations being more antagonistic. A third component of Ca current (N-type) is evoked with negative holding potentials and strong test pulses, and shows marked inactivation with a $\tau \sim 100$ ms. Unitary openings with a slope conductance of 11-16 pS show kinetics corresponding to whole-cell currents (panel B).

We have recorded activity of all three channel types on processes and somata of pyramidal-shaped neurons in culture, as well as acutely dissociated neurons from hippocampi of juvenile rats. This indicates that high-voltage activated Ca channels (L and N) are not confined to dendritic membranes as previously postulated.



281.4 CHARACTERIZATION OF BA^{++} CURRENTS THRU VOLTAGE-GATED CA^{++} CHANNELS IN IDENTIFIED MAMMALIAN CORTICAL NEURONS. K. Giffin*, J.P. Doyle* and J.M. Nerbonne, Dept. of Pharmacology, Washington University Medical School, St. Louis, MO. 63110.

In spite of the presumed importance of voltage-dependent Ca^{++} influx in the regulation and modulation of neuronal excitability, little is known about voltage-gated Ca^{++} channels in mammalian central neurons. The cortex, in particular, has remained largely intractable to cell physiologists apparently because of its complexity, cellular diversity and the difficulties associated with identifying specific cortical cell types. Here, we report the results of experiments aimed at characterizing the properties and distributions of voltage-gated Ca++ channels in cortical neurons, identified based on their projection targets. Callosal-projecting neurons are identified in vitro following retrograde labelling in vivo with rhodamine-labelled "beads". In neonatal (3-15 days) Long Evans rat pups, multiple bead injections are made into the primary visual cortex (Area 17). Following 2-15 days survival, neurons are dissociated from the contralateral area 17 and plated on monolayers of cortical astrocytes; dissociated cells prepared in this manner are viable for up to three weeks in vitro. Using the whole-cell variation of the patch-clamp recording technique, we have examined the waveforms of Ba^{++} currents through voltage-gated Ca^{++} channels in callosal-projecting cortical neurons within 6 hrs of isolation. Ba^{++} currents are recorded in bath solutions containing (in mM): NaCl, 140; $BaCl_2$, 5; KCl, 4; $MgCl_2$, 2; HEPES, 10; glucose, 5; pH 7.4 with 1 μM TTX added to suppress voltage-activated Na^+ currents; pipet solutions contain (in mM): CsCl, 140; EGTA, 10; HEPES, 10; glucose, 5; Tris-ATP, 3; Tris-GTP, 0.1; pH 7.4. In voltage-clamped cells, depolarizations, from holding potentials (hps) of -40 to -100 mV, evoke inward Ba^{++} currents which begin to activate at -30 mV, peak between 0 and +10 mV and are blocked by Co^{++} (5mM) or Ni^{++} (2mM). Ba^{++} current amplitudes in callosal-projecting neurons are uniformly small with peak amplitudes in the range 100-300 pA; this contrasts markedly with voltage-gated Na^+ and K^+ currents, which average 10 to 50 times larger. Although the amplitudes of Ba^{++} currents evoked from depolarized hps are reduced, normalized, peak current-voltage (IV) relations appear to be independent of hp over the range -40 to -100 mV. The waveforms of Ba^{++} currents evoked on depolarizations from -80mV suggest the presence of two kinetically-distinct components: one that is inactivating and another that is sustained during 250 ms depolarizations; no differences are observed, however, in normalized IV plots for these two components. In dissociated callosal-projecting neurons examined after 1-5 days in vitro, however, Ba^{++} currents peak at more hyperpolarized potentials. Present efforts are devoted to the further evaluation of developmental variations in voltage-gated Ca^{++} channels and to examining the possibility that these channels are regulated and/or modulated by cortical neurotransmitters. Support: Epilepsy Foundation of America, NIH (T32-HL07275) and NIMH (MH41705).

281.5 CROSSLINKING BY ^{125}I-ω-CONOTOXIN: EVIDENCE SUGGESTING MULTIPLE NEURONAL CALCIUM CHANNEL SUBTYPES. L. J. Cruz*, J. S. Imperial*, D. S. Johnson* and B. M. Olivera* (SPON: J. W. Conlee). Department of Biology, University of Utah, Salt Lake City, UT 84112.

The peptide toxin ω-conotoxin GVIA, isolated from the fish-hunting cone snail Conus geographus, has been shown to inhibit voltage-sensitive Ca channels. The receptor targets of this toxin are found in neuronal tissue; voltage-sensitive Ca channels from skeletal muscle T- tubules, or from cardiac muscle are not high affinity sites for the toxin (Cruz et al., Biochemistry 26: 820, 1987). Initial crosslinking studies indicated that a single band was radiolabeled by the toxin, with a M_r of 135,000-140,000 in chick brain membrane preparations.

We recently carried out more extensive crosslinking studies; these data suggest multiple ω-conotoxin receptor targets in chick neuronal tissue. Crosslinking of solubilized receptor preparations with bivalent crosslinking agents (such as disuccinimidyl suberate) yields two pairs of radiolabeled bands after SDS PAGE, one highly labeled pair with M_r = 140,000 and 193,000, and two lightly labeled bands with M_r of 160,000 and 216,000. Based on the recent results with purified T-tubule Ca channels, the two pairs suggest we may be crosslinking two different types of Ca channel complexes by this procedure. However, if photoactivatable derivatives of ^{125}I-ω-conotoxin are crosslinked to synaptosomes, instead of the two radiolabeled pairs of bands, three other bands are specifically crosslinked, with M_r = ~ 308,000, ~ 230,000 and 96,000. The high MW bands are rather broad.

At present, we are correlating these crosslinking data to the different types of Ca channels inhibited by ω-conotoxin. In addition to the physiological types of Ca channels that have been defined (L and N) as targets of ω-conotoxin (McCleskey et al., PNAS, in press), within the N class there may be two distinct subtypes. Inhibition of one of these (the N_A subtype) causes paralysis and death; inhibition of the second subtype (N_B) in the CNS causes a shaking syndrome. At present, we are attempting to assign which crosslinked bands correspond to the L, N_A, and N_B Ca channel subtypes. Crosslinking studies have been carried out in additional systems; the patterns in most vertebrates can be correlated to the pattern obtained with chick brain receptors. In Torpedo electric organ, two bands of significantly lower molecular weight (68 and 56 K) are crosslinked. (This research was supported by grant GM22737.)

281.6 ω-CONOTOXIN BINDING SITES IN DEVELOPING CHICK BRAIN. M. J. Litzinger, J. S. Imperial*, A. Azimi*. Depts. of Neurology and Biology, University of Utah, Salt Lake City, UT 84123.

ω-Conotoxin (ω-CgTx) binding in whole chick brain preparations as a function of development shows a relatively constant level until embryonic days 18-20 (the period just before hatching) when it increases dramatically and then levels off (Litzinger et al., Soc. Neurosci. Abstr. 12:1229, 1986). Similarly, ^{45}Ca uptake studies in synaptosomes from chick brain show a 4-fold increase between embryonic days 18-20; ω-CgTx blocks 38% of the uptake on embryonic day 17 and 86% on day 20 (Litzinger et al., Neurology 37, Supl. 1:346, 1987). On the other hand, nitrendipine (NTP) binding in chick brain preparations indicates a slow gradual increase during development (Marangoes et al., J. Neurochem. 42:1338, 1984) and NTP does not block synaptosomal ^{45}Ca uptake at any age (Litzinger et al., Neurology 37, Supl. 1:346, 1987). ω-CgTx has been shown to act at both the N- and L-type voltage-sensitive calcium channel (VSCC) while NTP acts only on the L-type (McCleskey et al., PNAS, In press). The N-type VSCC, believed associated with transmitter release, has been suggested as responsible for ^{45}Ca fluxes (Reynolds et al., PNAS, 83:8804, 1986).

Crosslinking of ^{125}I-ω-CgTx to chick membrane preparations using the bivalent crosslinker disuccinimidyl suberate (DSS) indicates the target to be a polypeptide with a M_r of 135,000-140,000 (Cruz et al., Biochemistry 26:820, 1987). Recent data on more extensive crosslinking using DSS suggest multiple ω-conotoxin receptor targets in chick neuronal tissue (Cruz et al., Soc. Neurosci. Abstr., 1987). With the modified method, brain preparations from chicks at embryonic day 16, post natal day 2 and adult stage all showed the same crosslinking pattern on SDS PAGE. However, preliminary data using photoactivatable derivatives of ^{125}I-ω-CgTx showed a difference in the relative intensities of specifically crosslinked bands for 16-day old embryos and 2 to 7-day old chicks. The most obvious difference is in a radiolabeled band at a M_r of ~ 96,000 which is very intense in preparations from newly hatched chick but barely visible in the embryo preparations. Whether the apparent increase in amount of the 96 K protein corresponds to the appearance of more N-type VSCC or not remains to be investigated. (Supported by a grant from Miles Laboratories, Inc.)

281.7 SOLUBILIZATION AND PARTIAL PURIFICATION OF ω-CONOTOXIN BINDING SITES FROM RAT BRAIN SYNAPTOSOMES. R.L. Rosenberg*, J.S. Isaacson*, B.M. Olivera*+, W.S. Agnew, and R.W. Tsien. Dept. of Physiology, Yale Univ. Sch. of Med., New Haven, CT 06510 and +Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

The peptide ω-CgTx VIA from Conus geographus blocks N- and L-type calcium channels of neuronal cells (McCleskey et al., PNAS 1987), and ^{125}I-labelled toxin binds specifically to high-affinity sites in chick brain synaptosomes (Cruz and Olivera, JBC 261:6230, 1986). We are using [^{125}I]ω-CgTx VIA as a marker for the solubilization and purification of Ca channels from rat brain.

The binding of [^{125}I]ω-CgTx to rat brain synaptosomes was measured in a standard glass fiber filtration assay. Saturable binding to a single class of sites was observed, with K_d 0.2-0.4 nM and B_{max} 0.4-0.5 pmol/mg protein.

Specific, saturable binding of [^{125}I]ω-CgTx was also observed in detergent extracts of rat brain synaptosomes using a rapid gel filtration assay. Efficient solubilization was obtained with a mixture of Triton X-100 and CHAPS (1:1) at a concentration of 0.5-1.0% (w/v) for 15 mg/ml membrane protein. Binding affinity was not markedly changed by solubilization (K_d 0.2-0.3 nM). B_{max} was 0.2-0.3 pmol/mg protein; overall recovery of binding activity in the detergent extracts averaged 40%. Binding to detergent extracts, unlike that to the intact synaptosomes, was inhibited by NaCl, with half-maximal inhibition at a concentration of 150 mM.

Partial purification of [^{125}I]ω-CgTx binding sites in detergent extracts was achieved with three different methods. When detergent extracts were fractionated on sucrose density gradients (12-30% w/v), [^{125}I]ω-CgTx binding peaked at 19% sucrose, well-separated from the bulk of protein (peak at 15% sucrose). Maximum specific activity was approximately 0.5 pmol/mg protein, indicating a purification of approximately 2-fold. Elution of detergent extracts through Sepharose 6B also gave a significant purification; [^{125}I]ω-CgTx binding activity eluted earlier than did the bulk of the protein, yielding a purification of 2-4 fold. A significant fraction of the binding sites eluted in the void volume, indicating a tendency for the sites to remain or become clustered upon solubilization. [^{125}I]ω-CgTx binding protein was bound by wheat germ agglutinin-Sepharose 6B, and could be eluted by addition of 200 mM N-acetylglucosamine to the column buffer, with a resultant 5-fold enrichment of binding sites. Unlike dihydropyridine binding activity from skeletal muscle, functional [^{125}I]ω-CgTx binding sites could not be recovered after adsorption to DEAE Sephadex.

These results show that the [^{125}I]ω-CgTx binding protein from rat brain can be solubilized from membranes without loss of activity, and that standard techniques for protein purification can lead to significant enrichment in [^{125}I]ω-CgTx binding activity.

281.8 FORMATION OF CELL-FREE PATCHES UNMASKS A LARGE, DIVALENT-PERMEABLE, VOLTAGE-INDEPENDENT CHANNEL IN APLYSIA NEURONS. J.A. Strong, A.P. Fox, R. W. Tsien, and L.K. Kaczmarek. Depts. of Pharmacology & Physiology, Yale Med. School, New Haven, CT 06510

Recent work has shown that excitable cells contain a number of different kinds of voltage-activated calcium channels, which differ in their conductances, voltage dependence, and kinetic properties. We now report that Aplysia neurons contain a voltage-independent divalent-permeable channel whose activity is prominent in cell-free patches but virtually undetectable in cell-attached recordings. For most experiments, we recorded from cell-free, inside-out membrane patches taken from bag cell neurons maintained in primary culture, using Ba as the extracellular charge carrier, and K-aspartate (with 15 mM EGTA, 15 mM HEPES (7.8)) on the intracellular side. Under these conditions, an inward channel is observed which has several unusual properties:

(1) a strong selectivity for divalent cations. In 370 mM Ba/535 mM K (or Na), the reversal potential is >75 mV, indicating a selectivity on the order of 1000:1 for Ba over K or Na.

(2) a surprisingly large single channel conductance. With 185 mM Ba, the single channel conductance is 85 pS; the open channel current at -70 mV is 8 pA. As has been found for several voltage-activated calcium channels, the open channel current-voltage relation becomes less steep near the reversal potential.

(3) a lack of voltage-dependent activation and inactivation. Although depolarization does increase the open time, channel openings can be readily observed within the entire voltage range studied (-120 to +150 mV).

(4) a ubiquitous distribution. Virtually every patch, taken from either the soma or from processes, contains at least 2-4 channels.

Based on these 4 characteristics, the channel is almost certainly the same as that which has been extensively characterized by Chesnoy-Marchais (J. Physiol. 367:457) in outside-out patches taken from Aplysia neurons in situ.

(5) We have found that, despite this ubiquitous distribution, channel openings are virtually never observed during cell-attached recordings. In a typical 20 minute cell-attached recording (used to study 2 smaller, voltage activated calcium channels present in these cells) we would see the channel open once or not at all. This suggests that an unknown cytoplasmic regulator acts either to keep the channel closed or to markedly alter its characteristics. We are presently attempting to characterize this regulation. Application of ATP or Mg.ATP (2 mM), GTP (0.2 mM), Ca (up to 10 uM) or glucose (5 mM) to the intracellular side of cell-free patches failed to close the channel. We have noted that openings are sometimes seen in cell attached patches in cells following prolonged exposure to low-Ca solutions. The channel is unlikely to be activated by cellular aspartic acid receptors, as we obtained similar results with K-methane sulfonate. We are also investigating the possibility that the channel subserves an influx of calcium into rapidly retracting processes. In 2 cell-attached patches we observed transient openings of the channel when a process just distal to the patch began to retract following acute mechanical injury.

281.9 LOW THRESHOLD CALCIUM CONDUCTANCE IN PARABRACHIAL RETICULAR NEURONS STUDIED *IN VITRO* AND ITS BLOCKADE BY 1-OCTANOL. C. S. Leonard and R. Llinás. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

The electrophysiological properties of dorsolateral pontine reticular formation neurons belonging to the lateral parabrachial and pedunculopontine nuclei were studied in guinea pig brain slices. Intracellular recordings from these neurons revealed two groups of cells. One group demonstrated action potentials followed by a long lasting afterhyperpolarization (AHP). Current clamp analysis and blockage of the AHP by bath application of 5 mM 4-aminopyridine indicated that an "A" current is responsible for this AHP. The other group of cells, in addition to fast, TTX-sensitive spikes, demonstrated "low-threshold" calcium-dependent spikes (LTSs) similar to those observed in thalamic (Jahnsen & Llinás: J. Physiol., 349: 205, 1984) and inferior olivary (Llinás & Yarom: J. Physiol., 315: 549, 1981) neurons. Indeed, the LTSs were insensitive to 10^{-4}M TTX, but were blocked by bath application of 0.5 mM Cd, suggesting that the LTSs are mediated by a Ca current. Also, in the above neurons the conductance underlying the LTS was usually inactivated at resting potential and became de-inactivated upon membrane hyperpolarization. In addition, 1-octanol which was recently reported to block low-threshold Ca currents in inferior olivary cells (Llinás & Yarom: Soc. Nsci. Abst., 12:174, 1986), almost completely blocked this low-threshold spiking when added to the bath at concentrations of 10^{-5} to 10^{-6}M. The location of these neurons overlapped that of neurons labelled retrogradely by injections of fluorescent microspheres into the thalamus *in vivo*. This finding is of interest as it is known that neurons of the lateral parabrachial nucleus projecting to the thalamus fire a burst of action potentials during the PGO waves and rapid eye movements of paradoxical sleep (Sakai: in Brain Mechanisms of Sleep, ed. D. McGinty et al., Raven, 1985). Since the LTS cells described here produce a burst of fast Na spikes when the LTS is activated, it is reasonable to hypothesize that the LTSs play an important role in the production and control of PGO waves and in the activation of thalamic systems involved with the generation of eye movements accompanying the dream state. [Research was supported by grants NS-07848 and NS-13742 from NINCDS.]

281.10 PHARMACOLOGICAL DIFFERENCES BETWEEN INFERIOR OLIVE AND THALAMIC LOW THRESHOLD CALCIUM CONDUCTANCES. E. Geijo-Barrientos* and R. Llinás (SPON: S. M. Simon). Dept. Physiology & Biophysics, New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

An *in vitro* study of guinea pig olivary neurons, using voltage clamp techniques, recently demonstrated that 1-octanol at doses of 1 μM reversibly and almost completely blocks the low-threshold Ca conductance in those neurons (Llinás & Yarom: Soc. Nsci. Abst., 1986). We designed a similar study to test whether the low threshold Ca conductance seen in thalamic neurons (Llinás & Jahnsen, Nature 297: 406, 1982; Jahnsen & Llinás, J. Physiol. 349: 205, 1984) is also equally affected by the alcohol. Adult guinea pigs weighing 150-200 gm were utilized and the cells in different thalamic nuclei (including nucleus reticularis thalami) were studied intracellularly using a single electrode voltage clamp (Axoclamp Instruments). Results demonstrated that 1-octanol at doses of 10-50 μM reduces only partly the low-threshold Ca current. Also, as previously reported for the inferior olive, it did not affect the high-threshold dendritic Ca conductance. These results suggest that two different low-threshold Ca conductances may in fact exist in CNS neurons. However, because the octanol blockage of the low-threshold Ca conductance may not occur by direct action on the low-threshold Ca channel, the possibility that octanol may be used to differentiate the two low-theshold Ca conductances must remain open. Nevertheless, the effects of octanol in the *in vivo* preparation also indicate that doses sufficiently high to block physiological as well as harmaline tremor are insufficient to block cortical spindling of thalamic origin (Llinás, Paré, Deschênes & Steriade, Soc. Nsci. Abst. 1987). This indicates that octanol may be utilized as a drug capable of differentiating these two types of neuronal oscillation. [Supported by a fellowship from Generalidad Valenciana (Spain) and Program Grant NS13742 from NINCDS.]

281.11 DIFFERENCES BETWEEN THALAMOCORTICAL SPINDLING AND PGO WAVE GENERATION DEMONSTRATED BY LOW-THRESHOLD CALCIUM CURRENT BLOCKAGE BY OCTANOL. R. Llinás, D. Paré, M. Deschênes and M. Steriade. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016, and Laval Univ. School of Med., Quebec G1K 7P4, Canada.

Recent studies *in vitro* have demonstrated that octanol at concentrations of 10^{-6} g/ml (7.6 μM) can specifically block the low threshold calcium conductance underlying calcium spikes in the inferior olive (Llinás & Yarom, Soc. Nsci. Abst. 1986). Further studies with this compound indicate that, by contrast, the thalamic low threshold spike is only partly blocked in concentrations of 10-50 μM (Geijo-Barrientos & Llinás, Soc. Nsci. Abst., 1987). However, neurons in the nucleus parabrachialis (which projects to the thalamus) seem to have a similar sensitivity to octanol *in vitro* (1-10 μM for almost complete block; Leonard & Llinás, Soc. Nsci. Abst., 1987) as do inferior olivary cells *in vitro*. The possibility exists, therefore, that octanol may differentiate the mechanisms which generate the thalamo-cortical oscillations, known as spindling, from the so-called PGO (ponto-geniculo-occipital) waves which accompany eye movement in the dreaming state. This hypothesis was tested in anesthetized cats after high collicular transection. Under these conditions, spontaneously occurring spindles and related spike bursts were abolished and replaced by tonic single spike discharge for 15 to 20 min by intravenous injections of octanol at a concentration of 15 to 18 mg/kg. On the other hand, PGO waves induced by Reserpine, as well as the PGO-like waves evoked in the lateral geniculate neurons, were blocked for 12 min by concentrations of only 5 mg/kg octanol. In agreement with these results is the fact that alcohol in general is known to reduce REM sleep in man (Gross et al., J. Nerv. Ment. Dis. 142:493, 1966). These results point out the relevance of biophysical studies *in vitro* in the understanding of the mechanisms that ultimately generate brain function. (Supported by Program grant NS13742 from NINCDS and MRC grants MT-3689 and MT-5877).

282.1 **NEUROTROPHIC ACTION OF GLYCYL-L-GLUTAMINE IN PRESERVING ACETYLCHOLINESTERASE OF PREGANGLIONICALLY DENERVATED CAT SUPERIOR CERVICAL GANGLION.** **G.B. Koelle, N.S. Thampi, and U.J. Sanville*. Dept. of Pharmacology, Med. Sch., Univ. of Pennsylvania, Philadelphia, PA 19104-6084.**

The fall in acetylcholinesterase (AChE; EC 3.1.1.7) of the cat superior cervical ganglion (SCG) that follows preganglionic denervation is opposed by intracarotid infusion of an aqueous extract of cat central nervous system or its dialysate, or of glycyl-l-glutamine (GlyGln), glycyl-l-glutamic acid, or l-glutamic acid (Koelle, G.B. et al., Proc. Natl. Acad. Sci. USA, 80:3106; 81:6539; 82:5213; 83:2751, 1983-1986). Infusions were given via the right common carotid artery from 24 to 48 hr following bilateral preganglionic denervation.

In contrast to the other preparations, the effect of GlyGln was confined to the circulatory remote left SCG and not detectable at the directly infused right SCG; this suggested that a metabolite of GlyGln, formed in the blood, is an active neurotrophic factor. An alternative explanation has now been tested: that GlyGln must combine slowly with some component of plasma to allow its penetration to the cytoplasm of ganglionic neurons.

GlyGln was incubated overnight at 5°C with fresh or heat-treated (60°C for 60 min) plasma, then infused as usual for 24 hr via the right common carotid artery; ganglia were excised and assayed 48 hr post-denervation. Infusion of GlyGln had a marked neurotrophic effect in preserving AChE in both right and left SCG at 3 µM following incubation with either fresh or heat-treated plasma; lower positive effects were detectable at 1 and 0.3 µM; at concentrations of 100 µM or higher, GlyGln had an inhibitory effect on AChE contents of SCG. Results indicate that GlyGln has a direct, potent neurotrophic action. Preliminary findings suggest that it acts by regulating the conversion of G_1 to G_4 AChE.

282.2 **DEOXYCYTIDINE MAY HAVE A CRITICAL ROLE IN THE SURVIVAL OF POSTMITOTIC PERIPHERAL NEURONS THAT IS INDEPENDENT OF DNA SYNTHESIS.** T. L. Wallace and E. M. Johnson, Jr. Center for Biotechnology, Baylor College of Medicine, and Houston Biotechnology, Inc., The Woodlands, TX 77381 and Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

The molecular mechanism by which neurotrophic factors maintain the survival of neurons is not well understood. We have found that deoxycytidine is critical for the KCl and insulin-stimulated survival of postmitotic ciliary parasympathetic ganglion neurons, and the nerve growth factor-stimulated survival of postmitotic dorsal root ganglion sensory neurons in vitro. Cytosine arabinoside (ARA C), a competitive inhibitor of 2'-deoxycytidine, caused a concentration-dependent inhibition of survival of both neuronal types with an $IC_{50} = 2 \times 10^{-8}$ M after four days in culture. This cytotoxic effect of ARA C was blocked by 2'-deoxycytidine and its 2'-deoxynucleotides, but not by cytidine, cytosine, 2'-deoxyadenosine, 2'-deoxyguanosine, or 2'-deoxythymine, showing that ARA C interfered with a deoxycytidine-specific survival process. 2'-Deoxycytidine, by itself, was not survival-promoting. That is, it acted to block the effects of ARA C, but could not by itself stimulate survival. Other antimitotic agents, such as adenine arabinoside, thymine arabinoside, 5-fluorodeoxyuridine, and aphidicolin had no effect on neuronal survival at a concentration 5,000 times the IC_{50} of ARA C, indicating that inhibition of deoxyribonucleic acid (DNA) synthesis was not the cause for the inhibition by ARA C of neuronal survival. This also showed that other 2'-deoxynucleosides were not involved in the survival process. Nitrobenzylthioinosine, an inhibitor of 2'-deoxycytidine and ARA C membrane transport in other cell types, inhibited the cytotoxic effect of ARA C, suggesting that ARA C entered the neurons through a similar transport mechanism and that ARA C needed to gain access to the inside of the neuron to be effective. These results indicate that ARA C, in addition to being an antimitotic agent for dividing cells, is also cytotoxic for postmitotic neurons. This inhibition of neuronal survival by ARA C is hypothesized to be due to inhibition of a 2'-deoxycytidine-dependent pathway, but one, however, that is independent of DNA synthesis. This suggests that 2'-deoxycytidine may be a common mediator of the survival-promoting effects of several trophic factors on peripheral neurons. Thus, 2'-deoxycytidine's role in cellular function appears to be not simply limited to that of a metabolic precursor of DNA. 2'-Deoxycytidine may have an important and previously unrecognized role in postmitotic neurons that is critical for neuronal survival.

282.3 PARTIAL PURIFICATION AND CHARACTERIZATION OF A MEMBRANE-DERIVED FACTOR REGULATING TRANSMITTER PHENOTYPIC EXPRESSION. J.E. Adler, L.S. Schleifer and I.B. Black. Division of Devel. Neurol., Cornell Univ. Med. Coll., New York, N.Y. 10021.

We have previously reported that cell membrane contact induces de novo expression of choline acetyltransferase (CAT) activity in virtually pure cultures of neonatal rat dissociated sympathetic neurons. To define underlying molecular mechanisms, we have begun to isolate and characterize the membrane-associated component(s) that stimulates CAT expression. Cultures were first exposed to membranes derived from a variety of adult rat tissues to define specificity. Membranes from spinal cord, as well as sympathetic and dorsal root sensory ganglia contained substantial CAT-inducing activity. Spinal cord membranes were employed as a source for purification due to the large quantities of tissue available and the high CAT-inducing activity.

After spinal cords were homogenized in low ionic strength buffer and centrifuged at high speed, CAT-inducing activity was predominantly localized to the membrane pellet. Using increased ionic strength, we were able to extract more than half of the total CAT-inducing activity from the membranes in a soluble form.

The solubilized extract was applied to a variety of chromatographic columns. Activity was eluted from a DEAE ion exchange column in a sharp peak containing less than 10% of the applied protein. Gel chromatography also resulted in a single sharp peak of activity containing 10% of the applied protein. Sequential application of the extract to these two columns yielded substantial purification. The activity peak from the gel column correlated with an approximate Mr of 27 KD. Activity eluted from a heparin affinity column at very low salt concentrations, suggesting that the factor is not a heparin binding protein. Our studies suggest that cell contact-mediated regulation of phenotypic expression may be governed by a discrete membrane factor. We are presently continuing isolation and characterization. (Supported by NIH grants HD 12108, NS 10259, the Dysautonomia Foundation Inc. and the American Heart Assoc. L.S.S. is a recipient of a Cornell Scholars Award. I.B.B. is a recipient of a McKnight Research Project Award).

282.4 SCHWANN CELLS SHED A TRUNCATED FORM OF THE NERVE GROWTH FACTOR (NGF) RECEPTOR. P.S. DiStefano and E.M. Johnson, Jr. Neuroscience Research Unit, Abbott Laboratories, Abbott Park, IL 60064, and Department of Pharmacology, Washington University, St. Louis, MO 63110.

Recent studies have demonstrated the rapid appearance of NGF receptors on Schwann cells due to the loss of axon-Schwann cell contact. This phenomenon has been demonstrated both in vivo and in vitro. We report here that cultured rat Schwann cells, as well as a schwannoma cell line (JS-1 cells), secrete a truncated form of the NGF receptor (NGF-Rt) into their culture medium. Cell surface NGF receptors and NGF-Rt from conditioned media were assayed by crosslinking specifically bound ^{125}I-NGF with EDAC. Cell surface receptors were then solubilized with octyl glucoside. NGF-NGF receptor species were immunoprecipitated from cell extracts or media with the anti-rat NGF receptor monoclonal Ab, 192-IgG. Labeled receptor species were visualized using SDS acrylamide gel electrophoresis/autoradiography. Conditioned media from primary Schwann cell cultures and JS-1 cells showed a distinct immunoprecipitable band at 65 kD (subtracting a monomer of NGF yielded a fragment of ~52 kD). Conditioned media from PC12 cells and brain septal neurons were devoid of NGF-Rt, as was naive medium.

To demonstrate an in vivo correlate of this phenomenon, urine and plasma samples were collected from various aged rats and assayed for NGF-Rt as described above. In urine, NGF-Rt levels were high in 1 day old rats and decreased approximately 50 fold by adulthood (4-8 wks). NGF-Rt was also detected in amnionic fluid of E15 and E19 rats. A similar developmental pattern of NGF-Rt was seen in plasma samples, although plasma concentrations appeared lower than urinary NGF-Rt levels. If the NGF-Rt observed in early development was a product of Schwann cells, we predicted that sciatic nerve section, which dramatically increases NGF receptor levels on Schwann cells, would result in increased urinary and plasma NGF-Rt levels. It was found that bilateral sciatic nerve transection increased NGF-Rt significantly in urine and plasma as early as 1 day post lesion; NGF-Rt levels remained elevated at 14 days post lesion.

The shedding of cell surface NGF receptors by Schwann cells may reflect a mechanism by which the cell eliminates or turns over NGF receptors. Alternatively, the NGF-Rt may subserve a secondary function once it is cleaved from the cell surface.

282.5 REDUCTION OF NATURALLY OCCURRING MOTONEURON DEATH IN VIVO BY A PUTATIVE TARGET-DERIVED NEUROTROPHIC FACTOR. R. W. Oppenheim, L. J. Haverkamp, D. Prevette* and S. Appel. Dept. of Anatomy, Wake Forest Univ. Sch. of Med., Winston-Salem, NC 27103 and Dept. of Neurology, Baylor Coll. of Med., Houston, TX 77030.

Between embryonic day 6 (E6) and E10 approximately one-half of the motoneurons (MNs) in the lumbar lateral motor column (LMC) of the chick embryo that normally innervate hindlimb musculature degenerate and die by a process known as naturally occurring neuronal death. It is generally believed that neurons in the LMC compete for a target-associated entity that is limited in amount relative to the total number of neurons present prior to cell death. A number of in vitro studies have, in fact, demonstrated that crude or partially purified factors derived from target muscles can influence the growth, differentiation and survival of dissociated spinal cord neurons. We now report that target derived factors can also markedly increase the survival of MNs in vivo.

In our initial experiments, we found that treatment of embryos for several days (E5-8) with 250 µl (3-4 mg protein/ml) of crude muscle homogenates taken from E8-9 hindlimbs reduced LMC-MN death by 20-30%. Kidney or lung extract or heat inactivated muscle extracts were ineffective. Embryonic motility, an index of neuromuscular activity, was normal in the treated embryos. Because most of the survival activity was retained in a 25-75% ammonium sulfate fraction ($AmSO_4$) all subsequent experiments were done with this partially purified extract. The survival effect of the $AmSO_4$ fraction on MNs was found to be dose-dependent. The partially purified extract had no effect on the survival of neurons in either dorsal root (DRG) or sympathetic ganglion (SG) whereas treatment with NGF (20 µg daily) increased the survival of DRG and SG neurons but had no effect on MN survival. Furthermore, whereas NGF treatment resulted in increased cell size of DRG and SG neurons (but not MNs), the muscle-derived factor did not affect MN, DRG or SG cell size. Treated embryos had reduced numbers of pyknotic MNs in the LMC. Enhanced MN survival occurred along the entire length of the lumbar LMC as well as in non-limb innervating (thoracic) regions. The massive MN death that occurs following early (E2) limb-bud removal was partly ameliorated by treatment with the $AmSO_4$ fraction. Preliminary results from treatment with three different components of the $AmSO_4$ fraction (< 30,000 MW, 30,000-75,000 MW and > 75,000 MW) indicate that most of the survival activity is contained in the < 30,000 MW component. Experiments are in progress to further purify and characterize this putative MN survival factor and to examine its primary or secondary effects on muscle development and innervation.

282.6 THYROTROPIN RELEASING HORMONE DE-AMINATION OBLITERATES ITS TROPHIC EFFECT IN NEURONAL CULTURES.

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The role of thyrotropin releasing hormone (TRH) in neuronal growth and survival has been the subject of several recent investigations. It has been localized to the ventral horn of the spinal cord in several mammalian species. We have shown in the past a trophic effect for this peptide in murine ventral horn neuronal cultures in terms of perikaryal size and complexity of neurites. We have also demonstrated its protective effect on neuronal loss following peripheral nerve section. The present study was undertaken to examine the possible effect of TRH-OH, the de-aminated metabolite of TRH formed via the proline endopeptidase pathway on neuronal cultures.

Ventral horn neurons from 12- to 14- day old mouse embryos were cultured using standard techniques. Two groups of cultures were fed daily a final concentration of 0.1 mM and 0.01 mM TRH respectively. Two more groups were similarly fed a final concentration of 0.1 mM and 0.01 mM TRH-OH. One group received vehicle only, and a final group was left untreated. At 18 days the cultures were fixed and stained with cresyl violet. Neuronal perikaryal area and maximal diameter were computed using a Leitz Dialux 22 microscope with camera lucida attachemnt and a Zeiss Videoplan semiautomatic image analyzer. Available software allowed for the construction of histograms and statistical analysis. The results revealed no significant difference in neuronal area and maximal diameter between the untreated controls, the vehicle treated controls and the TRH-OH treated cultures at both concentrations. As expected perikaryal area and maximal diameter were significantly increased in the TRH treated cultures at both concentrations employed ($p<0.01$).

These results suggest the lack of a neurotrophic effect for TRH-OH in this experimental paradigm. Whether the proline endopeptidase pathway leading to the formation of TRH-OH is significant in the inactivtion of TRH in these neuronal cultures merits further investigation.

282.7 AXONAL SPROUTING IN LISSAUER'S TRACT IN ANTI-NGF TREATED RATS. C.E. Hulsebosch, R.E. Coggeshall and J.R. Perez-Polo. Marine Biomedical Institute, Departments of Anatomy and Neurosciences, Physiology and Biophysics, Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550.

We previously reported more unmyelinated primary afferents in dorsal roots of the operated side compared to controls after unilateral spinal cord denervations. We interpreted this to mean that neurons emitted more branches as a result of spinal denervation. In an effort to manipulate this phenomenon, Nerve Growth Factor or antisera to Nerve Growth Factor (ANTI-NGF) were given in daily subcutaneous injections (1.5 µl/gm body weight of NGF at 10 ng/ml PBS and undiluted antisera, 3 µl/gm body weight, respectively) from birth for a period of one month. We previously reported a significant increase in the number of unmyelinated fibers in dorsal roots (T4, T5, T6) of the ANTI-NGF rats compared to denervated, NGF treated, preimmune sera treated or untreated littermate animals despite a concomitant 38% decrease in the number of small diameter dorsal root ganglion cells in the same segments of the ANTI-NGF rats. The present study presents preliminary data on the number of unmyelinated (UN) and myelinated (MY) fibers in Lissauer's tract, a tract in the white matter in which unmyelinated and small diameter myelinated primary afferents as well as propriospinal fibers are found, in the T3-T4 segment in ANTI-NGF rats compared to normal littermates.

	ANTI-NGF			NORMAL		
	MY	UN	TOTAL	MY	UN	TOTAL
	1833	15685	17518	1711	10259	11970
	1584	10256	11840	2226	10566	12792
	2037	13776	15813	1522	8289	9811
	1908	10171	12079	418	8007	8425
Mean	1841	12470	14310	1469	9280	10750
± S.E.	± 95	±1361	±1403	±380	±659	± 998

Since the same ANTI-NGF rats demonstrated a 38% decrease in small diameter neurons and small diameter neurons are thought to give rise to small diameter fibers, one would predict a significant decrease in the number of fibers in Lissauers tract unless sprouting occurred in either the primary afferents or the propriospinal neurons. Since the total means are 14,310 for the ANTI-NGF rats and 10,750 for the normal rats. This data supports the hypothesis of increased branches or axonal sprouting in the white matter as a result of NGF deprivation.

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282.8 NGF IMMUNOREACTIVITY AND BIOLOGICAL ACTIVITY IN THE PITUITARY GLAND. S. Soinila, J. Lakshmanan* and T. Lahtinen*
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A physiological role for NGF in the normal development of the peripheral sympathetic and sensory cells has been clearly demonstrated. There is also evidence that NGF has potent effects on various central neurons. However, the endogenous sources and routes of NGF supply to the neurons are not completely understood. We have studied various endocrine organs (pituitary, thyroid, parathyroid glands, adrenal medulla and gonads) as possible NGF sources and recently reported evidence that the pituitary gland might be one (Soinila et al., Soc. Neurosci. Abstr., 12, 1986).

In the present study we have investigated the biological NGF activity in the pituitary gland by coculturing each lobe at various ages with newborn rat sympathetic ganglia (for method see Lahtinen et al., Dev, Brain Res. 27:51, 1986).

Explants of the anterior, intermediate and posterior pituitary lobes taken from newborn, 2- and 3-week-old and adult rats stimulated nerve fiber growth from newborn superior cervical ganglia. This effect was completely abolished by antiserum to NGF. Freezing of the pituitary lobes for 7 days did not inhibit their growth-promoting effect, suggesting that biologically active NGF or an NGF-like factor is present in vivo in each lobe during the entire postnatal period.

NGF immunoreactivity in the adult gland is localized exclusively in the intermediate lobe (Soinila et al., Soc. Neurosci. Abstr.,12, 1986). We therefore tested the hypothesis that NGF levels in the anterior and posterior lobes remain low due to rapid retrograde axonal transport. The superior cervical ganglion, which is known to send axons to both anterior and posterior lobes, was bilaterally extirpated. This operation failed to produce immunohistochemically detectable NGF in either anterior or posterior lobe, but, surprisingly, it decreased the fiber growth stimulation by both lobes.

We suggest that pituitary NGF is a potential source of circulating NGF, and its levels are neurally controlled. It could also serve as a trophic factor for the innervating neurons or as a local neuromodulator.

282.9 NGF FACILITATES THE SURVIVAL OF RETINAL GANGLION CELLS AFTER OPTIC NERVE SECTION. G. Carmignoto*, P. Candeo*, R. Canella*, C. Comelli*, L. Bigon*, G. Vantini and L. Maffei*°. (SPON: G. Calderini). Fidia Research Laboratories, 35031 Abano Terme (PD), Italy. °Istituto di Neurofisiologia del CNR, Pisa, Italy.

In adult rats, intracranial transection of the optic nerve leads to degeneration of retinal ganglion cells, which is almost complete after 2 months. We here report that repetitive intraocular injections of βNGF (from male mouse submaxillary glands; 5 μg/injection every 48 hours) prevents, at least in part, the degeneration of a class of rat ganglion cells for a period as long as 2 months post-transection. Ganglion cells were retrogradely labelled with HRP pellets applied to the proximal stump of the optic nerve at 5-7-9 weeks after sectioning. After 24 hours, rats were perfused and the retinae dissected, post-fixed and reacted for HRP. The outlines of HRP-filled ganglion cell bodies were drawn with a camera lucida and measured with an IBAS-1 computer. In the same animals, the number of apparently normal myelinated axons of the optic nerve was estimated in semithin sections taken at 1 and 2 mm from the globe. Analysis of whole-mounted retinae shows that HRP filled ganglion cells of large size are more numerous in the retinae of the eyes treated with NGF, with respect to control retinae treated with cytochrome c. In particular, ganglion cells above 15 μm in mean diameter are present only in the retinae treated with NGF. Counting of myelinated optic nerve fibers shows that the number of morphologically intact fibers in the experimental optic nerves is about twice that of the controls. Hence, the intraocular injection of NGF has an effect on the survival of ganglion cells after section of the optic nerve. Whether this is due to a direct or indirect (via cholinergic amacrine cells) effect of NGF on the ganglion cells is currently under investigation.

282.10 MODULATION OF IN VITRO NEUROTROPHIC INTERACTION BY GANGLIOSIDE. F.J. Roisen, H. von Hoesslin*, S.P. Mahadik[1], M.M. Rapport[1], and G. Yorke*. Dept. of Anatomy, Sch. of Med., Univ. of Louisville, Louisville, KY 40292 and [1]Div. of Neurosci, New York State Psychiatric Inst., New York, NY 10032.

Gangliosides are glycosphingolipids that are more abundant in neuronal membranes than those of other tissues. They stimulate neuritic differentiation of Neuro-2a neuroblastoma and chick embryonic sensory ganglia (DRG) in culture. They potentiate some of the morphological and biochemical actions of Nerve Growth Factor (NGF) on DRG and the NGF-responsive rat pheochromocytoma cell line PC-12. We have demonstrated that monoclonal antibodies (mAbs) against the monosialoganglioside GM1 can diminish both NGF-mediated and NGF-independent neuritogenesis in DRG. The present study examines further the role of gangliosides in the regulation of neurotrophic interactions. Two neuritogenic models, DRG and PC-12, were cultured in the presence of either heart conditioned media (HCM) (Helfand et al, Exp. Cell Res., 113:39, 1978) or NGF. The effect of exogenous GM1 (150 ug/ml) on HCM or NGF mediated neuritogenesis was evaluated microscopically as neurite number and length and biochemically as changes in ornithine decarboxylase activity. For DRG and PC-12, the morphological and biochemical actions of NGF were potentiated by simultaneous treatment with GM1. Equivalent results were obtained by pretreating cultures with GM1 for 24 h prior to washing and refeeding with growth promoting medium. The neuritogenic action of HCM on DRG was enhanced by GM1. GM1 alone increased ODC levels, while HCM reduced ODC activity of DRG. Simultaneous exposure of DRG to HCM and GM1 resulted in further reduction of ODC activity. In contrast, although HCM elevated ODC activity of PC-12 cells, exogenous GM1 had no effect. Five mAbs against GM1 (Mahadik et al, J. Neurochem., 47:1172, 1986) were used to probe the role of endogenous ganglioside in DRG and PC-12 neurite formation. The antibodies were incorporated into the media at 20% and applied in the presence and absence of HCM or NGF. The mAbs reduced the actions of both HCM and NGF on DRG and PC-12. The mAb A2B5, which recognizes several neuronal gangliosides except GM1, had no effect. These studies suggest that GM1 can regulate neuronal development. Supported by NIH grant NS 24524.

282.11 SURVIVAL OF AXOTOMIZED SEPTAL CHOLINERGIC NEURONS: COMPARISON OF THE EFFECTS OF NERVE GROWTH FACTOR AND GM1 GANGLIOSIDE TREATMENTS. Lawrence F. Kromer, Dept. of Anatomy & Cell Biology, Georgetown University, School of Medicine, Washington DC 20007.

Several recent studies have indicated that the intraventricular administration of exogenous nerve growth factor (NGF) can prevent retrograde neuronal degeneration of septal cholinergic neurons after partial or complete transections of the dorsal septo-hippocampal pathways (Hefti '86, J. Neurosci. 6: 2155; Williams et al. '86, PNAS 83: 9231; Kromer '87, Sci. 235: 214). Peripheral administration of exogenous ganglioside GM1 also is reported to prevent retrograde changes after partial lesions of the basal forebrain cholinergic projections to hippocampal and neocortical areas (Cuello et al. '86, Brain Res. 376: 373; Sofroniew et al. '86, Brain Res. 398: 393). At present it is uncertain whether GM1 has a direct effect on cell survival or whether its effect is mediated by enhancing the ability of injured neurons to respond to low levels of their appropriate neurotrophic factor, such as NGF. The present experiments, therefore, were undertaken to compare the effects of exogenous GM1 and NGF on the survival of septal cholinergic neurons which received complete bilateral aspiration lesions of their axonal projections to the dorsal hippocampus. For this study lesioned animals were treated for 14 days beginning immediately after surgery with either a daily intraperitoneal administration of GM1 (30 mg/kg) or a continuous intraventricular infusion of NGF (2.5 ug of 2.5s NGF/day). Immunocytochemical staining for choline acetyltransferase (CAT) was used to identify surviving cholinergic neurons within the dorsal medial septum. Results from these experiments indicated that only about 20% of the CAT+ neurons in the medial septum survive bilateral lesions of the dorsal septo-hippocampal pathway. In contrast, approximately 85% of these neurons are present 2 weeks after this lesion when NGF is administered intraventricularly. Current data indicate that only 32% of the cholinergic neurons in the dorsal medial septal survive when GM1 is administered intraperitoneally. These preliminary results suggest that treatment with GM1 alone (in contrast to NGF) is not as effective in rescuing septal cholinergic neurons when there is a complete bilateral transection versus a partial lesion of the dorsal septo-hippocampal pathways. This difference in cell survival in the two paradigms may be associated with greater levels of endogenous trophic factors being available to the septal neurons after partial fornix/fimbria lesions. Further experiments are being conducted to resolve this issue. However, the present data are consistent with the hypothesis that the effect of GM1 on cholinergic neuronal survival requires the presence of at least low levels of endogenous trophic factor. (Supported by NIA grant #AG-06648)

282.12 GM1 GANGLIOSIDE FACILITATES NEURONOTROPHIC FACTOR EFFECTS BOTH IN VIVO AND IN VITRO. A. Leon, S.D. Skaper, G. Vantini and G. Toffano. Fidia Research Laboratories, 35031 Abano Terme (PD), Italy.

An increasing number of studies are now available indicating that the systemic administration of monosialoganglioside GM1 is effective in ameliorating outcome following experimentally induced brain damage to adult rodents. In addition, cultured neuronal cells, both primary and clonal, are known to respond to the ganglioside with pronounced morphological changes characteristic of cell differentiation. In cells with an absolute neuronotrophic factor requirement for survival and/or neurite outgrowth, the GM1 effects are associated with amplification of the trophic factor action on its target neuronal cells. For example, it has been reported that exogenously supplied GM1 is capable of potentiating the nerve growth factor (NGF)-induced neurite outgrowth of embryonic dorsal root ganglionic neurons and sympathetic ganglia. A similar situation was recently observed in vivo and suggests that GM1 effects in vivo, as in vitro, may be related to modulation of neuronal cell responsiveness to neuronotrophic factors. Studies conducted utilizing neuronal cell cultures indicate that the GM1 ganglioside stably associated to the cell surface is most probably responsible for the observed effects. There is now evidence that gangliosides may affect the production of second messengers involved in the membrane-mediated transfer of information. Hence, the capability of GM1 to facilitate neuronotrophic factor effects may involve amplification or modulation of the membrane-mediated transduction and/or translation of the trophic signal(s).

283.1 **HYPERALGESIA FOLLOWING NALOXONE-PRECIPITATED WITHDRAWAL FROM MORPHINE IS ASSOCIATED WITH INCREASED ON-CELL ACTIVITY IN THE ROSTRAL VENTROMEDIAL MEDULLA (RVM).** **J. B. Bederson*, N. M. Barbaro*, and H. L. Fields.**
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Two physiologically defined classes of cells in the rostral ventromedial medulla, on- and off-cells, have been implicated in modulation of nociceptive transmission and morphine (MS)-induced analgesia. On-cells burst just prior to withdrawal reflexes such as the tail flick or paw pinch withdrawal, and are inhibited by systemically administered MS. Off-cells pause just prior to tail flick and paw withdrawal, and are excited by MS. There is extensive evidence indicating that off-cell discharge inhibits spinal nociceptive transmission, but the role of the on-cell is less clear. On- and off-cells have reciprocal patterns of activity: on-cells are silent during periods of off-cell activity and vice-versa. Baseline tail flick latency (TFL) is shorter when on-cells are active and off-cells silent. The reciprocal relationship between on- and off-cell firing raises the possibility that the on-cell facilitates nociceptive transmission.

One sign of naloxone-precipitated opiate withdrawal is hyperalgesia. If MS-induced changes in on- and off-cell activity are important in analgesia then hyperalgesia may be the result of changes in the opposite direction in these cells. The present studies examine this hypothesis by studying the relationships between tail flick, paw withdrawal, and on- or off-cell activity after naloxone-precipitated withdrawal from MS.

Rats were anesthetized with chloral hydrate followed by continuous i.p. infusion adjusted to achieve stable TFL and paw withdrawal threshold (PWT), without signs of discomfort. Activity of on- and off-cells was continuously recorded. Saline or MS (1.25 mg/kg, i.v.) administration was followed by naloxone (1.0mg/kg, i.v.). TFL and PWT were correlated with cell activity.

Morphine eliminated tail flick and paw withdrawal (cutoff = 10 sec and 500 gm, respectively) as well as both spontaneous and reflex-related on-cell activity. Off-cell activity increased and the nocifensive-related pause was eliminated. When naloxone was given after MS, tail flick and paw withdrawal were consistently reduced to values significantly below baseline (hyperalgesia). On-cell activity was consistently increased above baseline during the period of hyperalgesia and returned to pre-MS levels 10-20 min later, along with the tail flick and paw withdrawal values. Off-cell activity abruptly decreased following administration of naloxone, returning to pre-MS levels as the period of hyperalgesia ended. In control (saline treated) animals, naloxone had no effect on any variable measured.

These results demonstrate a period of hyperalgesia after naloxone-reversal of morphine analgesia which is temporally correlated with increased on-cell activity. The hyperalgesia was observed following a single dose of morphine, and was not produced by naloxone alone. 1. The findings suggest that on-cells are part of a system which facilitates spinal nociceptive reflexes. 2. The results raise the possibility that certain aspects of opiate dependence and withdrawal are explainable by changes in the circuitry of the RVM.

Supported by PHS grant DA01949.

283.2 CHARACTERIZATION OF ANTINOCICEPTION PRODUCED BY INTRATHECAL SEROTONIN. G.F. Gebhart and R.E. Solomon. Department of Pharmacology, University of Iowa College of Medicine, Iowa City, IA 52242.

This study investigated the mechanisms by which intrathecal (i.t.) serotonin (5-hydroxytryptamine; 5-HT) produces antinociception by examining the effects of agents selective for 5-HT_1 receptor subtypes, the development of tolerance to 5-HT chronically administered i.t., and cross-tolerance between i.t. 5-HT and i.t. morphine. Rats were initially deeply anesthetized with pentobarbital (45 mg/kg i.p.) for placement of femoral arterial and venous and 7.5-8 cm i.t. catheters. Under light pentobarbital anesthesia (3-6 mg/kg/hr), the spinal nociceptive tail-flick (TF) reflex was evoked by radiant heating of the tail. Cumulative i.t. doses of drugs were administered in 7.5 µl volumes at 6 min intervals, and TF latencies were determined at 1, 3 and 5 min after each dose. Mean arterial pressure (MAP) was continuously recorded. Dose-response functions for 5-HT were determined 30 min after pretreatment with pargyline (5 mg/kg i.v.). 5-HT (10-320 µg) produced a dose-dependent inhibition of the TF reflex (ED_{50}=100.0 µg; 95% C.L.=47.6-210.0 µg), and a dose-dependent decrease in MAP (-17.0± 5.1 mm Hg at 320 µg). In the presence of the 5-HT antagonist methysergide (30 µg i.t.), the dose-response function for antinociceptive effects of 5-HT was shifted significantly to the right (ED_{50}=460.0 µg; 95% C.L.=164.2-1288.0 µg), whereas the depressor effects of 5-HT were not significantly changed. The selective 5-HT_{1A} agonist, 8-hydroxy-N,N-dipropyl-2-aminotetralin (8-OH-DPAT; 1-320 µg) and the selective 5-HT_{1B} agonist, 5-methoxy 3-(1,2,3,6-tetrahydro-4-pyridinyl) 1 H indole (RU 24969; 1-320 µg) did not increase TF latencies at any dose; rather, both agonists produced 10-20% decreases in TF latencies at the greatest doses tested (100-320 µg). 8-OH-DPAT and RU 24969 also produced dose-related depressor effects that were maximal at 320 µg (8-OH-DPAT: -46.0± 4.3 mm Hg; RU 24969: -28.7±5.0 mm Hg). These results suggest that the depressor, but not the antinociceptive, effects of i.t. 5-HT are mediated by spinal 5-HT_1 receptors. In rats treated chronically with i.t. 5-HT (320 µg/day x 7 days) and tested while awake, 5-HT initially produced significant increases in TF and hot-plate reaction latencies. These effects were significantly diminished on day 7, indicating the development of tolerance to the antinociceptive effects of i.t. 5-HT. Chronic i.t. morphine (32 µg/day x 7 days), which has been found to produce tolerance to the antinociceptive effects of i.t. morphine and cross-tolerance to i.t. clonidine, did not affect the dose-response function for antinociceptive effects of i.t. 5-HT in lightly-anesthetized rats. These results are not inconsistent with reports that opioids and α_2-adrenoceptor agonists, but not 5-HT, share the ability to inhibit release of putative nociceptive transmitters from primary afferents. Supported by DA 02879 and T32 GM 07069.

283.3 ROLE FOR THE HIGH-AFFINITY MU_1 OPIOID BINDING SITE IN CENTRAL OPIATE AND OPIOID ANALGESIA. R.J. Bodnar, C.L. Williams*, S.J. Lee* and G.W. Pasternak. Dept. of Psych. Queens College, CUNY, Flushing NY 11367 and Dept. of Neurol., Memorial Sloan-Kettering Cancer Ctr., NY, NY 10021.

The intracerebral microinjection technique has localized central sites of analgesic action for opiates and endogenous opioids, including the periaqueductal gray (PAG), locus coeruleus (LC), nucleus raphe magnus (NRM) and nucleus reticularis gigantocellularis (NRGC). Multiple opiate receptor subtypes (mu, delta, kappa, epsilon) have been implicated in opiate analgesia elicited from spinal and brainstem loci. Binding studies indicate that many of the ligands used in subtype determinations share a common high-affinity (mu_1) for which selective irreversible antagonists (e.g., naloxonazine) have been developed. Mu_1 sites have been implicated in a number of opiate actions, including supraspinal, but not spinal analgesia elicited by either systemic or intracerebroventricular administration of opiates or opioids. The present study evaluated whether the mu_1 binding site was involved in analgesia elicited by morphine injected into either the PAG, LC, NRM or NRGC. Rats were stereotaxically implanted with guide cannulae (28 gauge) aimed at one of the four loci and microinjected with morphine through a 33 gauge internal cannula 24 h following naloxonazine (20 mg/kg, IV) or vehicle. Latencies were assessed every 15 min for 60 min; naloxone (10 mg/kg, SC) was administered and latencies were then assessed 15 and 30 min after this injection. Morphine (5 ug) elicited analgesia from all four sites which was reversed by naloxone and naloxonazine. Naloxonazine produced a rightward shift in morphine analgesia dose-response curves from the PAG and LC. We then evaluated the analgesic effects of two delta receptor agonists, d-ser_2-thr_6-leucine enkephalin (DSTLE) and d-pen_2-d-pen_5 enkephalin (DPDPE); the former but not the latter interacts with mu_1 sites. Like morphine, DSTLE analgesia from all four sites was blocked by naloxone and naloxonazine. In contrast, DPDPE failed to produce and analgesic response at doses up to 15 ug when injected into either the PAG or LC. These data indicate that the high-affinity mu_1 opioid binding site mediates opiate and opioid analgesia elicited from these integral brainstem loci involved in opiate forms of pain inhibition. (Supported by ACS Grant PDT 169).

283.4 **THE RAT VISCERAL PAIN MODEL (VPM) IN THE ASSESSMENT OF TOLERANCE TO MORPHINE SULFATE (MS).** R.W. Colburn*, D.W. Coombs*, C.C. Degnan*, and L.L. Rogers*
(SPON: S. Velez) Dept. of Anesthesia Research, Dartmouth-Hitchcock Medical Center, Hanover, NH 03756

The Acetic Acid Writhing (AAW) test in the rat (Collier et al, Br J Chemother 32:295,1968) is a classic tool for visceral pain assessment. In the AAW model, intraperitoneal administration of noxious chemical agents induces an inflammatory effect and a quantifiable writhing response. We recently reported a new visceral pain model (VPM) to provoke a reproducible and reversible visceral insult in rats (Coombs et al, Anesthesiology: Submitted 1987) by duodenal distention via inflation of a surgically placed intraduodenal balloon catheter. This distention provokes a writhing-like activity (WLA) characterized by profound contraction of abdominal musculature, body stretching and posturing to right or left. Serial tests potentially allow acute and chronic assessment of analgesic agents and narcotic tolerance.

Under halothane anesthesia, 200 gram Sprague-Dawley rats were implanted with balloon catheters. The rats recovered 72 hours prior to testing nociception. A minimum threshold volume (MTV) to elicit WLA without destroying gut integrity was established (range 0.5-1.0 ml). Five MTV balloon pulses were given over 30 seconds. Rats were observed for 10 minutes to record WLA. Fifteen rats who exhibited WLA were grouped as follows: Group I = Saline Control and Group II = Morphine, 1 mg/ml. One ml/kg of saline or morphine was injected subcutaneously every 12 hours for 5 days. WLA was assessed on Days I and V, 30 minutes after the morning injection. Catheter position and gut integrity were confirmed at post-mortem. Association was analyzed by chi-square (see table below).

Results: On Day I, WLA was observed in 86% of controls and in no morphine-treated rats. On Day V, WLA was seen in 29% of control and 25% of morphine-treated rats.

	Saline Control (n=7)	Morphine (n=8)
Day I	6/7 (86%)	0/8 (0%) p 0.01
Day V	2/7 (29%)	2/8 (25%)p=NS

Conclusions: 1. Acutely morphine yields significant antinociception against mechanical visceral stimulation. 2. Reduced writhing (WLA) in controls at day 5 may result from stress-induced analgesia or from altered MTV over time; this requires further study. 3. On day 5 morphine-treated rats were not uniformly tolerant (25% WLA). This finding with 2 (above) may suggest inadequate morphine duration/magnitude to yield tolerance in the VPM or partial tolerance protection by stimulation/stress in the morphine group (Colpaert et al, Eur J Pharm 49:335, 1978). Further work is in progress.

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283.5 SPINAL AND SUPRASPINAL INTEGRATION OF THE VISCERAL AFFERENT INPUT TO THE THORACIC SPINAL CORD OF THE CAT. B.M. Lumb* and F. Cervero* (SPON: A.D.Smith). Department of Physiology, University of Bristol Medical School, Bristol BS8 1TD, U.K.

The diffuse nature of visceral sensations may be attributed, at least in part, to the relatively sparse visceral afferent input to the spinal cord and subsequent extensive central divergence. We have shown that the divergence of the visceral afferent input to the lower thoracic spinal cord of the cat involves supraspinal looped pathways under phasic and tonic descending inhibitory controls from the brain stem. We have now investigated further the extent of this central divergence by studying whether single thoracic neurones are influenced by bilateral visceral stimulation and whether this information is modulated by descending controls.

Extracellular recordings were made in the T9-T11 segments of the spinal cord of anaesthetized cats. Viscero-somatic neurones were distinguished by their responses to electrical stimulation of the dorsal roots and of the ipsilateral splanchnic nerve (iSPLN). These cells were then tested for bilateral visceral inputs with electrical stimulation of the contralateral splanchnic nerve (cSPLN). Descending influences were tested by reversible spinalization at T7 and by electrical stimulation in the nucleus raphe magnus (NRM) and the adjacent reticular formation (Ret.F.)

Viscero-somatic neurones could be divided into 2 groups on the basis of the presence or absence of a contralateral visceral input.

i) More than 90% of the neurones could be excited by stimulation of both the iSPLN and cSPLN, i.e. had bilateral visceral inputs. The majority of these cells were located in the ventral horn with the remainder distributed in the superficial and deep dorsal horn. The visceral responses of these cells were generally reduced or abolished in the spinal state which suggests that their visceral inputs were mediated or reinforced by supraspinal loops. More than 80% of these cells could be driven by stimulation in NRM and Ref.F. In every case tested the initial excitatory response to brain stem stimulation was followed by a period of inhibition.

ii) A small minority of units (less than 10%) showed no response to stimulation of the cSPLN, i.e. had an ipsilateral visceral input only. All these cells were located in the superficial dorsal horn and only 1 cell could be driven by electrical stimulation in NRM and Ret.F although, when tested, the visceral inputs to these cells were inhibited by brain stem stimulation.

These results show that the divergence of the visceral afferent input in the cat's thoracic cord includes a bilateral input to a large proportion of viscero-somatic neurones and that the visceral inputs to these cells may be mediated by supraspinal loops. We have also obtained evidence for the existence of a small population of viscero-somatic neurones in the superficial dorsal horn with an exclusively ipsilateral visceral input.

283.6 DIFFERENTIAL ENCODING OF PROLONGED NOXIOUS MECHANICAL STIMULI BY DORSAL HORN NEURONES OF THE RAT'S SACRAL SPINAL CORD. J.M.A. Laird*, F. Cervero* and H.O. Handwerker*+ (SPON: European Neurosci. Assoc.). Department of Physiology, University of Bristol Medical School, Bristol BS8 1TD, U.K. and (+) Institute of Physiology & Biocybernetics, University of Erlangen, D-8520 Erlangen, F.R.G.

When a fold of human skin is squeezed for 2 minutes at an intensity just above the pain threshold, the sensation becomes more and more painful throughout the stimulus period. However, microneurographic studies of human nerves and electrophysiological recordings from rat's single nerve fibres have shown that all types of cutaneous receptor, including nociceptors, exhibit adaptation to such stimuli (Adriaensen et al 1984, Human. Neurobiol. 3:53-58; Handwerker et al 1987, Exp. Brain Res. 65: 493-504). We have now examined the responses of dorsal horn neurones of the rat's sacral spinal cord to identical noxious mechanical stimuli in an attempt to establish the central mechanisms responsible for the increasing pain sensation that occurs in parallel with a decreasing afferent inflow from nociceptors.

Extracellular recordings were made from dorsal horn neurones in the S1-S2 segments of the spinal cord of rats anaesthetized with pentobarbitone. All neurones responded to noxious mechanical stimulation of the rat's tail and were tested with mechanical stimuli ranging from 4 to 8N (350-700 KPa) and lasting for 2 minutes. These stimuli were delivered by a feed-back controlled device previously used to examine the discharge patterns of cutaneous afferent fibres from the rat's tail.

All dorsal horn neurones studied were excited by these stimuli throughout the 2 min period and showed little evidence of adaptation. Most cells responded with a sustained discharge after an initial phasic response and in some cases increased their responses throughout the stimulus. Two broad categories of neuronal response could be distinguished:

i) Nociceptor specific neurones of the superficial dorsal horn encoded the intensity of the stimulus so that more intense stimuli produced greater sustained responses.

ii) Most multireceptive ("wide dynamic range") neurones of the deep dorsal horn did not show the same encoding properties. However, their excitability increased during and after the application of the stimulus and this resulted in an increase in the levels of background activity for several minutes after the end of the stimulus. The magnitude of their sustained discharges during stimulation was not correlated with the stimulus intensity.

These results provide initial evidence for a central component of the tonic pain sensation that results from prolonged noxious stimulation and show differential encoding properties between nociceptor specific and multireceptive neurones of the dorsal horn.

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283.7 OPIATES SUPPRESS CARRAGEENAN-INDUCED INFLAMMATION. K. Hargreaves* and J. Joris (SPON: S. Pretel). Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Although opiates inhibit neurogenic plasma extravasation (Lembeck & Holzer, Arch. Pharmacol. 312:175, 1979), their peripheral effects in models of actual inflammation have not been determined. Therefore, we examined the effects of opiates on suppressing carrageenan (CARRA)-induced edema, hyperthermia and plasma extravasation, in addition to their well-recognized effects on blocking hyperalgesia. The dorsal-plantar paw thickness, measured by a caliper to 0.1 mm, was taken as an index of edema. Local hyperthermia of the plantar surface of the inflamed paw was measured with a contact thermocouple. Plasma extravasation was quantitated by measuring (at 620 nm) Evans blue dye extracted (by formamide) from paw tissue (6 mm punch biopsies) after i.v. injection. For noxious thermal stimulation, rats in a chamber had their paws exposed to a beam of radiant heat applied through a glass floor; paw withdrawal latency (PWL) was taken as an index of the nociceptive threshold. All observations were collected by investigators blind as to treatment allocation. Six to twelve rats/group were employed with data analyzed by ANOVA and Duncan's test. In the first study, CARRA was injected into a hindpaw and 60 min later rats received either i.p. morphine (0.2, 1.0 or 5.0 mg/kg) or saline injection. Morphine caused a dose-related (ANOVA: $p<0.01$) blockade of CARRA hyperalgesia at 0.2 mg/kg (4.2 ± 1.5 sec), 1.0 mg/kg (5.1 ± 1.8 sec, $p < 0.05$) and 5.0 mg/kg (6.8 ± 1.3 sec, $p < 0.01$) as compared to the PWL of saline-treated rats (2.8 ± 1.4 sec). Inflammatory hyperthermia was also reduced by morphine at 0.2 mg/kg (29.5 ± 0.2°C, $p < 0.05$), 1.0 mg/kg (29.8 ± 0.3°C, $p < 0.01$) and 5.0 mg/kg (29.4 ± 0.3°C, $p < 0.01$) in comparison to saline-treated rats (31.6 ± 0.4°C). Similar results were observed with edema; morphine administered at 0.2 mg/kg (8.2 ± 0.4 mm, $p < 0.05$), 1.0 mg/kg (7.9 ± 0.3 mm, $p < 0.05$) and 5 mg/kg (7.7 ± 0.3 mm, $p < 0.05$) had significantly less edema than saline-treated rats (9.2 ± 0.4 mm). This effect is stereospecific, since 1 mg/kg levorphanol decreased edema (7.9 ± 0.1 mm, $p < 0.05$) as compared to saline (8.8 ± 0.3 mm), while 1 mg/kg dextrorphan had no effect (8.7 ± 0.5 mm). Administration of 2 mg/kg i.v. morphine significantly inhibited CARRA-induced plasma extravasation (10.4 ± 1.5 µg dye/biopsy, $p < 0.01$) as compared to i.v. saline (17.8 ± 2.5 µg dye/biopsy). These results indicate that systemic opiates exert anti-inflammatory effects in the CARRA model of inflammation either through a peripheral mechanism or via activation of centrifugal CNS processes. (J. Joris, supported by an FNRS Belgium Fellowship, is on leave from the Department of Anesthesiology, University of Liege).

283.8 INVOLVEMENT OF THE PERIPHERAL NERVOUS SYSTEM IN CARRAGEENAN-INDUCED INFLAMMATION. J. Joris*, R. Dubner and K. Hargreaves* (SPON: M.A. Ruda). Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Recent studies have demonstrated that substance P (SP) and calcitonin gene-related peptide (CGRP), 2 neuromediators present in C fibers, have peripheral inflammmatory activity (Lembeck et al., Eur J Pharmac 85:171, 1982; Brain & Williams, Br J Pharmac 86:855, 1985). We investigated whether the peripheral nervous system contributes to carrageenan (CARRA)-induced inflammation by examining the effect of denervation and a SP antagonist on this inflammation. Three signs of inflammation were assessed. Cutaneous hyperthermia was measured with a thermocouple. Paw thickness, measured with a caliper to 0.1 mm, was used as an index of edema. Hyperalgesia was determined using thermal stimulation: rats in a chamber had their paws exposed to a beam of radiant heat applied through a glass floor. Paw withdrawal latency (PWL) was taken as an index of the nociceptive threshold. All observations were collected by investigators blind as to treatment allocation. Six to eight rats/group were employed with data analyzed by ANOVA and Duncan's test. In a first experiment, one group of rats had a hindpaw denervated by sciatic and saphenous nerve section 8 days before testing. Another group was sham operated. In each of these 2 groups, half of the rats received either CARRA (2 mg) or saline (SAL) into the operated paw. Denervation significantly inhibited the inflammatory hyperthermia by 88.4% at 2 hr ($p<0.01$) and 38.1% at 3.5 hr. ($p<0.05$) after CARRA injection. Similar results were observed with edema: denervation inhibited CARRA-induced edema by 31.1% at 2 hr. ($p<0.01$) and 21.8% at 3.5 hr. ($p< 0.01$). On the other hand, denervation had no effect on the SAL-treated paw with respect to temperature and edema. In a second experiment, rats injected in one hindpaw with CARRA (2 mg) received 60 min later a second injection into the paw of either SAL or an SP antagonist, ($DPro^{2}$,$DTryp^{7,9}$)substance P (25, 50, 100 or 200 µg). As compared to the SAL treated paws (PWL = 2.6 ± 0.3 sec.), the SP antagonist produced a significant dose-related (ANOVA: $p<0.05$) blockade of CARRA-induced hyperalgesia at 25 µg (3.3 ± 0.8 sec.), 50 µg (3.4 ± 0.2 sec.), 100 µg (4.4 ± 0.6 sec.) and 200 µg (5.6 ± 1.3 sec.). These results indicate that CARRA-induced inflammation has a significant neurogenic component possibly involving the release of substance P. (J. Joris, supported by an FNRS Belgium fellowship, is on leave from the Department of Anesthesiology, University of Liege).

283.9 D-BACLOFEN ANTAGONIZES L-BACLOFEN AT LOW DOSES BUT NOT AT HIGH DOSES. G. H. Fromm, T. Shibuya*, C. F. Terrence*. Dept. of Neurology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

D-baclofen (D-BCF) antagonizes L-baclofen (L-BCF) in the feline trigeminal nucleus [Terrence CF et al., Pharmacology 27:85, 1983] and in the rat spinal cord [Sawynok J, Dickson C. Pharmacology 31:248, 1985], but not in hippocampal slices [Haas HL et al. Neurosci Let 55:1, 1985] . We have also found that L-BCF is more effective than five times as much racemic baclofen in the treatment of trigeminal neuralgia, suggesting that D-BCF antagonizes L-BCF in humans too [Fromm GH, Terrence CF. Neurology in press].

We have now investigated the effect of iontophoretic application of L-BCF and D-BCF to single neurons in the trigeminal nucleus of Sprague-Dawley rats anesthetized with halothane. 10-20 nA of 0.1 M L-BCF depressed excitatory transmission similar to the effect of 0.1-0.4 mg/kg L-BCF given i.v. The concommittant application of 10-20 nA 0.1 M D-BCF antagonized the effect of L-BCF. However, 40-50 nA D-BCF facilitated the action of L-BCF. We also found that 30-40 nA L-BCF had a much stronger effect than previously obtained with parenteral L-BCF, and D-BCF was not able to block it. The iontophoresis of 240-350 nA D-BCF produced an effect similar to that 10-20 nA L-BCF.

An antagonistic action by one enantiomer of a drug on the effect of the other is rather unusual, but has been reported for apomorphine and some Ca channel blockers. Our observations indicate that D-BCF has a similar effect as L-BCF but is one to two orders of magnitude less potent. The weak effect of D-BCF therefore probably interferes with the stronger effect of L-BCF when both are administered in equimolar low concentrations, comparable to those used in the treatment of trigeminal neuralgia. The failure to observe an antagonistic effect in the hippocampal slice experiments was probably due to the fact that a high dose of L-BCF was administered, as well as that the dose of D-BCF was twice that of L-BCF. Our experiments therefore also indicate that drug action in humans can only be predicted when therapeutic concentrations are administered in an appropriate model.

283.10 DIFFERENT TYPES OF PAIN ARE REGULATED DIFFERENTLY IN THE CNS. A NEW MODEL OF PAIN REGULATION IS NEEDED. K.Hole, P.K.Eide*, O.B.Fasmer* and O.-G.Berge*. Dept. of Physiol., University of Bergen, N-5000 Bergen, Norway.

It is well established that the serotonergic raphe-spinal pathways may tonically inhibit nociception. When the tail flick test is used as a measure of nociception in rats and mice, lesioning of these pathways with intrathecal 5,6-dihydroxytryptamine (5,6-DHT) or blocking spinal 5-hydroxytryptamine (5-HT) receptors with receptor blockers injected intrathecally, reduce the tail flick latency. On the other hand, antinociception is observed when spinal 5-HT receptors are stimulated with 5-HT agonists or when 5-HT is released from spinal terminals using parachloroamphetamine injected intrathecally.

However, when the formalin test was used in mice, 5,6-DHT induced lesion reduced pain sensitivity in the early phase of the test, while the late phase remained unchanged. Thus, one may conclude that the serotonergic raphe-spinal pathway may tonically inhibit, tonically enhance, or have no tonic activity depending on the test used to measure nociception. The early phase in the formalin test is probably related to a direct chemical stimulation of nociceptors, the late phase related to inflammatory pain, while the tail flick test measures a spinally integrated nociceptive reflex.

Similarly, the putative 5-HT receptor antagonist metitepin injected systemically in mice increased the sensitivity in the tail flick test and reduced the sensitivity in the increasing temperature hot plate test.

These and similar observations show that the role of the 5-HT system in control of nociception is complex, possibly different for different types of pain (different simulus modalities, intensities and duration). As a working model one may postulate that the nociceptive control system has a set point value, similar to several other control systems. This set point may vary, during the night-day cycle, depending on behavioral state and stress, and may be influenced by a variety of external and internal stimuli. The consequences of manipulations of serotonergic functions may thus depend on the stimulus and the set point value and the state of the control system at the time of stimulation.

283.11 GONADAL STEROIDS MODULATE SWIM ANALGESIA IN INTACT AND GONADECTOMIZED RATS. M.-T. Romero, M.L. Cooper*, B.R. Komisaruk and R.J. Bodnar. Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367 and Inst. of Animal Behavior, Rutgers Univ., Newark, NJ 07102.

Female rats a significantly smaller magnitude of analgesia following morphine, opioid-mediated intermittent cold-water swims (ICWS) and nonopioid-mediated continuous cold-water swims (CCWS) than age-matched and weight-matched males. Normal gonadal function modulates these gender-specific effects since castration reduces CCWS and ICWS analgesia to levels observed in female rats, and ovariectomy reduces these analgesic magnitudes further in females. The present study evaluated the roles of the gonadal steroids, testosterone proprionate (TP) and estradiol benzoate (EB) upon CCWS and ICWS analgesia in intact and gonadectomized male and female rats. Age-matched rats received castration, ovariectomy or sham surgery. Four weeks later, intact and gonadectomized males and females (range: 8-10 rats/group) received either TP (2 mg/kg, SC) for 14 days, EB (5 ug/kg, SC) for seven days or sesame oil vehicle (VEH) for 14 days; each treatment continued through behavioral testing. All animals were exposed to CCWS (2°C for 3.5 min) and ICWS (2°C, 18 10-sec swims and 10-sec rests) in a counterbalanced fashion with a one-week interval elapsing between conditions. Tail-flick latencies, jump thresholds and core body temperatures were assessed for up to 2 h following each swim. Gonadectomy reduced both CCWS and ICWS analgesia. The magnitude of swim analgesia was reinstated by TP treatment in castrated males and ovariectomized females for CCWS analgesia on both tests and for ICWS analgesia on the jump test. TP potentiated CCWS analgesia in intact males on the tail-flick test. EB attenuated CCWS analgesia in intact females and reinstated ICWS analgesia in ovariectomized females on the jump test. Hypothermia could not account for the altered analgesic effects or the effects of the steroids. These results suggest that TP, but not EB plays an important role in the gonadal modulation of both opioid-mediated and nonopioid-mediated forms of swim analgesia, but supplements of neither steroid to intact rats consistently alters these analgesic responses. (Supported by PSC/CUNY Grant 6-66351 and NIH BRSG RR 07064).

283.12 HISTAMINE AND CONTINUOUS COLD-WATER SWIM ANALGESIA IN RATS: EFFECTS OF CIMETIDINE. J.A. Robertson, L.B. Hough and R.J. Bodnar. Dept. of Psych., Queens College, CUNY, Flushing, NY 11367 and Dept. of Pharmacol. & Toxicol., Albany Medical College, Albany, NY 12208.

Cimetidine, a histamine H_2 receptor antagonist, produces differential effects upon analgesic responses. While cimetidine fails to affect morphine analgesia, it reduces a nonopioid form of footshock analgesia, and potentiates an opioid form of footshock analgesia. The antagonist effects upon footshock analgesia were presumed to occur centrally since neither of the two forms of footshock analgesia was mediated by neurohormonal factors. Since it was not known how cimetidine affected neurohormonally-mediated forms of environmentally-induced analgesia, the present study evaluated the effect of this H_2 receptor antagonist upon the nonopioid and neurohormonal analgesic response elicited by acute exposure to continuous cold-water swims (CCWS) in rats. Sprague-Dawley rats received cimetidine (10, 50 and 100 mg/kg dissolved in phenol (5 mg/ml), IP) or a phenol vehicle 30 min prior to CCWS (2°C for 3.5 min). Tail-flick latencies, jump thresholds and core body temperatures were assessed 30, 60 and 90 min thereafter. CCWS analgesia on both nociceptive measures was significantly potentiated by 50 to 100% across the post-swim time course by the 100 mg/kg, but not the 50 or 10 mg/kg doses of cimetidine. The 100 mg/kg dose of cimetidine also potentiated CCWS hypothermia; the two lower cimetidine doses were ineffective. In contrast, none of the cimetidine doses affected baseline tail-flick latencies jump thresholds or core body temperatures. The associated potentiations of both CCWS analgesia and CCWS hypothermia by the same cimetidine dose range suggests that H_2 antagonist effects upon this manipulation may be altering the stressful consequences of the swim rather than specific effects upon the activated nonopioid neurohormonal pain-inhibitory system. (Supported by PSC/CUNY Grant 6-66351 and BRSG NIH Grant RR 07064).

283.13 POTENTIATION OF CONTINUOUS COLD-WATER SWIM ANALGESIA BY YOHIMBINE. K.L. Kepler and R.J. Bodnar. Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367.

The analgesic response following continuous cold-water swims (CCWS) is mediated through a nonopioid, but neurohormonal and hypothalamo-hypophysial mechanism of action. Norepinephrine (NE) has been postulated as a possible modulator of CCWS analgesia since this stressor promotes NE release. In this regard, CCWS analgesia is reduced following lesions placed in the noradrenergic locus coeruleus and is potentiated following pretreatment with either clonidine, an alpha$_2$-NE receptor agonist or desipramine, a NE reuptake blocker. The present study evaluated whether yohimbine (YOH) an alpha$_2$-NE receptor antagonist altered CCWS analgesia on the tail-flick and jump tests in rats, and whether any effects correlated with YOH-induced changes in CCWS hypothermia or basal thresholds. YOH (0.1-2.0 mg/kg, IP) dose-dependently increased jump thresholds, but failed to alter basal tail-flick latencies or core body temperature. YOH (0.1 and 2.0 mg/kg) potentiated CCWS (20C for 3.5 min) analgesia for 60 min on the jump test; this potentiation was additive relative to YOH and CCWS effects alone. YOH dose-dependently potentiated CCWS analgesia on the tail-flick test at 30 min after the swim, an effect not accountable by basal changes. Since CCWS hypothermia failed to be affected by YOH, the analgesic potentiations could not be explained by this variable. Together with our previous determination that clonidine pretreatment potentiated CCWS analgesia, these data indicate that both agonists and antagonists of the alpha$_2$-NE receptor potentiate this form of analgesia, suggesting that such NE-induced effects may be orthogonal to intrinsic pain-inhibitory mechanisms subserving CCWS. These data confirm previously-established analgesic efficacies of alpha$_2$ NE antagonists and agonists administered peripherally. Further, these effects dissociate CCWS analgesia from the YOH-induced decreases of neurally-mediated autoanalgesia and neurally and opioid-mediated footshock analgesia. These results may be explained by differential NE-induced changes in nociception at different levels (e.g., brainstem, spinal cord) of the neuraxis. (Supported by PSC/CUNY Grant 6-66351 and NIH BRSG RR07064).

PEPTIDE: RECEPTORS I

284.1 NEUROPEPTIDE MODULATION OF PHOSPHOLIPASE C ACTIVITY IN BRAIN SLICES AND NG108-15 NEUROBLASTOMA-GLIOMA HYBRID CELLS. S.J. Fluharty, C.V. Nicchitta & J.R. Williamson. (SPON: C.R. Gallistel) Depts. of Animal Biology and Biochemistry, Institute of Neurological Sciences, University of Pennsylvania, Phila., PA 19104.

Several neurotransmitter receptors are coupled to the hydrolysis of inositol phospholipids through the stimulation of a polyphosphoinositide specific phospholipase C (PLC) in neural cells. This receptor-mediated release of inositol phosphates (IPs) and the subsequent rise in cytosolic calcium is presumed to play an important role in the regulation of neuronal function. The present report compares the effects of angiotensin II (ANG II), a neuropeptide which is known to stimulate PLC in a variety of peripheral cells, with Substance P (SP) and the alpha-adrenergic agonist phenylephrine (PE) in brain slices, and with bradykinin (BK) in a culutred neuron-like cell line, NG108-15.

Male SD rats were sacrificed and slices of the hypothalamus-thalamus-septum (HTS) region were prepared. Slices were incubated under constant gassing in KRH containing 10 uCi/ml of (^{3}H)-myoinositol for 1 hr in the presence of 10 mM LiCl. After labelling, ANG II (10 uM), SP (1 uM) or PE (100 uM) was added and samples were quenched with 1 M TCA 30 min later, neutralized and applied to Dowex 1X-8 columns. Experiments involving cultured NG108-15 cells were similar except that labelling was for 24 hr and samples were quenched 30 sec after addition of ANG II or BK (10 uM). IPs were eluted sequentially with 0.2 - 1.0 M NH_4 formate/0.1 M formic acid. In some cases, membranes were prepared from HTS or NG108-15 cells for radioligand binding studies using (^{125}I)-ANG II.

In HTS slices, both PE and SP increased IPs by 140% and 74%, respectively. In contrast, ANG II had no effect on IPs nor did it alter the response to PE despite the presence of ANG II receptors in this region (B_{max} = 7.4 fmols/mg prot: K_D = 548 pM). ANG II receptors also are present in NG108-15 cells (B_{max} = 12.8 fmols/mg prot; K_D = 331 pM) yet this peptide did not appear to stimulate PLC in these cells. On the other hand, BK increased IP_1, IP_2 and IP_3 levels from 200-400%.

In summary, our results are consistent with the suggestion that the properties of neuronal and peripheral ANG II receptors may differ particularly with regard to their coupling to inositol phospholipid metabolism. Therefore, the precise intracellular second messenger system(s) utilized by these receptors in the brain remains to be established. Supported by NS 23986, DK 15120 and the University of Pennsylvania Research Foundation.

284.2 PHOSPHOLIPASE A2, A KEY ENZYME IN THE PRODUCTION OF ARACHIDONIC ACID, BINDS BRADYKININ (BK) WITH HIGH AFFINITY AND SPECIFICITY. C. R. Mantione Miami Valley Laboratories,
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Quinacrine, a phospholipase A2 (PLA2) inhibitor, prevents BK-induced prostaglandin formation without affecting basal activation of prostaglandins in guinea pig lung (Vargaftig and Dao Hai, J. Pharm Phamacol 24, 159, 1972). Also, quinacrine blocks BK-stimulated cGMP formation in murine neuroblastoma cells (Snider, M. R. and Richelson, E., J. Neurochem 43, 1749, 1984). This led to an investigation of whether quinacrine was acting by inhibiting BK binding directly to a site on PLA2.

For these experiments, bee venom PLA2 (25-250 ng) was incubated with [3H]BK in 25 mM TES buffer, pH 6.8, with 0.05% BSA for 90 min at 25° C. [3H]BK bound with 85% specific binding over the concentration range of 0.05-10 nM. The pH optimum lies between 6.5-7.2, then slowly declines at higher pH. The association rate constant was determined to be 0.0074 min-1 at 25° C, whereas the dissociation T1/2 was greater than 180 min. Under binding conditions described above, a Scatchard analysis yielded a Kd of 3.8 nM and a Bmax of 1328 pmoles/ng enzyme. Pharmacologically, this binding site represents neither a B1 or a B2 receptor. Both the B1 specific antagonist, Leu8,desArg^9-BK, and the B2 antagonist, [Thi5,8 D-Phe7]-BK, inhibits binding with a Ki between 7-9 nM. The agonist, MetLys-BK is about 3 times more potent than BK. Substance P, a neurokinin, was equivalent to that of BK and Lys-BK. The algesic agent capsaicin was inactive. Quinacrine had a Ki of 62 µM. L-Phosphatidylcholine, a PLA2 substrate, was inactive, as were PAF and various prostaglandins.

These data provide evidence that PLA2 and other phospholipases has a high affinity site which specifically binds the kinin family of peptides. PLA2 may represent an important in vivo binding site for regulating inflammation and pain generation mediated by bradykinin, and possibly certain neurokinins.

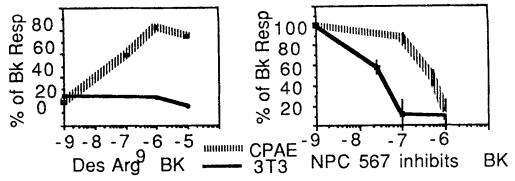
284.3 **TWO DISTINCT BRADYKININ RECEPTORS DISTINGUISHED BY DIFFERENTIAL COUPLING TO PHOSPHOLIPASE A_2 AND PHOSPHOLIPASE C**

B.R. Conklin, R.M. Burch, L.R. Steranka, J. Axelrod, Laboratory of Cell Biology, National Institute of Mental Health, Bethesda MD 20892 and Nova Pharmaceutical Corp., Baltimore MD 21224

Bradykinin (BK) is a nonapeptide with potent actions in mediating vasodilation, pain, and smooth muscle contraction in a variety of biological systems. Many of these actions are thought to be mediated by arachidonic acid and its metabolites. Phospholipase A_2 (PLA_2) and Phospholipase C (PLC) are rate limiting enzymes in receptor mediated arachidonate release and its metabolism to eicosanoids. We have investigated the coupling the BK receptor to these two lipases in Swiss 3T3 fibroblasts and CPAE bovine pulmonary artery endothelial cells, and have found striking differences in their respective sensitivity to several bradykinin analogs and their receptor coupling to PLA_2 and PLC.

The BK receptor in CPAE cells has been shown to be coupled to PLC while PLA_2 activation was not observed (Clark et al JBC, 261:23,10713, 1986). In contrast, we have evidence that in the 3T3 cells, BK receptor mediated arachidonate release is coupled to PLA_2 (Burch, Axelrod, PNAS in press). We have measured receptor function by release of ^{3}H-arachidonic acid as well as RIA for the primary eicosanoid end product of BK stimulation in each cell line (prostaglandin E_2 in the 3T3 cells, and 6-keto-prostaglandin $F_{1\alpha}$ in the CPAE cells). The dose response curves for bradykinin in the two cell lines are similar with EC50s of 3T3: 200 pM and CPAE: 210pM. Des-Arg^9-BK is a full agonist in the CPAE cells with a EC50 of 40nM while it is completely inactive in the 3T3 cells up to a concentration on 10μM. NPC 334 (p-chlorophe^6-Pro^7-BK) is a full agonist in the 3T3 cells but is only a partial agonist in the CPAE cell reaching maximum of 56% of the bradykinin response at 250 nM and dropping to 10% at 10μM. NPC 567, (DArg-(Hyp^3,DPhe^7)-BK a BK antagonist, is 10-fold more potent in blocking the effects of 10 nM BK the 3T3 cells than in the CPAE cells. Des-Arg^9-Leu^8-BK is a weak antagonist with effects seen at 10 μM in both cell lines.

These findings indicate that there are at least two distinct bradykinin receptors, one represented in the CPAE cells coupled to PLC and the other in the 3T3 cells coupled to PLA_2 for release of arachidonate.



284.4 VASOPRESSIN-STIMULATION OF PHOSPHATIDYLINOSITOL HYDROLYSIS IN THE RAT BRAIN: ENHANCED RESPONSIVENESS TO VASOPRESSIN IN THE HOMOZYGOUS BRATTLEBORO RAT. L.M. Shewey* and D.M. Dorsa, (SPON: W. Catterall). GRECC, VA Medical Center, and Depts. of Medicine and Pharm., Univ. of Washington, Seattle, WA 98108.

We have previously reported characterization of vasopressin (AVP) receptors in the septum of the Brattleboro (BB) rat. Animals homozygous for this trait have a near complete inability to synthesize AVP in the brain. Binding studies of AVP receptors using membranes prepared from the septum of heterozygous (HE) and homozygous (HO) Brattleboro rats revealed an increased number of AVP receptors with a lower affinity for [^{3}H]-AVP in HO animals when compared to those in the HE animals. The present studies were undertaken to determine the effect of this apparent "up-regulation" in the absence of endogenous AVP in the HO-BB rat septum on the post-receptor responsiveness of the tissue to AVP. We have recently reported that AVP stimulates phosphatidylinositol hydrolysis as measured by the accumulation of [3H]-inositol-1-phosphate (IP_1) in septal slices from Long-Evans rats. This response was maximal at 0.5μM AVP with a 64.9±6.6% (n=7) stimulation of [3H]-IP_1 levels relative to basal values, and could be completely inhibited by d(CH_2)$_5$ Tyr(Me)AVP, a specific V_1 antagonist. For the present study, septal slices from age-matched, adult male HE- and HO-BB rats were prelabeled with [3H]-inositol in the same assay, and AVP-stimulated accumulation of [3H]-IP_1 was measured in the presence of lithium. A dose-dependent accumulation of [3H]-IP_1 in response to AVP was seen in both HE and HO septal slices, but at all concentrations, the response was greater in the HO than in the HE. In the HO, a maximal response of 48.4± 5.9% stimulation over basal was observed at a concentration of 5μM AVP (n=7), while in the HE, only a 17.9±4.6% response was seen at a comparable protein concentration (n=7). Interestingly, the HO-BB septal slices also demonstrated an increased [3H]-IP_1 accumulation in response to oxytocin (0.1μm) (26.4± 3.3%, n=7) when compared to HE (3.8±2.1%, n=8). These results indicate that BB rats do possess functional AVP receptors in the septum that are able to hydrolyze inositol phospholipids in response to AVP. In accordance with their altered receptor characteristics compared to the HE animal, the post-receptor response in the septum of HO-BB rats is much larger than in HE, and the maximal response occurs at a concentration of AVP that is one order of magnitude higher than for HE or LE animals. Thus, the Brattleboro rat represents a potentially useful model for the study of CNS AVP receptor regulation and subsequent alterations in target cell sensitivity.

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284.5 BINDING OF A TRITIATED VASOPRESSIN ANTAGONIST TO BRAIN VASOPRESSIN RECEPTORS: FURTHER EVIDENCE FOR A V_1 RECEPTOR. M.D. Brot*, L.M. Shewey*, K. Myers*, C. Blamick*, and D.M. Dorsa, (SPON: T. KENNEDY) GRECC, VA Medical Center, and Depts of Medicine, Pharmacology, Psychology and Psychiatry, Univ of Washington, Seattle, WA 98108.

[^{3}H]-arginine^8-vasopressin (AVP) receptors have been localized in various regions of the rat brain, including the septum. Functionally, septal AVP receptors have been implicated in mediating vasopressin's effects on memory, thermoregulation, and neuronal excitation. Pharmacologic studies have suggested that these AVP binding sites have similar characteristics to peripheral V_1 receptors present in liver and smooth muscle.

For this study, a specific V_1 antagonist, [^{3}H]d(CH_2)^5-Tyr(Me) AVP, (Manning Compound, MC) was used to further characterize septal vasopressin binding sites. Saturation experiments using both [^{3}H]-AVP and [^{3}H]-MC (over a concentration range of 0.25 to 10nM) were performed using membranes prepared from septal tissue from Long-Evans rats. The specificity of [^{3}H]-MC for the binding sites was assessed by measuring the displacement potency of various competitor peptides including oxytocin (OXY), AVP, MC, and dDAVP (deamino-D-arginine^2-vasopressin), a specific V_2 agonist, and compared to that for [^{3}H]-AVP.

The results from saturation experiments using [^{3}H]-AVP as a ligand indicated the presence of a single class of binding sites with a Kd of 1.7 ± 0.34nM, and B_{max} of 22.6 ± 4.2fmol/mg protein in septal membranes. When [^{3}H]-MC was used, saturation analyses on the same membrane preparations yielded a Kd of 0.72 ± 0.12nM and a B_{max} of 23.9 ± 10.25fmol/mg protein, suggesting a higher affinity interaction of the antagonist with the binding site.

The results of studies of the specificity of [^{3}H]-AVP and [^{3}H]-MC binding to septal membranes are summarized in the table below. The numbers represent the inhibition constants (Ki, nM) for each of the competing ligands tested.

Labeled Ligand	AVP	MC	OXY	dDAVP
[^{3}H]-AVP	0.40	0.25	289.5	>1000
[^{3}H]-MC	1.46	0.18	>1000	>1000

For both tritiated ligands, the displacement potencies of OXY and dDAVP are much lower than those of AVP and MC. These findings indicate that both [^{3}H]-AVP and [^{3}H]-MC label a binding site which is similar in specificity to peripheral V_1-type receptors and provide additional support for the existence of these receptors in the brain. Furthermore, [^{3}H]-MC does not appear to label oxytocin receptors in this brain region. These data also suggest that MC may be a useful ligand for studies of brain vasopressin receptors.

Supported by NIH NS 20311 and the Veterans Administration.

284.6 DIFFERENT DISTRIBUTIONS OF INSULIN-LIKE IMMUNOREACTIVITY AND INSULIN RECEPTOR IN THE MAMMALIAN SPINAL CORD. N.B. Reddy,* W.K. Engel, and V. Askanas, USC Neuromuscular Center, USC Sch. Med., Los Angeles, CA 90017.

The distribution patterns of insulin-like immunoreactivity (ILI) and insulin receptor (IR) have been previously mapped in mammalian brain but not in spinal cord.

We have isolated and frozen immediately on dry ice, spinal cords from rat, cat and monkey. Cryostat transverse sections of cervical, thoracic and lumbar levels were collected on gelatin-subbed glass slides. ILI was identified utilizing a PAP immuno-histochemical reagent kit containing polyclonal anti-human insulin antibodies (DAKO Corp.). As controls, adjacent sections were incubated either in normal serum or in preabsorbed primary antibody solution. IRs were identified by autoradiography using 0.1-0.5 nM ^{125}I-insulin. Nonspecific ^{125}I-insulin binding in the presence of 1 uM unlabeled insulin was determined in adjacent sections. Autoradiographs were quantitatively analyzed with computerized densitometry (RAS System, Amersham Corp.) using optical density standards obtained from Amersham.

ILI was observed in the cytoplasm of neurons of both the dorsal and ventral horns. In the large ventral horn neurons, the amount of staining per neuron was much more prominent than in the small dorsal horn neurons, and probably more per unit of cytoplasmic area, although the latter comparison was difficult to quantitate in the sections. The nuclei and extracellular spaces did not have any significant staining. ILI was also observed in the axonal processes originating from neurons, especially in the ventral horn, suggesting possible transport of insulin-like substance (ILS) from the cell body. When the sections were incubated with normal serum or with preabsorbed primary antibody, no ILI was observed in the neurons, suggesting the specificity of immunocytochemical procedure for insulin-like substance. The patterns of ILI distribution was similar in cervical, thoracic and lumbar segments of spinal cord, and between the 3 species studied.

The distribution of IRs was distinctly different in various regions of the spinal gray matter. Computerized densitometry of the monkey spinal cord revealed that the density of IR in dorsal horn (laminae 1-3, +4) was 45+2 fmoles/mm^2 compared to 19+4 fmoles/mm^2 in ventral horn (laminae 7-9), and 24+1 fmoles/mm^2 in dorsal part of lamina X. Corresponding nonspecific binding values in the dorsal and ventral horns were, respectively, 2+0.6 and 3+1 fmoles/mm^2. Similar patterns of IR distribution were observed in the cervical, thoracic and lumbar segments, and between the 3 species. (Neither ILI nor IR was found in the spinal cord white matter.)

Thus, IR density is more than twice as high in the dorsal horn as it is in the ventral horn, but ILI is greater in ventral horn neurons, indicating that ILI and IRs are differently distributed. Physiologic roles of the spinal ILS and IRs remain to be defined. Speculatively, ILS might be a transported-exported trophic peptide interacting with post-synaptic IRs; e.g., ventral horn neuronal ILS may interact with muscle IRs and dorsal horn IRs may receive ILS from axons of dorsal root ganglia and intraspinal afferents.

284.7 PEPTIDE MAPPING AND NORTHERN BLOT ANALYSIS OF INSULIN RECEPTORS IN BRAIN AND ADIPOCYTES. K.A. Heidenreich, P.R. Gilmore*, and E. Hatada* Department of Medicine, Division of Endocrinology and Metabolism, M-023E, University of California, San Diego, La Jolla, CA 92093.

We have previously shown that insulin receptors in the central nervous system are structurally distinct from insulin receptors of more classic target tissues like adipocytes and liver. The receptor subunits in brain have smaller apparent molecular weights (M_r) than the subunits in other tissues and the size discrepancy involves differences in N-linked glycosylation of the proteins. In this study, we examined the degree of homology in the protein backbones of insulin receptors in rat brain and adipocytes by peptide mapping and compared the mRNAs encoding the receptors by Northern blot analysis. Insulin receptors in both tissues were photoaffinity labeled by ^{125}I-B2(2-nitro-4-azido-phenylacetyl)-des-Phe-B1-insulin and isolated by SDS gel electrophoresis. The labeled receptors were removed from the gel by electroelution and treated with endo-B-N-acetyl-glucosaminidase F, an enzyme that cleaves N-linked oligosaccharides at the core. The deglycosylated receptors were then subjected to partial proteolysis by five proteases with differing substrate specificities, and the intact receptors and proteolytic fragments were analyzed by electrophoresis. In brain, the intact α-subunit had an apparent M_r of 115,000; whereas, in adipocyte, the α-subunit had an apparent M_r of 125,000. After deglycosylation, both subunits were reduced to 100kDa. Treatment of the deglycosylated subunits with each of the five enzymes yielded a unique pattern of fragments ranging from 70kDa to 11kDa. For each enzyme, there was a striking similarity in the peptide maps generated from receptors in brain and adipocytes. Thus, both subunits contain similar protease-sensitive sites exposed at discrete intervals from the labeled hormone binding site including loci for aromatic amino acids (chymotrypsin data), glutamic acid residues (S. aureus V8 protease data), and lysine and arginine residues (trypsin data). Northern hybridization experiments were carried out using poly(A)$^+$ RNA isolated from rat brain, rat adipocytes and human hepatocarcinoma (HEPG2) cells, and an Eco R1 fragment of the human insulin receptor cDNA encoding most of the α-subunit and all of the β-subunit. In rat brain, two bands of 9.5 and 7.4kB were detected. In rat adipocytes, the same two bands were observed. The mRNA bands observed in rat tissues represented only two of the five mRNA species seen in human HEPG2 cells. The results indicate that the protein domains and the mRNAs encoding insulin receptors in brain and adipocytes are very similar, if not identical. We conclude that the structural heterogeneity of insulin receptors may result from differences in post-translational processing of the same gene product. The variation in receptor structure may contribute to the tissue-specific differences in insulin action.

284.8 INSULIN-LIKE GROWTH FACTOR-1 RECEPTORS IN BOVINE RETINA: HIGH LEVELS OF IGF-1 BINDING ARE ASSOCIATED WITH LOW LEVELS OF BETA SUBUNIT AUTOPHOSPHORYLATION. R. J. Waldbillig, NEI, D. LeRoith, NIDDK and G. J. Chader, National Eye Institute, NIH, Bethesda, MD 20892

It was found that bovine retina contains high affinity specific receptors for insulin-like growth factor-1 (IGF-1). Tracer binding studies with WGA-purified receptors reveal that ^{125}I-IGF-1 binding exceeds ^{125}I-insulin binding by a factor of 10-20. Scatchard plots indicate that, in large part, this difference is attributable to differences in the affinity of each receptor for its ligand. In competition-inhibition studies with unlabeled IGF-1, the half-maximal inhibition of ^{125}I-IGF-1 binding occurred at 0.08 nM. Unlabeled insulin was approximately 400 times less effective in inhibiting IGF-1 binding. It was also found that the IGF-1 receptor exhibits a kinase activity that autophosphorylates the receptor's beta subunit in a concentration and time dependent manner. Interestingly, however, at equal levels of IGF-1 and insulin binding, the IGF-1 receptor autophosphorylation response is markedly weaker than the autophosphorylation seen with the insulin receptor. In addition to an autophosphorylation response, IGF-1 receptors also exhibit kinase activity towards the retinal G-protein, transducin. The incorporation of ^{32}P into transducin was concentration and time dependent and the alpha-GDP form of transducin was a more effective substrate than was the alpha-GTP form. IGF-1 receptors purified from retina rod-outer segments exhibit a tyrosine-specific kinase activity and incorporate ^{32}P into poly (glu, tyr) 4:1 in a concentration (half maximum = 0.25 nM) and time dependent manner. SDS-PAGE analysis of retinal crude membranes crosslinked to ^{125}I-IGF-1 and then treated with neuraminidase, revealed the existence of two differentially glycosylated IGF-1 receptor alpha-subunit subpopulations. Specifically, it was found that neuraminidase increased the mobility of subunits in the upper half of the alpha-subunit radiographic band while smaller receptors, in the lower half of the radiographic band, were relatively unaffected. Further work will be required to determine the function of IGF-1 receptors in retinal physiology.

284.9 **INSULIN-LIKE GROWTH FACTOR-II (IGF-II) BINDING SITES IN THE RAT BRAIN: LOCALIZATION BY QUANTITATIVE AUTORADIOGRAPHY.** **D.G. Baskin, N.J. Bohannon*, M.G. King*, and R.G. Rosenfeld*. University of Washington and Veterans Administration Medical Center, Division of Endocrinology/Metabolism, Seattle, WA 98108, and Dept. of Pediatrics, Stanford University, Stanford, CA 94305**

Receptors for IGF-II, a growth promoting peptide (MW ca. 7500), which is made in the liver and has insulin-like structure and biological activity, have been identified in brain membrane preparations. This finding has raised interest in the hypothesis that IGF-II may influence the functions and development of the CNS. The precise locations were IGF-II acts within the CNS are largely unknown, however. We approached this problem by identifying brain IGF-II binding sites in situ with quantitative autoradiography. Slide-mounted cryostat sections (20 um) of brain were immersed in a solution containing 0.1 nM [125-I]-IGF-II, which was mixed with 100 nM [Thr-59]-IGF-I (Amgen) to block binding of the labeled IGF-II to brain IGF-I receptors. After incubation for 2 hrs at 22C, the sections were rinsed at 0C, dried, and placed in contact with LKB Ultrofilm for 5 days. Relative concentrations of bound iodoIGF-II within labeled regions were measured by video densitometry of the autoradiographic images, using the Drexel DUMAS/BRAIN computer/software system for quantitative autoradiography. Visual examination of the autoradiographic images showed that binding sites for iodoIGF-II were highly concentrated in discrete loci. The locations of major iodoIGF-II binding densities delineated well known anatomical landmarks, particularly certain neuronal laminae and nuclei. Highest binding (69-85 dpm/sq mm) was present in the dentate gyrus and Ca1, Ca2, Ca3 and Ca4 regions of the hippocampus, in the supraotic nucleus, choroid plexus, olfactory pyriform cortex, mitral cell layer of olfactory bulb, and habenula. Moderate binding (50-68 dpm/sq mm) was present in the subfornical organ, caudate-putamen, lateral hypothalamus, ventromedial hypothalamus, hypothalamic paraventricular nucleus, red nucleus, median eminence, pontine nuclei, external plexiform and inner granule cell layers of the olfactory bulb, and suprachiasmatic nucleus. Low binding (<49 dpm/sq mm) was present in the thalamus, superior colliculus, medial geniculate, and arcuate nucleus, as well as anterior and posterior pituitary glands. Binding was similar to background levels when iodoIGF-II was mixed with unlabeled IGF-II (from Dr. R. Humbel), rat IGF-II (MSA), or antibodies against the rat IGF-II receptor. The data are consistent with the conclusion that binding sites with characteristics of IGF-II receptors are associated with cells in some (but not all) brain regions containing high densitites of neuronal cell bodies. Many of these regions are characterized by pyramidal-type cell bodies, which suggest that IGF-II binding sites are associated with axosomatic synapses. These results suggest that cells which are sensitive to and regulated by IGF-II are found within specific, highly localized neural pathways and neuroendocrine centers of the brain.

284.10 CHARACTERIZATION AND DISTRIBUTION OF BINDING BY ANTIBODIES TO RAT INSULIN-LIKE GROWTH FACTOR-II (TYPE II) RECEPTOR IN CNS AND OTHER TISSUES. I. Ocrant*, K.L. Valentino, H. Pham*, R.G. Rosenfeld*, R.L. Hintz*, and D.M. Wilson*. Depts. of Pediatrics and Psychiatry, Stanford Univ. School of Medicine, Stanford, CA 94305.

Insulin-like growth factors (IGFs) I and II are peptides homologous to insulin and mitogenic for a variety of mammalian cells. Important in somatic growth and probably important in differentiation, both types of IGFs and their receptors are found in the CNS from fetal through adult life. A description of the distribution of IGF-II receptors at the organ and cellular level was undertaken in order to further an understanding of the possible role of this peptide in the CNS.

A rabbit antiserum was raised by primary immunization with lectin affinity purified receptors from a rat cell line (18,54-SF) rich in type II receptors, followed by booster immunizations with rat IGF-II affinity purified receptors from the same cell line. A monoclonal antibody was produced by immunization of mice with the latter preparation. The specificities of these antibodies were demonstrated by their ability to immunoprecipitate rat type II receptors, by similar patterns of immunostaining in sections of brain and other tissues, and by inhibition of immunostaining by homologous ligand. The polyclonal antiserum was further characterized by its ability to specifically block the binding of IGF-II and by Western blot.

Adult rats were perfused with fixative. Brains and other tissues were removed, frozen, and sectioned. Antibodies were applied and then detected with rhodamine or fluorescein conjugated second antibodies. Type II receptors were localized primarily in ependymal cells, choroid plexus, and mesenchymal cells surrounding small and large blood vessels in adult rat brain sections. Likewise, immunostaining of rat muscle, kidney, and liver disclosed primarily mesenchymal staining. Studies in fetal tissues are in progress.

When immunostaining was performed on 18,54-SF cells and B104 rat neuroblastoma cells, the type II receptors were primarily localized to the plasma membrane and also in the Golgi apparatus following permeabilization with Triton X-100.

Primary, dispersed, adult rat brain cell cultures were established which included many viable neuronal cells at day 14 of culture. Immunohistochemical localization of type II receptors was confined to nonneuronal cells. Cells which were positively stained showed the same pattern of cellular localization as 18,54-SF and B104 cells.

We conclude that type II (IGF-II) receptors are present in significant amounts in the CNS, and are primarily localized to nonneuronal cells. This provides important information for future research into the possible actions of this peptide in the CNS and other tissues.

284.11 FUNCTIONAL ANGIOTENSIN RECEPTORS INDUCED IN XENOPUS OOCYTES BY INJECTED mRNA. L.J. Greenfield, Jr., K.R. Lynch* and J.T. Hackett. Neuroscience Program and Depts. of Physiology and Pharmacology, Univ. of Virginia School of Medicine, Charlottesville, Va. 22908.

The Xenopus oocyte can synthesize and insert foreign receptors and ion channels into its membrane when injected with exogenous messenger RNA. Oocytes injected with poly $(A)^+$ RNA from mouse liver, cow adrenal medulla, rat kidney or rat brain synthesize functional angiotensin receptors, detected by ^{125}I-Angiotensin II (I-AII) binding or voltage clamp electrophysiology. RNA was isolated by guanidinium thiocyanate/cesium chloride centrifugation and purified by oligo-dT cellulose chromatography. Intact oocytes injected with 20-30 ng of mRNA were incubated 48 hrs later for 2.5 hrs in 10^5 cpm/oocyte carrier-free I-AII (2200 Ci/mmol), competing peptides, and ^{14}C sucrose to monitor internalization of medium. Cells were then washed and counted individually for ^{125}I and ^{14}C. Mouse liver mRNA induced 305±26 cpm bound/oocyte (n=11), compared to 15±4 cpm/oocyte (n=9) for vehicle-injected cells. Incubation with 10^{-6} M Sar^1Ile^8AII reduced binding to 56±10 cpm/oocyte. Bovine adrenal medulla mRNA gave maximal binding of 176±15 cpm/oocyte (n=9). IA-II binding was blocked by 10^{-8} M AII and by AI, AIII and saralasin (10^{-6} M), but not by unrelated peptides. Mouse liver mRNA also induced expression of the mouse H_2D^d major histocompatibility antigen, detected by iodinated antibody, which confirms the expression of foreign membrane proteins. Oocytes injected with mRNA from mouse liver, rat kidney, brainstem or diencephalon and voltage clamped to -70 mV responded to superfused human AII (1 to 100 nM) with a fluctuating inward current. Cells injected with vehicle or mRNA from caudate nucleus or cerebellum did not respond to AII (1 uM). The magnitude of the currents was highly variable between cells, ranging from 10 to several hundred nA. The reversal potential (determined by intersection of I/V curves obtained by ramp voltage commands given before and during the AII response) was -21.8 ± 3.7 mV (n=5), consistent with the chloride equilibrium potential. Slope conductance increased from 888±89 nS to 2480±580 nS, or over 300%. The shape of the response was also variable; after an initial delay of 30 to 90 seconds, a large inward current "spike" lasting 5 to 15 seconds was often observed, followed by oscillations of smaller amplitude at about 0.3 Hz. In some cells, a smooth inward current was seen; the reversal potential of this response was similar to that of fluctuating currents. Reapplication of AII often evoked no response, even one hour or more after initial application. The inward Cl^- current is similar to that evoked by acetylcholine, glutamate, and 5-HT on endogenous or mRNA-induced receptors, and may involve the same ion channels and second messengers. Localization of brain A-II receptor mRNA to brainstem and diencephalon suggests that these responses may represent neuronal A-II receptors. (NIH HL33513 & NSF BNS840629)

284.12 ^{125}I-ANGIOTENSIN III BINDING IN THE RAT BRAINSTEM. R.C. Speth, T. Balestreri*, J. Erickson and J.W. Harding. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

Angiotensin III, the des asp^1 heptapeptide fragment of angiotensin II (Ang II), is an active metabolite of Ang II. In the brain it has been shown to cause dipsogenic and pressor responses with a potency similar to that of Ang II. Administered iontophoretically into the brain, Ang III is a more potent excitant of neuronal cell firing than Ang II (Harding and Felix, Brain Res. In press). We are using in vitro receptor autoradiography to investigate the binding of radioiodinated Ang III. Rat brains were frozen sectioned at 10-20 µm, thaw mounted to subbed slides, and stored at -20°C. Sections were thawed and preincubated for 30 min at 22°C in a medium containing 150 mM NaCl, 10 mM $MgCl_2$, 5 mM EGTA, 5 mM dithiothreitol, 5 µM bacitracin, 5 µM puromycin, aprotinin 0.2 U/ml, 50 mM $NaPO_4$, pH 7.1, and 0.1% bovine albumin. Sections were next incubated for 40 min at 22°C in the same medium containing 0.7 nM ^{125}I-Ang III. Adjacent sections were incubated in the presence of 0.7 nM ^{125}I-Ang III plus 1 µM Ang II. Sections were then rinsed in water and buffer and quickly dried under a stream of hot air. For comparison, additional brainstem sections were incubated with ^{125}I-Ang II (0.6 nM, 60 min at 22°C) and ^{125}I-Sar^1,Ile^8Ang II (0.3 nM, 120 min at 22°C) in the presence and absence of 1 µM Ang II. Sections were apposed to x-ray film to visualize radioligand binding. Incubation medium was subsequently analyzed for metabolism of the radioligands by the brain sections using HPLC. The incubation medium virtually abolished metabolism of the radioiodinated angiotensins by sections of rat brainstem. Deletion of bacitracin, puromycin, or aprotinin from the incubation medium revealed metabolism of ^{125}I-Ang III but did not affect metabolism of ^{125}I-Sar^1, Ile^8Ang II. Examination of autoradiograms from the brainstem indicated high specific binding of ^{125}I-Ang II and ^{125}I-Sar^1,Ile^8Ang II to the solitary tract nucleus, dorsal vagal motor nucleus, spinal trigeminal nucleus, locus coeruleus, and inferior olivary nucleus. In contrast, ^{125}I-Ang III binding was observed only in the inferior olivary nucleus and locus coeruleus. This disparity suggests that Ang III may not act at all of the same sites in the brain as Ang II and that there is a heterogeneity of brain Ang II receptors. Since Ang III causes pressor and dipsogenic responses similar to Ang II when administered intraventricularly, determination of the specific binding sites for ^{125}I-Ang III in the brain may identify the brain areas mediating the pressor and dipsogenic responses to Ang II in the brain. Grant support NIH NS21305 and HL32063 and AHA 831145.

284.13 PURIFICATION OF PITUITARY CRF RECEPTORS. Erica Nishimura*, Nils Billestrup*, Marilyn Perrin* and Wylie Vale. The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.

Corticotropin releasing factor (CRF), the primary hypothalamic neurohormone involved in the regulation of pituitary ACTH secretion, initiates its effects by binding to specific cell surface receptors. As a means of eventually understanding the molecular basis of CRF action, pituitary CRF receptors were solubilized and purified by affinity chromatography to apparent homogeneity.

The zwitterionic detergent CHAPS was used to solubilize functional CRF receptors from bovine anterior pituitary membranes. The radioligand [Nle^{21}, ^{125}I-Tyr]-ovine CRF (^{125}I-oCRF) was employed to measure specific soluble CRF binding sites. The apparent Kd of the solubilized form of the receptor (100 nM) was greater than that measured for membrane bound CRF receptors (1 nM). This decrease in affinity following solubilization has been observed for other cyclase coupled peptide hormone receptors and has been attributed to the possible disassociation of regulatory proteins from the receptor during detergent extraction. The solubilized material was incubated 17 hr at 4° C with wheat germ agglutinin (WGA)-agarose, then washed with 12 column volumes of 50 mM Tris-HCl, pH 7.4 buffer. The solubilized receptor was retained on the WGA-agarose column and could be eluted with 0.3 M N-acetylglucosamine, resulting in an approximate 4-fold increase in specific activity. This material was applied to CRF-agarose which was prepared by conjugating [$Nle^{21,38}$,Arg^{36}]-rat CRF to Affi-gel 10 (Bio-Rad). Following overnight incubation at 4° C the gel was washed with 10 column volumes of Tris buffer and the CRF receptor was eluted with 0.5 N acetic acid, pH 5.0 containing 0.5 mM CHAPS. Overall, a 2,500-5,000 fold purification was achieved with a 6-12% recovery in activity.

SDS gel electrophoresis of the affinity purified receptor, examined by silver staining, revealed a single protein of apparent Mr = 70,000. This is in agreement with the relative molecular weight of the CRF receptor identified by cross-linking of ^{125}I-oCRF to bovine anterior pituitary membranes (Nishimura et al., submitted).

285.1 RETINOTECTAL W-CELL PLASTICITY: EXPERIMENTALLY INDUCED RETINAL PROJECTIONS TO AUDITORY THALAMUS IN FERRETS. A.W. Roe*, P.E. Garraghty, and M. Sur. (SPON: R.D.G. McKay). Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

In ferrets in which visual cortex and superior colliculus have been ablated and auditory thalamus deafferented at birth, an aberrant visual pathway is produced that in the adult mediates visual input from the retina to the auditory thalamus and, in turn, to auditory cortex (Sur and Garraghty, Soc. Neurosci. Abstr. 12:592, '86; cf. Schneider, Brain Beh. Evol. 8:73, '73; Frost, J. Comp. Neurol. 203:227, '81). We have now tested the hypothesis that the retino-auditory thalamus projections are mediated by a "W-cell" pathway that normally projects to the superior colliculus.

Newborn ferret pups underwent the following surgery: the superior colliculus was ablated unilaterally and the ipsilateral brachium of the inferior colliculus was cut to deafferent the auditory thalamus (in particular, the medial geniculate nucleus, MGN) and thus provide alternative target space for retinal afferents. After these animals had matured, HRP-WGA injections were made into the eye contralateral to the lesioned hemisphere. In addition to the normal retinal-recipient zones in thalamus, we found label in the dorsal, ventral, and medial divisions of the MGN, as well as the lateral division of the posterior nuclear complex (PO_2) and the lateral posterior nucleus (LP) of the thalamus. This projection was sparsely and nonuniformly distributed across these targets, appearing in clumps. Physiologically, the retino-MGN pathway exhibited W-like properties: cells were poorly driven visually, had responses that waxed and waned, and had long latencies to chiasm stimulation.

To examine the retinal source of these projections, we injected HRP into: 1) the thalamus of normal adult animals, 2) the superficial superior colliculus of normal adults, and 3) the thalamus of neonatally operated animals. Whereas retinal ganglion cells back-filled from thalamus of normal animals included those of large (>350 um^2), medium (200-350 um^2), and small (<200 um^2) soma size, those projecting to superficial superior colliculus were only of small soma size. Importantly, thalamic injections in the operated animals revealed a greater percentage of small sized back-filled retinal cells than that resulting from thalamic injections in normal animals. The presence of additional small retinal ganglion cells projecting to the thalamus in our operated animals suggests that cells normally projecting to superior colliculus have rerouted their axons to visual thalamus (LP) and auditory thalamus (MGN and PO_2).

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285.2 TRAJECTORIES AND BRANCHING PATTERNS OF OPTIC TRACT AXONS THAT PROJECT TRANSIENTLY TO SOMATOSENSORY THALAMUS IN THE NEONATAL HAMSTER. R.B. Langdon, J.M. Freeman, and D.O. Frost. Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Retinofugal axons project transiently to the thalamic somatosensory (ventrobasal, VB) nucleus from the day of birth (PO) to the third postnatal day (P3) in hamsters (Frost, 1984, J. Comp. Neurol. 230:576). The region of VB receiving this projection is contiguous with the overlying dorsal lateral geniculate nucleus (LGd). In rodents, retinofugal axons pass through the thalamus to the mesencephalon in the superficial optic tract (SOT) as a sheet that covers the LGd, and in the internal optic tract (IOT) that courses parallel to the SOT in fascicles distributed through the thickness of the LGd. In adult hamsters, SOT axons send collaterals to the thalamus, whereas IOT axons do not (Schneider & Jhaveri, 1983, Neurosci. Abst. 9:809). In this study, we examined, on PO, P1, P2 and P4, the trajectories and branching patterns of individual SOT and IOT axons. We concentrated on axons that projected transiently to VB. Golgi-like axonal filling was achieved in vitro by lodging pellets of HRP in the optic tract just ventral to the LGd. After incubation, brains were fixed, sectioned, reacted with DAB/Co/Ni, osmicated, and plastic-embedded.

SOT and IOT axons were consistently labeled anterogradely and retrogradely for ca. 2 mm from the injection sites. Prior to P4, collaterals of SOT axons were confined to the outer half of LGd. In contrast, neonatal IOT axons sent long, unbranched, radially oriented collaterals through the LGd into VB. The distribution of these collaterals was congruent with that of retino-VB projections labeled by intraocular HRP injection; like the intraocularly labeled projections, they disappeared between P2 and P4 (Frost, ibid). Starting on P4, the collaterals of SOT axons extended deeper into LGd and elaborated their terminal arbors.

The development of retinofugal axons is viewed as occurring in 3 distinct stages: (1) elongation of the main axon trunk; (2) extension of collaterals into target nuclei; and (3) elaboration of terminal arbors (Jhaveri, Edwards & Schneider, 1983, Anat. Rec. 205:225A; Frost, ibid.). The present data show that the retino-VB projection is due to exuberance in stage (2), namely, transient extension of thalamic collaterals by IOT axons.

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285.3 SYNAPSE FORMATION BY OPTIC TRACT AXONS THAT PROJECT TRANSIENTLY TO SOMATOSENSORY AND AUDITORY NUCLEI IN THE NEONATAL HAMSTER. J.M. Freeman and D.O. Frost. Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Retinofugal axons project transiently to multiple non-visual nuclei in the neonatal hamster including the thalamic somatosensory (ventrobasal, VB) nucleus and the ventro-caudal part of the midbrain auditory nucleus, the inferior colliculus (IC; a small retinal projection to dorsal IC persists permanently; Frost, J. Comp. Neurol., 230:576, 1984). The transient retino-VB and retino-IC projections are prominent during the first few days of life, but absent after days P3 and P8, respectively (PO = day of birth). In order to understand the mechanisms controlling the stabilization/elimination of immature neural connections, we have investigated whether the transient axons form synapses before withdrawing.

Retinal projections were labeled at various ages from PO to P8 by anterograde transport of intraocularly injected HRP. 100um sections were cut parasagittally in the midbrain and coronally in the thalamus. HRP was demonstrated using the Hanker-Yates technique with Co/Ni intensification. Following the HRP reaction, sections were embedded in plastic for electron microscopy (EM).

The transient projection to IC was visible in the 100um sections as one or more clearly defined fascicles which coursed superficially around the convexity of IC and sometimes extended almost to its ventral extremity. Selected 100 um sections were re-sectioned at 8um so that the most distal portion of the projection could be isolated and thin sectioned. Using this technique, we identified by EM many labeled axon trunks, growth cones and structures that appeared to be presynaptic elements making contacts satisfying the criteria for synapses (presence of pre- and post-synaptic membrane thickenings and clear vesicles concentrated in the presynaptic profile).

The transient projections to VB were diffuse, not fasciculated. By EM, labeled profiles were scarce and so far only one has been seen that makes contacts meeting the criteria for synapses. The scarcity of such profiles reflects a sampling problem due to their diffuse distribution in VB. A similar sampling problem also occurs in the thalamic visual (lateral geniculate) nucleus. We have begun to use an in vitro labeling technique that allows the isolation of single, HRP-filled axons in VB and should increase our yield of labeled elements making synapses.

These data demonstrate that transient retino-IC- (and probably retino-VB) axons make synapses before they withdraw. Thus, activity-dependent mechanisms may be responsible for the elimination of these axons.

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285.4 VISUAL RESPONSES OF SOMATOSENSORY CORTEX NEURONS IN HAMSTERS WITH STABILIZED RETINAL PROJECTIONS TO SOMATOSENSORY THALAMUS. C. Metin* and D.O. Frost. Institut des Neurosciences, Université Pierre et Marie Curie, Paris, France. Section of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

Ablation of the superior colliculus and dorsal lateral geniculate nucleus (LGd) and partial deafferentation of the thalamic somatosensory (ventrobasal, VB) nucleus in newborn hamsters, permanently stabilize the normally transient, neonatal retino-VB projection. We have found visually evoked multi-unit responses in the first and second somatosensory cortices (SI and SII, respectively) of operated- but not of normal, adult hamsters. Here, we quantitatively compare the visual response properties of single neurons in SI and SII of operated hamsters and in primary visual cortex (area 17) of normal animals.

Adult hamsters were anesthetized with urethane and paralysed. In normal hamsters, area 17 neurons had receptive fields (RFs) with areas of ca. $90°^2$. 60% of the neurons were "asymmetric" - they responded best to stimuli moving in one or both directions along a specific axis; 25% of the neurons were "symmetric" - they responded equally to all directions of stimulus movement. The cells were heterogeneous in their velocity preferences (range 8-180°/sec). 40% of the neurons were orientation tuned. All orientations and movement directions were represented. Symmetric cells were usually ON-center, OFF-center or ON/OFF, while asymmetric ones were simple or complex. Mean response latency was ca. 200 msec. Neurons were recorded at all cortical depths. Most visual neurons in SI and SII of operated hamsters had RF's with 2 response areas separated by 20-40°, each covering ca. $100°^2$. 25% of the neurons were asymmetric; 50% were symmetric. The neurons generally preferred rapidly moving stimuli (range 10-200°/sec; 60% of cells preferred 50-200°/sec). All movement directions were represented. Orientation preference, when present, was weak. Among neurons that responded to stationary stimuli, ON/OFF responses predominated. Mean response latency was ca. 200 msec. 50% of visually responsive neurons were recorded near the layer III/IV border. 25% of visually responsive neurons also responded to somatosensory stimulation.

Thus, visually responsive neurons in SI and SII of operated hamsters have many essential features of neurons in area 17 of normal hamsters. Our results suggest two hypotheses that are not mutually exclusive: 1) thalamic or cortical structures that normally process information of different modalities perform similar transformations on their inputs so that they can process information from other modalities; 2) the functional differentiation of specific sensory structures depends on their afferents. Supported by grants EY03465 & NS22807 from the NIH, 5-417 from the March of Dimes and UA1199 from the CNRS.

285.5 VISUAL CORTEX RECEIVING AN ABERRANT SOMATOSENSORY INPUT MAINTAINS NORMALLY TRANSIENT PYRAMIDAL TRACT AXONS. Dennis D.M. O'Leary and Brent B. Stanfield, McDonnell Center for Studies of Higher Brain Function, Dept of Neurosurgery, Washington Univ. Sch. of Med., St. Louis, MO 63110, and NIMH, Poolesville, MD 20837.

During the first two postnatal weeks neurons in the rat occipital (visual) cortex transiently extend axons through the pyramidal tract (PT). We have investigated factors that may be involved in the selective elimination of these PT axons which occurs during the third week. We first examined the effect of removing permanent targets of the occipital PT neurons (which we previously identified as the superior colliculus - SC, and basilar pontine nuclei - BPN) on the elimination of occipital PT axons. At birth we removed the cerebellum (which resulted in the degeneration of the BPN) and the SC bilaterally. On P34 we injected the pyramidal decussation (PD) with lul of 2% Fast Blue (FB). The animals were perfused on P40. Even in the complete absence of the SC and BPN, few if any FB labeled neurons are seen in the occipital cortex. Thus events occurring in the definitive targets of the early occipital PT neurons do not appear to precipitate the normal loss of their PT axon collateral.

However, in a second series of rats in which we removed at birth a large part of right rostral cortex, neurons in right occipital cortex could be labeled by FB injected into the PD on P34. Both the number and the tangential distribution of occipital PT neurons are increased in rats that, in addition to the cortical lesion, were bilaterally enucleated at birth. In similarly lesioned rats, we find an aberrant medial lemniscal projection to the right dorsal lateral geniculate nucleus (dLGN). In these additional experiments we injected bilaterally on P34 0.2 ul of 2.5% WGA-HRP/10% HRP in the lower medulla of normal rats, rats with a right rostral cortical lesion, and bilaterally enucleated rats with a right rostral cortical lesion. One day later, the rats were perfused and their brains processed by the TMB protocol. No label is present in the dLGN of the normal rats, but in the lesioned rats labeled fibers can be followed into the right dLGN where they ramify. In rats with only a cortical lesion the label in the dLGN is largely confined to its more ventral and lateral parts, while in the enucleated and cortical lesioned rats the amount of dLGN label is greater and is spread over much of the nucleus. These differences in the aberrant medial lemniscal projection to the dLGN appear to correlate with the number and distribution of occipital PT neurons. Our results indicate that the transient occipital PT axons can be maintained. Further, they suggest that the input relayed through the thalamus influences which subcortical projections initially extended by the visual cortex will be retained. Supported by NIH Grants EY70205 and NS18506.

285.6 THE DEVELOPMENT OF TRANSITORY AUDITORY-TO-VISUAL CORTEX PROJECTIONS IN THE CAT. G.M. Innocenti, P. Berbel* and P. Melzer*, Institute of Anatomy, University of Lausanne, CH-1005 Lausanne.

In newborn kittens, visual areas 17 and 18 receive intra- and inter-hemispheric projections not only from other visual areas but also from auditory areas, including A1 and A2, in both hemispheres. Projections from auditory areas originate from supragranular layers 2 and 3, and much less densely from 5 and 6 (Innocenti and Clarke, Dev. Brain Res., 14:143, 1984). The postnatal fate of this projection has now been studied in i) normal kittens of different ages (2, 7.5, 8, 14, 14.5, 22, 30, 40 days) and 3 adults; ii) 2 adult cats which had been binocularly enucleated at birth; iii) 5 kittens of different ages (11, 14, 18, 27, 41 days) and 7 adult cats which all received injections of the "axon sparing" excitotoxin ibotenic acid (ibo; 1-2 µl; 30 µg/µl) in their visual areas 17 and 18 on postnatal days 2 or 3. The two latter experiments were meant to investigate, respectively, the role of the retina and of the target cortex in the development of auditory-to-visual projections. Connections were traced retrogradely (fast blue, diamidino yellow, rhodamine latex beads, WGA-HRP) from areas 17-18 or anterogradely (WGA-HRP) from A1-A2. In addition, in selected cases, ibo-injected cortex was characterized by Nissl and Golgi staining, cytochrome oxidase histochemistry, glial fibrillary acidic protein immuno-histochemistry, electron microscopy, (^{14}C)-2-deoxy-D-glucose autoradiography and single-unit recordings.

Normally, the auditory-to-visual projections have almost completely disappeared in the adult; a few layer 5 and 6 neurons can nevertheless be labeled in ipsi- and contralateral A1 and A2, especially after large injections involving together areas 17, 18 and 19. Transitory axons are eliminated by day 30, apparently without having ever entered the visual areas to any significant extent. Bilateral enucleation at birth does not prevent the loss of the projection from the supragranular layers, but possibly a few more neurons remain in the infragranular layers. In contrast, neonatally ibo-injected areas 17 and 18 maintain the juvenile intra- and inter-hemispheric projections from supra- and infragranular layers of A1-A2. What causes this abnormal stabilization is unclear. Ibo-injected visual areas have lost their deep layers and show cytoarchitectonic changes similar to those found in microgyria, a congenital malformation of human neocortex; nevertheless they retain several normal connections and functional properties. The juvenile, exuberant projection from medial area 17 (Innocenti et al., Neurosci. Lett., 4:237, 1977) to the ibo-injected cortex is eliminated as normally. Axons from auditory cortex, however, have access to the "microgyric" cortex, and form terminal arbors and probably synapses in it.

285.7 IDENTIFICATION OF LATERAL GENICULATE CELLS TRANSIENTLY PROJECTING TO THE POSTEROMEDIAL LATERAL SUPRASYLVIAN AREA (PMLS) IN KITTENS. L.L. Bruce[1] and B.E. Stein[2]. Dept. Rehab. Med.[1] and Physiol.[2], Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Lateral geniculate cells in laminae A, A1, and C of the newborn kitten have connections with PMLS that begin retracting at about the time of eyelid opening. The retraction is nearly completed by 2 months postnatal (Bruce, L.L. and Stein, B.E., Soc. Neurosci. Abstr., 12:438, 1986). However, cells in laminae C1-3, where only W-cells are found, retain their connections with PMLS into adulthood. Although X- and Y-cells are located solely in the laminae that have transient connections with PMLS, it was not clear whether only X-, only Y-, or both X- and Y-cells have transient connections with PMLS. Since geniculate X-, Y-, and W-cells have different average somal sizes, the distribution of somal sizes can be used to evaluate the cell types involved in the transient projection.

In each kitten, an injection of fast blue (a long-lasting retrogradely transported dye) was made in PMLS at 6-7 days postnatal, prior to the onset of retraction. At 4 weeks of age, a second injection of horseradish peroxidase was made in striate cortex (area 17) that labeled X-, Y-, and W-cells in all geniculate laminae. The next day, the kittens were perfused with saline followed by 3% paraformaldehyde in citrate buffer and the tissue was processed for the HRP reaction. Fast blue- and HRP-labeled cells in each geniculate lamina were traced using a drawing tube. Somal areas were measured using a digital graphics tablet interfaced to a computer.

The distribution of the sizes of PMLS-projecting cells in laminae A and A1 was almost identical to the distribution of geniculate cells in the same laminae that project to area 17. Thus the transient geniculo-PMLS projection appears to be derived from both X- and Y-cells. Within the C complex, the size distribution of PMLS-projecting cells includes a population that is smaller than those in the population projecting to area 17. This suggests that within the C complex there may be a population of cells with very small somata that project to PMLS but not to area 17.

These data indicate that X-, Y-, and W-cells project to PMLS for a short period following birth. During maturation, X- and Y-cells, but not W-cells, lose their axon collaterals with PMLS. The reasons why X- and Y-cells are at a competitive disadvantage for maintaining connections with PMLS are not yet clear.

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285.8 CELLULAR CONSTITUENTS OF THE INTERMEDIATE ZONE DURING PRENATAL DEVELOPMENT OF THE CEREBRAL CORTEX. A. Ghosh and C.J. Shatz. Dept. Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

During the development of the mammalian cerebral cortex the white matter- called the intermediate zone (IZ) at these early ages- is a complex region that contains radial glia and migrating neurons en route from the ventricular zone (VZ) to the cortical plate (CP) (Rakic, J. Comp. Neurol. 145:61, 1972), as well as waiting thalamocortical axons that will eventually grow into the CP. A special transient type of neuron, the subplate neuron, is also present (Chun et al, Nature 325:617, 1987). To learn more about the organization of the IZ, and the morphology of the waiting axons, we made localized injections of HRP within the IZ between embryonic day 45 (E45) and E62 during the development of the cat's visual cortex (gestation is 65 days).

At each age studied, HRP injections at several radial distances from the VZ were made into the IZ of 2mm thick parasagittal slices through the telencephalon that were removed and maintained in vitro for 5 hrs. All injections labeled radial glia and migrating neurons. However, the labeling of other cellular elements was a function of the depth of the injection. Injections just below the CP always labeled a small group of subplate neurons immediately surrounding the injection site. In contrast, deeper injections labeled rather smooth, radially aligned processes that ended in terminal arbors well above the injection site, frequently within the vicinity of the subplate cells. With increasing age, many of these arbors were located progressively closer to the CP, and by E57, some of them were present in future cortical layer 6. While some of these processes could be arborizing radial glial cells (Schmechel & Rakic, Anat. Embryol. 156:115, 1979), we think it more likely that many are the waiting thalamocortical axons by virtue of their axon-like appearance, their location and their presence within the CP by E57- a time when transneuronal autoradiographic labeling experiments indicate that the geniculocortical afferents begin their ingrowth into the CP (Shatz & Luskin, J. Neurosci. 6: 3655, 1986). Moreover, in order to label these arborizing processes, the injection site had to be close to the optic radiations, as verified by transneuronal autoradiography in conjunction with in vitro HRP labeling.

These observations indicate that within the IZ different cellular populations are distributed at different depths. Further, if the arborizing processes are indeed the thalamocortical axons, then during the waiting period axons need not end in simple growth cones but rather can elaborate terminal arborizations in locations distant from their final destinations. In that case, these axons may participate in complex interactions, perhaps with the subplate cells which are known to receive synaptic contacts at these ages. Supported by NIH grants EY02858 to CJS and MH17047 to AG.

285.9 **PATTERNS OF CYTOCHROME OXIDASE ACTIVITY AND ACETYLCHOLINESTERASE STAINING IN THE VISUAL CORTEX OF THE DEVELOPING FERRET.**

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Previous studies from our laboratory (Jackson, Peduzzi and Hickey, 1984; Jackson and Hickey, 1986) have defined the birthdates and time-course of migration for neurons destined to reside in each of the layers of the ferret's visual cortex. Just as the location of neurons born on a given day changes during development, the pattern of cytochrome oxidase activity in the developing visual cortex differs markedly from the pattern observed in the visual cortex of the adult ferret. At postnatal day 10 (P10), the only intense cytochrome oxidase activity is present at the most superficial part of the cortical plate. Cells located at the top of the cortical plate at P10 are found in layer IV of the adult animal. At P15, the only intense cytochrome oxidase activity is again located at the top of the cortical plate. However, the cells located in this region at P15 ultimately reside in layers II and III of the adult visual cortex. At P24, there is some cytochrome oxidase activity at the uppermost part of layer II (a region containing immature neurons). At this age, there is also an intense band of cytochrome oxidase activity in layer IV which is characteristic of the adult primary visual cortex. The difference in the patterns of cytochrome oxidase activity in the visual cortex of the developing and mature animals may reflect the increased metabolic activity of neurons during their rapid differentiation at the uppermost part of the cortical plate.

The pattern of acetylcholinesterase (AChE) histochemical staining in the ferret visual cortex also varies at different times in development. AChE staining at P7 reveals a region containing fewer reactive fibers (compared to surrounding regions) at the caudal pole of the cortex. At P10, the cortical plate lacks staining except for a few fibers crossing perpendicular to the pial surface. Light staining is present above and below the cortical plate. The pattern at P15 is similar to that seen in the P10 animal, with the exception that the staining is generally more intense, with the greatest density of reactive fibers being in layer V. By P24, all the cortical layers are stained except for layer IV; i.e., the general pattern found in the adult primary visual cortex. Although the AChE pattern changes during development, it provides a convenient method to identify the extent of the primary visual cortex.

Further studies are needed to determine if there is a correlation between the arrival of the geniculocortical fibers and the changing patterns of cytochrome oxidase activity and AChE histochemical staining in the primary visual cortex during development.

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285.10 **DEVELOPMENT OF LAMINAR AND COLUMNAR PATTERNS OF CYTOCHROME OXIDASE ACTIVITY IN GALAGO VISUAL CORTEX.**

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The distribution of cytochrome oxidase (CO) staining is a useful marker for identification of functional modules within the primate visual cortex. In the prosimian primate galago previous reports have shown that in striate cortex, zones which receive input from Y-like, X-like, and W-like LGN layers stain darkly with CO, i.e. cortical layers IVα, IVβ, and blobs in III. We were interested in determining the appearance of this pattern during development when the galago visual system is known to be most susceptible to visual experience. CO-staining patterns were examined in area 17 in 55 galagos, *Galago crassicaudatus* (GC) and *Galago senegalensis* (GS). CO-stained sections from flattened cortices, and parasagittally or coronally sectioned cases were compared to adjacent Nissl and fiber stained sections. Our results confirm that in adults the darkest CO-staining occurs in the LGN recipient zones. In addition, there is also evidence of periodic staining in layer IVα, faint periodic staining in the upper 2/3 of layer VI, and staining of some large pyramidal cells in layer V. The periodicity of CO staining in layers IVα and VI is aligned with the CO blobs in layers II/III. The frequency of CO-blobs per mm^2 in GC is on average 2.6 in cortex representing central vision and 2.9 in cortex representing peripheral vision. By contrast, area 17 in GS is smaller, with 4.4 and 5.3 blobs per mm^2 in central and peripheral visual field representations, respectively. At birth (P0) in both species, the CO pattern is similar to that of the adult with the exception that blobs in layers II/III are lighter staining, blobs in layer VI are more distinct, the number of highly reactive pyramidal cells per unit area in layer V is greater, and layer IV in some cases appears to have three sublaminae. As in adults, at P0 there are more blobs per mm^2 in layers III and IV in cortex representing peripheral vision than in cortex representing central vision. From P0 to adulthood the only changes that occur (other than possible changes in blob frequency) are that blobs in layers II/III become more heavily stained, the frequency and intensity of CO-reactive pyramidal cells in layer V declines, and the blobs in layer VI become very faint. In GC the supragranular layers appear adult-like by 6 weeks and the infragranular layers appear adult-like by 12 weeks. Our results show that the galago is born with basically the same CO pattern seen in an adult; the main difference is a shift in staining emphasis from the infragranular to the supragranular layers. In addition our results suggest some interesting relationships. First, since magnocellular LGN cells terminate within layer IVα, the light/dark periodicity (which roughly matches that of a L/R ocular dominance column pair) must mean that the Y-like LGN pathway from the two eyes does not drive cortex equivalently. Likewise the CO periodicity in layer IV suggests that feedback from layer VI to the LGN may be dominated by one eye. Second, concerning the immature cortex, it is noteworthy that the size of LGN axonal arbors innervating layer IV at P0 is larger relative to the total area of striate cortex than in the adult, indicating that the forces which organize the frequency distribution of the blobs may not be related directly to LGN input.

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285.11 **POSTNATAL DEVELOPMENT OF INTRINSIC AXONAL ARBORS OF PYRAMIDAL NEURONS IN CAT STRIATE CORTEX.**

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Within striate cortex, distinct patterns of axonal collaterals provide defined avenues for communication within layers (horizontal connections) and between layers (vertical connections). Pyramidal cells in layers 2/3, for example, project vertically and horizontally within layers 2/3 and to layer 5, but lack appreciable collaterals within layer 4. Nothing is known about how this laminar specificity arises. We therefore examined the development of this intrinsic circuit, from postnatal day (PND) 5 to PND 110, using intracellular fluorescent dye injections in brain slices.

At the youngest ages, laminar specificity was even more precise than in the adult. Until PND 12, no sprouts, spines, or collaterals were present in layer 4. Although rudimentary, both layer 3 and layer 5 collaterals were present, and of equivalant lengths, suggesting that they emerged from the parent axon in rough synchrony. From PND 14-28 about half the cells had one or two small spine-like processes (< 10 μm long) in layer 4. At PND 50-110, in contrast, the axons of many layer 2/3 cells had numerous spine-like processes, and 2-4 collaterals, sometimes 100 μm long and branched, within layer 4, which are also seen in adult cats. Therefore the laminar specificity of this connection results from highly precise initial outgrowth, not removal of inappropriate branches.

Within layer 2/3, axon arbor development appeared conservative. At PND 5, 80% of cells had collaterals, an average of 3.5 per cell. No processes emerged within 50 μm of the soma. The cell's collaterals emerged closely spaced, suggesting that certain regions of the main axon are specialized to support collateral outgrowth. By PND 7 each cell averaged 4.5 collaterals: this number remained constant through PND 110. Thus a cell's complement of collaterals appears specified early, prior to extensive collateral outgrowth. Cells strengthen their vertical and horizontal connections by repeated branching of existing collaterals, not by growth of new ones: at PND 5, few collaterals branched, by PND 13, over 40% branched, and by PND 20, 75%. Long horizontal collaterals within layer 2/3 were observed by PND 13 in about half the filled cells. Some travelled over 1 mm, but without further branching. Clustered horizontal connections within layer 2/3 only emerged at about PND 50. In contrast, by PND 20, the vertical axonal arbor of many cells was already well-developed, with collaterals branching repeatedly near the cell's apical dendrites. This implies that vertical intralaminar connections develop considerably before distant horizontal connections.

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285.12 **DEVELOPMENT OF LAMINA SPECIFIC CORTICAL CONNECTIONS.**

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Previously we have shown using retrograde tracing of the connection between area 17 and 18a in the developing rat cortex that primary visual cortical neurons in lower layers are labeled before cells in upper layers. To identify the laminar targets of area 17 neurons in area 18a and compare their termination patterns during development we used anterograde tracing with Phaseolus vulgaris leucoagglutinin (PHA-L) in neonatal and adult Long Evans rats.

PHA-L was injected into area 17 of neonatal rats at postnatal day 5 (P5) and into adult animals. After 2-7 days survival, animals were perfused, the brains sectioned and the lectin visualized immunocytochemically.

The laminar pattern of connections in adult animals has been investigated by concentrating injections in either the superficial or deep layers. In adult animals deeper cortical layers of area 17, (layers 4, 5 and 6), project to layers 1-6 in area 18a. Superficial layers (layers 1, 2/3), in contrast, have their terminals limited to superficial layers.

Fibers labeled after an injection on postnatal day 5 (P5) and visualized after a 2 day survival, appear immature. Growth cones are seen on many fibers. These intracortical fibers do not have abundant varicosities nor do they show dense ramifications in target areas, features typical of cortical fibers in adult animals.

The injections in neonatal animals involved all cortical layers of area 17, but fibers in area 18a are only seen in layers 6, 5, and 1. Occasionally fibers are seen in the still differentiating superficial layers, but these fibers do not extend outside of area 17.

The absence of fibers in the superficial layers at P7 leads us to two related hypotheses. The first is that the ability of cortical neurons to receive synapses is expressed in a sequence which is determined, like the gradient of axon outgrowth, by the sequence of generation of cortical cells. The second takes note of the fact that the earliest innervated layers, layers 6, 5, and 1, are exactly those layers which are not innervated in the adult by fibers from superficial layers. We suggest that the early arriving fibers, from early generated cells, inhibit fibers from superficial layers from terminating in layers 6, 5, and 1, by a competitive process.

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286.1 NICOTINIC ACETYLCHOLINE RECEPTORS REGULATE CHLORIDE CHANNELS IN CULTURED NEURONS FROM THE CHICK EMBRYO CEREBRUM. M.H. Jalilian Tehrani and E.M. Barnes, Jr. Depts. of Biochem. and Physiol. and Mol. Biophys., Baylor Col. of Med., Houston, TX 77030.

The expression in vitro of neurotransmission systems by neuronal cultures provides preparations which are amenable to biochemical and biophysical investigations. We have shown previously that cultured neurons from the chick embryo cerebrum contain all of the GABAergic synaptic elements with properties similar to those in vivo. We report here that these cultures also express high levels of cholinergic synaptic elements.

Cultured neurons permeabilized with Triton X-100 contained choline acetyltransferase activity (7.0 ± 1.8 nmol/hr•mg protein) at levels which are 76% those of the adult brain. Acetylcholine esterase was also found at high levels (134 ± 5 nmol/min•mg) representing 106% of the activity in the chicken cerebrum. Specific binding of [^{3}H]-N-methylscopolamine (NMS) and [^{3}H]nicotine to membranes was used to assay muscarinic and nicotinic receptors, respectively. The Kd value for [^{3}H]NMS binding was 0.25 ± 0.05 nM, similar to the value (0.17 ± 0.08 nM) for chicken cerebrum; whereas the Bmax value for cultures (210 ± 50 fmol/mg) represented 37% of the receptor density of adult tissue). Likewise, neurons bound [^{3}H]nicotine with a Kd = 15 ± 1 nM which was comparable to the cerebrum (Kd = 25 ± 2 nM); the nicotinic receptor density (29 ± 2 fmol/mg) was 58% of that found for the adult tissue. These findings confirm the preponderance of muscarinic receptors over nicotinic receptors in the vertebrate brain. The culture system may also prove useful in investigating the physiological functions of central ACh receptors.

Without exogenous ligands, cerebral neurons possess a persistent ^{36}Cl ion permeability. Less than 20% of this ^{36}Cl uptake is inhibited by bicuculline, strychnine, stilbene sulfonates, loop diuretics, or by omission of K or Na from the medium. However, 60% of this ^{36}Cl flux was blocked by 100 μM carbachol or nicotine, but not by 100 μM muscarine. The chloride channel blocker t-butylbicyclophosphorothionate at a 10 μM concentration also inhibited over 50% of the ^{36}Cl flux. The effect of nicotine and carbachol was reversed by the nicotinic antagonists, hexamethonium (10 μM) and d-tubocurarine (50 μM), but 10 μM scopolamine was ineffective. These results suggest a novel coupling between nicotinic receptors and Cl channels in the vertebrate brain.

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286.2 EFFECT OF BASAL FOREBRAIN INJECTION OF CARBACHOL ON ACETYLCHOLINE (ACH) TURNOVER AND 8-ARM RADIAL MAZE PERFORMANCE. A. Sullivan,* K.L. Hambrecht,* L.G. Farley*, B.G. Lyeth and S.E. Robinson, (SPON: D.A. Brase) Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298

Intrastriatal injection of soman (14.85 nmol) elevates the concentration of ACh in the striatum without significantly affecting the content of ACh in other brain regions. However, ACh turnover, as measured by a mass fragmentographic technique, is decreased in the parietal cortex following such treatment. It appears that the effect of intrastriatal soman on cortical ACh turnover results from stimulation of basal forebrain cholinergic cell bodies by elevated ACh produced in the striatum, as ACh turnover was significantly reduced in the parietal cortex after unilateral injection of carbachol (5nmol, 1 μl) in the basal forebrain through stereotaxically-implanted chronic guide cannulas (AP + 6.06, L 2.4 and V-1.6, according to the atlas of Konig and Klippel). This area has been identified as the source of cholinergic innervation of the parietal cortex (Bigl et al., Brain Res. Bull. 8: 727, 1982). The turnover rate of ACh was reduced by 46% in the ipsilateral cortex and by 29% in the contralateral cortex, but was not affected in the striatum 20 min after the carbachol injection.

In order to determine if this reduction in ACh turnover had behavioral relevance, the effect of bilateral injection of carbachol was studied on performance in the 8-arm radial maze, which has been used to measure short-term, or working, memory. Sixteen male Sprague-Dawley rats (beginning at 150g) were maintained at 85% of their free-feeding weight. Rats were adapted to the maze and trained to a criterion of one or fewer working memory errors per trial. Rats were tested each weekday and the trials were restricted to the rats entering each of the 8 arms or a maximum of 10 min. Rats not reaching criterion after 25 sessions were removed from the study. Bilateral guide cannulas aimed at the basal forebrain were implanted. On the third day after surgery, rats were again performing at criterion. Injection of artificial CSF did not significantly affect performance in the radial maze. However, bilateral injection of carbachol significantly slowed performance in the radial maze (9.8 ± 0.2 min versus 6.0 ± 0.9 min run time) and, in most cases, prevented the rats from entering all the arms. With the exception of one subject, the animals did not appear to exhibit impaired movement. Additional studies must be performed to determine if the disruption of radial maze performance involves cognitive or motor deficits. However, it must be pointed out that intrastriatal injection of soman at doses that inhibit cortical ACh turnover does not affect locomotor behavior (Lynch et al., Pharmacol. Biochem. Behav. 24: 941, 1986).

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286.3 CIRCULATING CHOLINE LEVELS DO NOT REFLECT THE ABILITY OF CENTRAL CHOLINERGIC NEURONS TO SYNTHESIZE AND RELEASE ACETYLCHOLINE. L.Wecker, G. Cawley* and S. Rothermel*, Louisiana State Univ. Medical Center, New Orleans, LA 70112.

Whether or not chronic alterations in circulating levels of choline alter the availability of precursor with consequent changes in acetylcholine (ACh) synthesis is a question of fundamental importance to both basic scientists and clinicians. Thus, the objective of these studies was to determine the effects of alterations in the dietary availability of choline on serum choline levels and on the synthesis and release of ACh in striata. Rats were maintained on either choline deficient (0% choline chloride), control (0.2% choline chloride), or choline supplemented (2.0% choline chloride) diets for 30-40 days. Serum levels of choline in rats fed the control diet were 15 uM and choline deficiency reduced levels by 20%, whereas supplementation increased levels by 52%. The total choline pool in the circulation, which is composed primarily of lipid esters, was 1.4 mM in the control animals, and was not altered by either deficiency or supplementation. To ascertain the effects of the diets on ACh turnover, striatal slices (0.4mm) were incubated in choline-free buffer, and the synthesis and release of neurotransmitter were measured. The initial tissue content of ACh in slices from the control group was 3.5 nmoles/mg pt, and values did not differ among dietary groups. The amount of ACh synthesized was 1.4 nmoles/mg pt/60 min for control samples and ACh was released in a linear fashion at a rate of 6.8 pmoles/mg pt/min. ACh synthesis and release from tissue from supplemented rats did not differ from controls and was 1.2 nmoles/mg pt/60 min and 6.9 pmoles/mg pt/min, respectively. In contrast, ACh synthesis and release from slices from deficient rats were not as vigorous as in the control group; release proceeded at a rate of only 46% and synthesis was 77% of control values. To determine whether this difference reflected alterations in the availability of precursor for ACh synthesis, the concentration and release of choline from the slices were determined. The initial tissue content of choline in slices from the control group was 4.4 nmoles/mg pt, and values did not differ among dietary groups. Choline was released linearly from slices in the control group at a rate of 18 pmoles/mg pt/min. Slices from the deficient group exhibited a 20% reduction in choline release, whereas release from striata in the supplemented rats increased by 51%. Results indicate that dietary choline supplementation does not alter ACh turnover in brain, despite an increase in precursor availability. In contrast, choline deficiency limits available precursor and depresses ACh turnover. (Supported by NIMH-33443.)

286.4 CONTROL OF ACETYLCHOLINE SYNTHESIS AND SECRETION BY LA-N-2 HUMAN NEUROBLASTOMA CELLS IN CULTURE. U.I. Richardson*, J.K. Blusztajn*, M. Liscovitch*, M. Irwin*, and R.J. Wurtman (SPON: M. Karnovsky) Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 01239.

The LA-N-2 strain of human neuroblastoma cells has been shown to contain large amounts of acetylcholine (ACh). We have investigated the rates of ACh synthesis and release in LA-N-2 cells in order to characterize them as a potential model of cholinergic neurons. When grown in a serum-containing medium the cells do not extend many neurites. In the absence of serum most cells develop neurites that can reach a length of 0.1 mm. Electron microscopic examination reveals cytoskeletal structures in these processes. The cells elaborate an extensive Golgi apparatus whose vesicles may contain ACh.

ACh content of the cells (determined by a radioenzymatic assay) varies with extracellular choline concentration in a saturable fashion, reaching a maximum of approximately 1 nmol/mg protein. Radiolabeled choline is taken up by the cells [and then converted to ACh or phosphocholine (PCh) as determined by purification from cell extracts by high performance liquid chromatography] in a saturable manner which is described by a single rectangular hyperbola. Hemicholinium-3 (HC-3)(0.1 mM) inhibits this uptake as shown in the table:

	Km (μM)		Vmax (pmol/mg protein/5 min)	
	control	HC-3	control	HC-3
Choline	35.5±1.8	53.4±10.9	821.3±14.5	730.2±44.3
Acetylcholine	28.4±2.1	62.0± 2.1	623.8± 8.6	394.0±62.0
Phosphocholine	7.9±1.7	66.7±15.0	106.8± 6.2	73.7± 7.3

Turnover rates of ACh and PCh (half-lives of 4 and 25 hours, respectively) were determined by pulse-chase experiments with [^{3}H]choline. Most of the radioactivity lost from the ACh pool was recovered in the medium, suggesting that ACh turnover is primarily associated with its release. The radioactivity lost from the PCh pool was quantitatively recovered in the form of phosphatidylcholine (PtdCho) suggesting that the PCh is protected from intracellular hydrolysis and is used as precursor of PtdCho.

The cells release ACh spontaneously and this release is enhanced upon depolarization with potassium or veratridine (the latter effect is blocked by tetrodotoxin), or by a muscarinic agonist, carbachol. LA-N-2 cells contain acetylcholinesterase and release this enzyme into the medium upon depolarization with potassium in a calcium-dependent manner.

Taken together the data demonstrate that LA-N-2 cells exhibit properties of differentiated cholinergic neurons and promise to be a useful in vitro model for studies of ACh synthesis and release.

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286.5 **CHOLINERGIC - GABAERGIC RECIPROCAL SYNAPTIC INTERCONNECTION IN THE RAT SEPTAL AREA AND ITS HIPPOCAMPO-SEPTAL AFFERENTS: EM DOUBLE IMMUNOSTAINING COMBINED WITH DEGENERATION.** C.Leranth and M.Frotscher Yale University, School of Med. Section of Neuroanatomy and Dept. of OB/GYN, New Haven, CT. 06510 and (M.F.) Dept. of Anatomy, Johann Wolfgang Goethe Univ. Frankfurt a.M. F.R.G.

The cholinergic innervation of rat hippocampus pyramidal, Golgi identified granule cells, and using EM double immunostaining (EMDI), the cholinergic innervation of dentate hilar area GABAergic and somatostatin immunoreactive inter- and commissural neurons has been reported in our laboratory (Frotscher and Leranth, 1985, 1986; Leranth and Frotscher, 1987). In recent studies we analyzed the synaptic interconnections of these septo-hippocampal cholinergic neurons with GABAergic neurons and hippocampo-septal afferents in the rat septal area.

Preembedding EMDI using immunoperoxidase and avidinated colloidal gold as two contrasting electron dense immunolabels revealed cholinergic innervation of septal GABAergic neurons and GABAergic innervation of septal cholinergic neurons. The combination of fimbria-fornix transection with immunostaining for GAD demonstrated that degenerated hippocampal afferents to the septum terminate on GABAergic and non-GABAergic septal neurons. In contrast, following the same operation and immunostaining for ChAT, synaptic connections between degenerated hippocampal afferents and septal cholinergic neurons were not found.

These observations led us to the conclusion that septal projective cholinergic neurons receive inputs from GABAergic neurons, while septal GABAergic neurons are innervated by both cholinergic and hippocampal afferents. However, the question still arises whether the same septal GABAergic neurons are targets of both hippocampal and cholinergic inputs.

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286.6 Tonically active nucleus basalis neurons in the awake monkey project to cerebral cortex. Russell T. Richardson and Mahlon R. DeLong, Depts. of Neurology and Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21205.

Neurons within the nucleus basalis of Meynert (NBM) in awake monkeys have previously been described as having a characteristic tonic discharge pattern (DeLong, J. Neurophysiol. 34:414, 1971). Neurons displaying this discharge pattern have the same anatomical distribution as the cortically projecting cholinergic neurons of the NBM in that they are consistently found ventral to the globus pallidus and within the medullary laminae. We now confirm that at least some of these spontaneously active neurons project to cerebral cortex.

Three pairs of stainless steel stimulating electrodes were implanted near the cingulum bundle of a male rhesus monkey. The cingulum bundle contains numerous axons of NBM cells that project to cortex (Kitt et. al., Brain Res. 406:192, 1987). Therefore, antidromic activation of an NBM neuron from the cingulum bundle would indicate that the neuron projects to the cortex. Neurons were considered to be antidromically activated if the driven action potentials had a constant latency, followed paired pulse stimulation of 200 Hz, and collided with orthodromic action potentials. Stimulation pulses were 300 msec square waves up to 3 mA.

To date, seven tonically firing NBM neurons have been antidromically activated in the awake monkey. Spontaneous discharge rates ranged from 7 to 18 Hz (mean: 12 Hz), and the coefficients of variation of the interspike interval ranged from 46 to 78% (mean 61%). The duration of the initial negativity of the action potential (200-10KHz filtering) ranged from 240 to 320 usec (mean: 264 usec). The discharge rates, interspike interval variances, and spike durations of these antidromically driven cells are similar to those of tonically active NBM neurons recorded previously (Mitchell et. al., Exp. Brain Res., in press).

The activity of three of the seven neurons was recorded while the monkey performed a go/no-go task as described previously (Richardson & DeLong, Soc. Neurosci. Abstr. 12:356, 1986). One cell did not respond, and two cells had significant ($p < .01$) changes in firing only in the choice phase of the task, with no differences between responses on go trials and no-go trials. In the earlier study of 82 NBM neurons, the most common response occurred in the choice phase of the go/no-go task, with no significant response differences between go and no-go trials in 89% of the cases. Thus, despite the small number of antidromically driven neurons, their activity in this task was comparable to that of other tonically discharging NBM neurons.

These findings indicate that the previously characterized population of tonically active NBM neurons project to cerebral cortex.

286.7 CARBACHOL CONJUGATED MICROSPHERES: A NEW PHARMACOLOGICALLY ACTIVE RETROGRADE PROBE FOR MAPPING BEHAVIORALLY EFFECTIVE DRUG INJECTION SITES IN THE MAMMALIAN BRAIN. J. Quattrochi, H.A. Baghdoyan, R. Madison, T. Hensch*, and J.A. Hobson. Laboratory of Neurophysiology, Harvard Medical School, Boston, MA 02115.

Microinjection of cholinergic agonists into the dorsolateral pontine tegmentum has been shown to elicit desynchronized (D) sleep signs and a D sleep-like behavioral state in adult cats. However, a consistent methodological problem underlying the injection of pharmacological agents in these studies is the extent of diffusion from the injection site. Also, it is impossible to know from such experiments which cholinergic neurons project to the injection site. In order to restrict diffusion and retrogradely label neurons which project to a more precisely defined anatomic site in the cat brainstem, we now report the conjugation of the cholinergic agonist, carbachol, to fluorescent latex microspheres.

Carbachol was covalently linked to rhodamine fluorescent microspheres. Pharmacological effectiveness was assessed by scoring polygraphic recordings of behavioral state after carbachol microspheres in 250 nl saline were administered through chronically implanted guide tubes stereotaxically aimed at the anterior dorsolateral pontine reticular formation. All injections were unilateral into the same site and consisted of the conjugate reaction solution extensively dialyzed without microspheres, carbachol alone (4 microgram dose), or carbachol microspheres. Measures of behavioral state include latency to onset of the first D episode (D latency) and percentage of the total recording time (4 hours) spent in D sleep (D percentage).

D latency after administration of carbachol microspheres was 9.2 min compared with 4.2 min after carbachol alone and 108.2±48.5 min in control conjugate dialysis solution and baseline recordings. D percentage was similar between carbachol microspheres (61%) and carbachol alone (62%) compared to 4.3±1.2% in controls. D percentage was markedly greater during the first two hours (75% and 68.1%) for carbachol microspheres than for carbachol (50.7% each hour). However, by the fourth hour D percentage was greater with carbachol (74.3%) compared with carbachol microspheres (21.5%).

This micropharmacologic application of fluorescent microspheres now facilitates the precise in vivo characterization of cholinergic sleep-generating neurons with a specificity unattainable with present methodology.

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286.8 BRAIN BINDING SITES FOR THE ACETYLCHOLINE UPTAKE BLOCKER AH5183: PHARMACOLOGY AND EFFECTS OF LESIONS. M. R. Marien+, D. McHugh* and C. A. Altar. +Douglas Hospital Research Center, 6875 Lasalle Blvd., Verdun, Canada, and Neuroscience/Cardiovascular Res., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ, 07901, USA.

AH5183 is a noncompetitive inhibitor of high-affinity acetylcholine transport into cholinergic vesicles. Using quantitative autoradiography, we have shown that [3H]AH5183 binds specifically, saturably and with high affinity to sites in rat brain which are located in cholinergic nerve terminal regions (Marien, Parsons and Altar, PNAS 84:876, 1987). The present study investigated the pharmacology and the cholinergic terminal localization of the [3H]AH5183 binding site. The binding of [3H]AH5183 (kindly supplied by Dr. S. Parsons) to 20 um-thick brain sections of male Sprague-Dawley rats (Tac:SD) was performed and analyzed by quantitative autoradiography as previously described (Marien et al, 1987; Altar et al, J. Neurosci. Meth. 10:173, 1984).

[3H]AH5183 binding was not displaced by 10 µM concentrations of hemicholinium-3 or by cholinergic, adrenergic, dopaminergic, GABAergic, glutamatergic, sigma, phencyclidine, or calcium channel receptor ligands. Of 60 compounds tested, the active displacers of [3H]AH5183 binding were (IC50, nM): quinacrine (3600), ketanserin (500), decahydro-3-(4-phenyl-1-piperidinyl)-2-naphthalenol (100), haloperidol (43), and 3-(4-phenyl-1-piperidinyl-2-hydroxyaminotetralin (14).

Cholinergic afferents to the neocortex or hippocampus were unilaterally lesioned 14 days before sacrifice by injection of 150 nmoles of quinolinic acid into the nucleus basalis magnocellularis or by transection of the fimbria. Respective reductions of choline acetyltransferase activity in the frontal cortex (-52%) or hippocampus (-80%) were accompanied by decreases in the Bmax (but not the Kd) values of [3H]AH5183 binding in the frontal cortex (-29%, $n = 6$, $p < 0.01$) and hippocampus (-25%, $n = 10$, $p < 0.001$).

These findings further demonstrate the unique pharmacology of the [3H]AH5183 recognition site in brain, and indicate a presynaptic localization for a proportion of these sites on neocortical and hippocampal cholinergic afferents.

286.9 A SINGLE RNA SPECIES INJECTED IN XENOPUS OOCYTE DIRECTS THE SYNTHESIS OF ACTIVE CHOLINE ACETYLTRANSFERASE. R.E. McCaman, L. Carbini*, V. Maines* and P.M. Salvaterra. Div. Neurosciences, Beckman Research Institute/City of Hope, Duarte, CA 91010.

We have recently isolated and characterized a cDNA clone containing a 728 amino acid coding sequence for Drosophila choline acetyltransferase (ChAT; EC 2.3.1.6) (Itoh et. al., PNAS 83:4081, 1986). In this study we have tested the ability of this clone to produce active enzyme. We have sub-cloned the lambda gt11 insert of the Drosophila ChAT cDNA into the pGEM2 plasmid (Promega Biotech Inc.) that allows us to synthesize in vitro a single species of complementary RNA (cRNA).

Stage 5 oocytes from Xenopus laevis were microinjected with 25-50 nl of diethylpyrocarbonate-treated water containing approximately .02-50 ng of capped ChAT RNA in the sense orientation. Control oocytes were injected with either an equal volume of water or 50 nl of water containing antisense RNA. Control and experimental oocytes were assayed for ChAT activity and endogenous levels of acetylcholine (ACh) and choline.

The measured levels of ChAT activity in several different experiments range from 40 to 1300 pmole/min/oocyte. The level of ChAT activity observed in control oocytes was below the level of detection (i.e. <1 pmole/min/oocyte. RNA injected oocytes also showed a high level of endogenous ACh (up to 350 pmole/oocyte). Control oocytes produced no detectable (i.e. <.5 pmole/oocyte) endogenous ACh. The steady state level of choline was reduced during synthesis of ACh. Analyses using the Western blot technique showed that the extracts from Xenopus injected with sense RNA contained a single 75 kd polypeptide recognized by anti bodies to the Drosophila ChAT fusion protein produced by the cDNA clone. The size of this polypeptide agrees well with the major polypeptide recognized by these same antibodies in SDS homogenate of fresh Drosophila heads. No immunoreactive proteins were produced by control oocytes.

We conclude from these results that enzymatically active ChAT protein is produced by oocytes injected with the ChAT cRNA. In addition, the protein is very similar, if not identical to the major form of ChAT present in Drosophila.

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286.10 MOLECULAR FORMS OF ACETYLCHOLINESTERASE FROM ADULT LAMPREY BRAIN AND AMMOCOETE SKELETAL MUSCLE. Leo Pezzementi, Hugh C. Nickson,* Robert C. Dunn,* and Ronald J. Bradley. Birmingham-Southern College and University of Alabama at Birmingham, Birmingham, Al.

Lampreys belong to the most primitive class of vertebrates, the Agnatha. To learn more about the evolution of acetylcholinesterase (AChE) in the vertebrates, we studied the cholinesterase activity from the brain of the adult lamprey and from the skeletal muscle of the larval form, the ammocoete. We found that the brain enzyme is true AChE and that 98% of it is present in the G_4 globular form. Only 1% of the AChE was found distributed among the asymmetric forms, A_4, A_8, and A_{12}; an additional 1% of the activity could not be extracted. The identity of the asymmetric forms was confirmed by collagenase digestion. These data demonstrate that asymmetric AChE is present in the brains of organisms representing all classes of vertebrates. However, our results raise questions about a proposed phylogenetic relationship concerning vertebrate brain AChE.

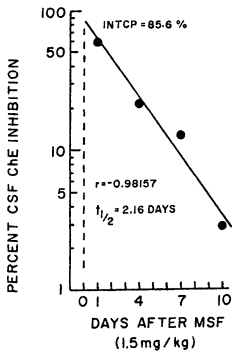
The ammocoete is a relatively non-motile filter-feeder, which, in terms of anatomy and behavior, superficially resembles the cephalochordate amphioxus. Although the molecular forms of AChE from amphioxus are not known, urochordates and echinoderms possess only globular esterase. In addition to providing insights into the evolution of AChE, a study of esterase in the ammocoete could provide information about developmental changes in the enzyme. We found that cholinesterase activity of the ammocoete is also due to AChE and that 85% of the esterase is present in globular enzyme. The predominant globular from detected was G_4. In contrast to skeletal muscle from spawning adult lamprey, considerable amounts of G_1 and G_2 were also observed. Additionally, approximately 5% of the esterase was found distributed among the asymmetric forms A_4, A_8, and A_{12}. Ten percent of the esterase could not be extracted. Thus, AChE from ammocoete skeletal muscle is qualitatively similar to adult enzyme since both exist in globular and asymmetric forms; however, at the same time, the larval enzyme is quantitatively different in its complement of globular forms. Since G_1 and G_2 are probably precurors of G_4, the lack of the smaller globular forms in muscle from spawning adult may be related to the deteriorating condition of the spawning lamprey. This research was supported by a Cottrell College Science Grant From Research Corporation to L. P.

286.11 METHANESULFONYL FLUORIDE: AN ACTIVE CNS CHOLINESTERASE INHIBITOR IN PRIMATES. D.E. Moss, P.Z. Manderscheid, R. Palacios*, and R.G. Perez*. Lab. of Psychobiochemistry, Dept. of Psychology, Univ. of Texas at El Paso, El Paso, TX 79968.

Methanesulfonyl fluoride (MSF) can produce up to 90% inhibition of rat brain ChE with less than 35% inhibition of peripheral enzyme measured in smooth muscle, skeletal muscle and heart (Moss et al., 1985). Because MSF, a CNS selective ChE inhibitor with low general toxicity, may be therapetically valuable in dementia in Alzheimer's disease or other CNS diseases, it was important to determine if MSF would produce similar effects in primates.

M. fasicularis were injected i.m. with increasing doses of MSF in sterile peanut oil until a dose of 1.0 mg/kg was used for 11 injections given 3 times per week and then a dose of 1.5 mg/kg was maintained for 12 injections given 2 times per week. At the end of this treatment, RBC AChE was inhibited over 80% while plasma BChE was inhibited about 30%. Cortical biopsies taken 2 or 3 days after the last injection showed that treated monkeys were inhibited 80% relative to controls. At no time during treatment did any monkey show toxic effects as shown by blood chemistry, behavior, or loss of weight or vigor.

At least six months after the completion of the previous experiment, inhibition of CSF ChE by MSF was studied. In this experiment, a single injection of 1.5 mg/kg MSF produced 85% inhibition of CSF ChE which also showed a half-time for the recovery of enzyme activity of 2.16 days. A single injection of 3.0 mg/kg produced virtually 100% inhibition. This dose produced a general malaise but no toxic or respiratory crisis.



Because of the remarkably low toxicity associated with CNS ChE inhibition produced by MSF, it appears that the extreme toxicity of other ChE inhibitors may not be due entirely to inhibition of ChE in the CNS but may also involve other mechanisms of toxicity. MSF may be safe and efficacous in human therapeutics.

Moss, D.E. et al. In: Senile Dementia of the Alzheimer Type, New York, Liss, 1985, pp. 337-350.

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287.1 PHENCYCLIDINE RECEPTOR LIGANDS ATTENUATE CORTICAL NEURONAL INJURY FOLLOWING HYPOXIA OR N-METHYL-D-ASPARTATE EXPOSURE. M.P. Goldberg, V. Visekul* and D.W. Choi. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The dissociative anesthetic phencyclidine (PCP) and the sigma opiate SKF 10,047 (SKF) have been reported to antagonize neuroexcitation at N-methyl-D-aspartate (NMDA) receptors. We investigated the possibility that these and related compounds might systematically reduce the cortical neuronal injury produced either by hypoxia, or by toxic exposure to NMDA.

Cultures of dissociated mouse neocortical neurons and glia were exposed to a hypoxic atmosphere of 5% CO_2 / 95% N_2 at 37° for 10 hr. 24 hr later, control (untreated) cultures showed widespread neuronal damage and substantial efflux of lactate dehydrogenase (LDH) to the bathing medium. In contrast, addition of the following compounds at 100 µM to the exposure medium (during and after hypoxia) led to preservation of neuronal morphology and marked reduction of LDH efflux: PCP, ketamine, (+)-SKF, (-)-SKF, (±)-pentazocine (PENT), dextrorphan, and levorphanol. Quantitative concentration-response studies showed half-maximal reduction (ED_{50}) of LDH release in the range of 1 µM PCP, 10 µM (+)-SKF, and 30 µM PENT.

In separate experiments, these drugs were tested for ability to attenuate the neurotoxicity induced by direct application of NMDA itself. Cultures were exposed to 500 µM NMDA for 5 min, either alone, or in the presence of an added antagonist drug, before being returned to normal medium and assessed 1 d later. In this paradigm, each of the compounds listed above at 100 µM yielded substantial reduction of NMDA neurotoxicity. Quantitative concentration-protection studies with PCP, (+)-SKF, and PENT showed ED_{50} values in the range of 3 µM PCP, 10 µM (+)-SKF, and 30 µM PENT.

The observed potency order for reduction of both NMDA- and hypoxia-mediated cortical neuronal injury (PCP > SKF > PENT) corresponds to that previously reported by Olney et al. (Neurosci Lett 68: 29) for antagonizing acute NMDA excitotoxicity in the chick retina. This order also corresponds to that obtained in binding studies of the phencyclidine receptor site, and differs from that alternatively expected at the high-affinity SKF / sigma receptor site (Largent et al., J Pharm Exp Ther 238: 739), thus favoring the notion that the former is the site associated with the NMDA receptor channel. In addition, the close correlation between the pharmacology of hypoxic neuronal injury and NMDA neurotoxicity observed here provides additional evidence that these two processes are related.

Supported by NIH NS12151 and a Hartford fellowship to D.W.C.

287.2 METHADONE SELECTIVELY AND NON-STEREOSPECIFICALLY ATTENUATES N-METHYL-D-ASPARTATE RECEPTOR-MEDIATED NEUROTOXICITY: A GENERALIZABLE PROPERTY OF OPIOIDS? D.W. Choi and V. Viseskul*. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

N-methyl-D-aspartate (NMDA) receptor-mediated toxicity may participate in the pathogenesis of neuronal cell loss in several acute and chronic neurological diseases. We have recently shown that the opioid agonist levorphanol and the opioid antagonist naloxone, both can selectively antagonize NMDA neurotoxicity in cortical cultures. The present experiments further explored the ability of methadone and other opioid compounds to antagonize excitatory amino acid neurotoxicity.

Exposure of murine neocortical cell cultures to a 5 min pulse of 500 µM NMDA resulted by the following day in widespread neuronal disintegration and a substantial efflux of lactate dehydrogenase (LDH) to the bathing medium. Addition of 10 µM - 1 mM (±)-methadone to the NMDA exposure solution produced a concentration-dependent reduction (ED_{50} about 100 µM) in both the morphological evidence of neuronal cell damage, and the efflux of LDH, with near complete blockade at higher concentrations. In contrast, 1 mM (±)-methadone produced little attenuation of quisqualate neurotoxicity (500 µM for 5 mins). 30 - 100 µM (±)-methadone also markedly attenuated the neurotoxicity of the endogenous NMDA agonist, quinolinate (1 mM for 20 - 24 h) without affecting the neurotoxicity of kainate (100 µM for 20 - 24 h).

The potent opioid isomer (-)-methadone, and the weak opioid enantiomer (+)-methadone, appeared grossly equipotent (and similar to the racemate) at blocking the neurotoxicity of NMDA. Other experiments showed that high (200 µM - 10 mM) concentrations of morphine itself (and somewhat lower concentrations of (+)-morphine), as well as 1 - 3 mM concentrations of codeine, meperidine, fentanyl, and nalorphine, also produced some attenuation of NMDA-induced neurotoxicity.

Thus methadone and several other opioids exhibit novel antagonist activity against NMDA receptor-mediated neurotoxicity in vitro. In the four cases where stereospecificity was tested, this activity was found to an equal or greater degree in the non-opioid dextrorotatory enantiomers [(+)-methadone, dextrorphan, (+)-naloxone, and (+)-morphine], suggesting that the neuron-protective effect is not mediated by conventional opiate receptors. Many opioids are clinically available and cross the blood-brain barrier easily. The more potent members of this class of drugs - and perhaps especially their enantiomers, which would offer reduced opiate receptor-related side effects - may have potential as clinical therapeutic agents.

Supported by NIH NS12151. DWC is a Hartford fellow.

287.3 GLUTAMATE NEUROTOXICITY AND ASTROCYTE SWELLING: LACK OF INVOLVEMENT OF NMDA RECEPTOR. P. H. Chan, L. Chu*, A. Yu* and S. Chen* (SPON: R. Fishman). Department of Neurology, Brain Edema Research Center, University of California, San Francisco, CA 94143

Excitatory neurotransmitter L-glutamic acid (GLU) and its agonists N-methyl-D-aspartate (NMDA) have been implicated in selective vulnerability and neuronal death following ischemia, hypoglycemia and epileptic seizures. NMDA receptor antagonists have been shown to reduce the ischemia or hypoglycemia-induced neuronal death of hippocampus in vivo. Furthermore, these NMDA receptor antagonists also reduce the GLU-induced swelling and death in neuronal cell culture in vitro. We have reported previously that GLU and its agonists including homocysteic acid, kainic acid, aspartic acid and NMDA caused significant cellular swelling and cation changes in brain slices (Chan et al, J Neurochem 33:1309, 1979). However, the role of GLU and the NMDA antagonists on glial cell swelling and injury was not delineated in those studies. We now study the excitotoxic mechanisms of glial swelling using intact cerebral cortical astrocytes of newborn rats. The intracellular water space (IWS) (measured by 3-O-methyl [^{14}C]-D-glucose) was increased (control = 3.4 + 0.2 ul/mg protein) by GLU (1 mM) by 175%, 213% at 1 hour and 4 hours respectively and was returned to baseline at 24 hours. The GLU-induced IWS changes were dose-dependent. Among the GLU agonists, homocysteic acid at equal molar concentration exhibited similar potency in inducing astrocytic swelling (214%), followed by L-aspartate (ASP) (160%) and quisqualate (152%), whereas NMDA, kainate and quinolinate were not effective. Unlike GLU and ASP, both homocysteic acid and quisqualate caused a persistant increase in IWS of astrocytes with prolonged incubation time (e.g. 24 hours). DL-2-amino 5-phosphonovaleric acid (APV) and 2-amino-7-phosphonoheptanoic acid (APH), antagonists of NMDA-preferred receptor, and kynurenic acid, a non-specific GLU receptor antagonist at a concentration of 1 mM were not effective in inducing cellular swelling of astrocytes. Furthermore, pretreatment with APV, APH, or kynurenic acid failed to reduce the GLU-induced astrocytic swelling. These data indicate that GLU may exert its excitotoxic effects on astrocytic swelling through mechanisms other than those mediated by NMDA receptor. Supported by NS-14543.

287.4 ISCHEMIC BRAIN DAMAGE IS REDUCED BY SYSTEMIC ADMINISTRATION OF THE N-METHYL-D-ASPARTATE (NMDA) ANTAGONIST, MK-801. C. Park*, D.G. Nehls*, E. Ozyurt*, D.I. Graham*, J. McCulloch. Wellcome Surgical Institute, University of Glasgow, Glasgow, G61 1QH, Scotland.

Previous studies have indicated that N-methyl-D-aspartate (NMDA) receptor antagonists are capable of reducing the amount of damage caused by cerebral ischemia when administered by direct intraparenchymal injection (Simon et al., Science, 226:850, 1984). MK-801 is a new potent non-competitive NMDA antagonist which readily penetrates into the CNS (Wong, E.H.F., et al., Proc. Natl. Acad. Sci. USA, 83:7104, 1986). We have studied the protective effects of this agent in models of permanent focal cerebral ischemia in cats and rats.

Animals underwent microsurgical occlusion of the middle cerebral artery (MCA) under general anesthesia. In cats, saline or MK-801 (5 mg/kg i.v.) was given 30 minutes prior to occlusion or 2 hours after occlusion. In rats, saline or MK-801 (0.5 mg/kg i.v.) was given 30 minutes prior to or 30 minutes after occlusion. Anesthesia was maintained for 3 hours (rats) or 6 hours (cats) at which time the animals were sacrificed and the brains perfusion fixed. Ischemic area was determined at multiple coronal planes and the volume of the area showing early ischemic cell change was computed.

In cats, with pre-treatment, MK-801 significantly reduced the volume of the hemisphere with early ischemic cell change from 3231 ± 394 mm^3 (control) to 1602 ± 445 mm^3 (MK-801) ($P<0.01$). The protective effect occurred predominantly in the cortex where the ischemic volume was decreased by 57% ($P<0.01$), whereas there was no significant reduction in ischemic volume in the caudate nucleus. In rats pretreated with MK-801, the volume of the hemisphere showing early ischemic cell change was reduced by 38% ($P<0.005$). Throughout all the levels of sections, there was a consistent decrease in ischemic volume suggesting that protection occurred throughout the ischemic area and was not limited to the ischemic penumbra. This contrasts with the effects of nimodipine in the same model in which protection was limited to the ischemic penumbra.

MK-801 is extremely effective in reducing the amount of ischemic damage in a model of focal ischemia using pre-treatment. The results of pre-treatment will be compared to those of treatment with MK-801 administered after MCA occlusion.

287.5 COMPARATIVE EFFICACY OF VARIOUS AGENTS IN PREVENTING GLUTAMATE-INDUCED OR ISCHEMIC NEURONAL DEGENERATION IN CHICK RETINA. J.W. Olney, M.T. Price, J. Labruyere*, E. Silverman*, M. Mueller*. Department of Psychiatry, Washington University Medical School, St. Louis, MO 63110.

Recent evidence suggests that certain neurodegenerative conditions, including anoxic-ischemic brain damage, may be mediated by the neurotoxic (excitotoxic) action of excitatory amino acid (EAA) transmitters. Glutamate (Glu), the most abundant EAA in the CNS, is released in toxic concentrations under ischemic conditions and is presumed to be the endogenous pathogen primarily responsible for ischemia-induced neuronal degeneration. Three subtypes of Glu receptors are thought to mediate excitotoxic events, each being named after an agonist to which it is differentially sensitive--N-methyl aspartate (NMA), quisqualate (Quis), kainate (KA). Recent evidence suggests that NMA-specific antagonists may be effective in vivo as neuroprotective agents in ischemia. It should be noted, however, that this evidence pertains exclusively to CA-1 hippocampal neurons which may be unique in having an exceedingly high ratio of NMA to non-NMA receptors. Neurons having a larger complement of non-NMA receptors might be less well protected by NMA antagonists since Glu, a mixed EAA agonist that acts at all EAA receptor subtypes, is not prevented by NMA antagonists from activating non-NMA receptors.

To explore this issue we have comparatively evaluated in the ex vivo chick embryo retina the ability of various EAA antagonists to protect against the neurotoxic actions of prototypic EAA agonists (NMA, KA, Quis or Glu) with their ability to protect retinal neurons against ischemia (oxygen-glucose deprivation). We found NMA antagonists quite potent in blocking NMA neurotoxicity but weak in blocking KA, Quis, Glu or ischemic damage. Of all agents tested, the most effective in blocking ischemic degeneration was the thiobarbiturate, Thiamylal, which behaves in the retina as a broad spectrum antagonist that blocks NMA as well as KA, Quis and Glu toxicity.

Our findings support the interpretation that endogenous Glu mediates ischemic neuronal degeneration in the chick retina and that many retinal neurons have non-NMA receptors where Glu exerts excitotoxic action in the presence of NMA receptor blockade. Since many CNS neurons may have a substantial complement of non-NMA as well as NMA receptors, NMA antagonists may not adequately protect such neurons against anoxic-ischemic degeneration. Whether broad spectrum EAA antagonists such as thiamylal might be more effective warrants evaluation. Supported by RSA MH38894 (JWO) and a grant from the Washington Univ/Monsanto Biomedical Res Fund.

287.6 A DELAYED EXCITOTOXIC MECHANISM MEDIATES THE DEGENERATION OF RAT STRIATAL NEURONES CAUSED BY N-METHYL-D-ASPARTATE AND QUINOLINATE. A.C. Foster, R. Gill* and G.N. Woodruff*. Merck Sharp and Dohme Research Labs., Terlings Park, Eastwick Road, Harlow, Essex, U.K.

Intra-cerebral injection of excitotoxins has been used extensively to produce neuronal lesions and provide animal models of human neurodegenerative disorders. However, the degree to which such acute neurotoxicity experiments can mimic chronic neurodegenerative events has been questioned. Using the selective, non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (Wong et al, PNAS 83: 7104, 1986), we now provide evidence that the degeneration of rat striatal neurones caused by local injection of NMDA receptor agonists is mediated by a delayed mechanism more akin to a chronic degenerative event than was previously suspected.

Male Sprague-Dawley rats (250-350g) were anaesthetised with equithesin and stereotaxic injection of quinolinate (QUIN, 200nmol), NMDA (200nmol) or kainate (5nmol) in 1µl of phosphate-buffered saline made into the right striatum over a period of 2 min. Animals were injected with a single dose of 10mg/kg MK-801 (i.p.) at various times and killed 7 days following excitotoxin injection, their striata dissected and neuronal degeneration assessed by measuring cholineacetyl transferase (CAT) and glutamate decarboxylase (GAD) activities.

We have previously reported that neuronal degeneration caused by NMDA or quinolinate was prevented by 10mg/kg MK-801 when the drug was administered i.p. 1 hour prior to excitotoxin injection (Foster et al, Br. J. Pharmac. Proc. Suppl. 89 87OP, 1986 and 90 7P, 1987). As shown below, the degeneration of striatal neurones caused by NMDA or QUIN was also prevented by MK-801 given i.p. up to 5 hours after excitotoxin injection. The protection of cholinergic neurones was virtually complete when MK-801 was administered at 1 and 2 hours and still significant at 5 hours. GABAergic neurones were partially protected at 1 and 2 hours.

% decrease of enzyme activity (mean ± SEM, N = 3-10 per group)

Excitotoxin		Untreated controls	10mg/kg MK-801 given at times after excitotoxin 1h	2h	5h	24h
QUIN	CAT:	78.7 ± 4.5	0.8 ± 0.5**	4.6 ± 2.2**	41.8 ± 6.8**	67.9 ± 12.9
200nmol	GAD:	84.2 ± 2.6	23.0 ± 4.0**	42.2 ± 5.0**	79.4 ± 1.9	70.5 ± 12.1
NMDA	CAT:	52.6 ± 9.0	5.7 ± 2.2**	2.9 ± 1.6**	9.3 ± 2.4**	47.9 ± 11.1
200nmol	GAD:	59.4 ± 6.9	15.4 ± 4.1**	33.9 ± 15.0*	51.9 ± 4.0	65.5 ± 9.0

* P < 0.05, ** P < 0.01 Dunnett's Multiple Range Test

In contrast, MK-801 (10mg/kg, i.p.) administered 2 hours following intra-striatal injection of kainate (5nmol) was unable to prevent reductions in striatal CAT and GAD activities induced by this excitotoxin.

The ability of MK-801 to prevent the local neurotoxicity caused by NMDA agonists when administered after their injection indicates that these excitotoxic effects are mediated by a delayed mechanism which requires activation of NMDA receptors. Delayed degenerative events are characteristic of certain types of ischaemic-induced neuropathology (Kirino, Brain Res. 239: 57, 1982) and may be a reflection of neurotoxic sequalae resulting from an overstimulation of NMDA receptors.

287.7 DIFFERENTIAL SPARING OF NADPH-DIAPHORASE NEURONS IN QUINOLINIC LESIONED RAT AND PRIMATE STRIATUM

Joseph B. Martin, Neil W. Kowall, Robert J. Ferrante, P. Ben Cipolloni and M. Flint Beal
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Quinolinic acid is an endogenous excitotoxin present in human brain that reproduces the neurochemical profile of Huntington's disease when injected into rat neostriatum. In the present study we have made lesions with quinolinic acid in both rat and primate striatum to further evaluate the neuronal selectivity of the lesions. A dose of either 120 or 240 nmol of quinolinic acid or 10 nmol of kainic acid was injected into the anterior rat striatum over 2 minutes. Similarly lesions were made in 3 rhesus monkeys by injecting either 240 nmol in 1 µl or 360 nmol in 1.5 µl. These lesions were made at 5 anterior-posterior levels in both the caudate and putamen. Both rats and monkeys were sacrificed two weeks after the lesions. Sections were stained for NADPH-diaphorase (NADPH-d) and cresyl violet. Some rats were pretreated with diisopropyl fluorophosphate and later double stained for both acetylcholinesterase (AChE) and NADPH-d. In both species there was a lesion core in which there was almost total neuronal loss and dense gliosis. Within these areas there were no preserved NADPH-d neurons. To evaluate possible selective neuronal sparing, both NADPH-d and Nissl stained neurons were counted within the transition zone (TZ) bordering the lesion core. This zone is characterized by partial neuronal depletion and moderate gliosis. Control cell counts were made in identical regions in the contralateral unlesioned striatum. The total number of Nissl stained neurons was depleted 50% within the TZ, as compared to the control side. In rats, the percent of NADPH-d neurons was significantly increased in the TZ as compared to the control side (control: 3.5%, lesion: 8.0%). In rhesus monkeys, the percent of NADPH-d neurons was also significantly increased from 1.7% to 3.4%. In contrast to these findings, kainic acid lesions in rats resulted in a significant reduction of NADPH-d neurons (control: 2.2%, lesion: 0.9%), in a region in which there was 28% total neuronal depletion. In the AChE/NADPH-d stained quinolinic rat lesions, all NADPH-d and AChE neurons were counted throughout the striatum in tissue sections at 200 um intervals. AChE neurons could often be seen and were relatively preserved in the core of the lesion, as compared to NADPH-d neurons. At more posterior levels, 2 mm from the center of the lesion, sparing of NADPH-d neurons relative to AChE was seen. These findings indicate that NADPH-d neurons are spared relative to Nissl stained neurons in quinolinic acid lesions in both rat and monkey.

287.8 SYSTEMIC APPROACHES TO PREVENTING QUINOLINIC ACID NEUROTOXICITY IN RAT STRIATUM

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If quinolinic acid or another endogenous excitotoxin is involved in the pathogenesis of Huntington's disease (HD), then a systemic therapy capable of blocking its neurotoxicity might be useful therapeutically in HD in an attempt to halt the disease process. In the present study we have examined the ability of a wide variety of compounds to modify quinolinic acid neurotoxicity in rat striatum. Compounds were administered intraperitoneally 30 minutes before stereotactically injecting 240 nmol of quinolinic acid into the anterior striatum. One week after the injections the animals were sacrificed and the lesioned and contralateral striatum were dissected. The ability of agents to block neurotoxicity was assessed by comparison both with saline injected controls and with the contralateral side. The extent of the lesions was measured biochemically with determinations of substance P, GABA, somatostatin and neuropeptide Y. Histologic examination of lesions was also made. The free radical blockers vitamin C, vitamin E and beta-carotene had no beneficial effect and buthionine sulfoxine, a glutathione depleter, also had no detrimental effect. Allopurinol has been shown to ameliorate ischemic damage felt to be associated with free radical formation. Neither pretreatment with 50 nor 100 mg/kg had any beneficial effect. Nimodipine is a blocker of voltage-dependent calcium channels. Since increased calcium concentrations have been implicated in excitotoxin mediated neuronal death we examined the effects of this compound. Pretreatment at doses of 2.0, 4.0, or 10.0 mg/kg had no beneficial effect.

The effects of quinolinic acid are known to be blocked by co-injection with N-methyl-D-aspartate blockers APV and APH. Neither of these compounds administered systemically at a dose of 50 mg/kg had a beneficial effect. Baclofen has been reported to block release of glutamate. Pretreatment with 10 mg/kg did not modify toxicity. Recently Iversen and colleagues have reported that MK-801 is a systemically effective N-methyl-D-aspartate blocker. A dose of 1.0 mg/kg had no effect however there was partial protection at 2.0 mg/kg which showed a dose-response to complete protection at a dose of 4.0 mg/kg. These data therefore show that MK-801 can prevent neuronal toxicity in the quinolinic acid model of HD.

Supported by NS 16367. Dr. Iversen of Merck, Sharp and Dohme generously provided MK-801.

287.9 **STUDIES OF EFFECTS OF CHRONIC LOW-LEVEL QUINOLINIC ACID EXPOSURE IN ORGANOTYPIC CULTURES OF THE RAT CORTICOSTRIATAL SYSTEM.** W.O. Whetsell, Jr. and Robert Schwarcz. (SPON: M.D. Johnson) Vanderbilt University School of Medicine, Nashville, TN and Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD.

The endogenous amino acid, quinolinic acid (QUIN), has been shown to produce specific post-synaptic neurodegenerative changes in rat CNS tissue both in vivo and in vitro. This excitatory amino acid ("excitotoxin") has received increasing investigative attention as an endogenous agent which may produce a slowly-developing specific neurodegeneration in certain neurological disorders. For that reason, we have begun to examine the effects of chronic administration of low levels of QUIN in CNS tissues which have been shown to undergo acute neurodegenerative changes after exposure to relatively high levels of QUIN. Organotypic cultures of rat corticostriatal system, which have been of particular value in examining the QUIN effect (Whetsell and Schwarcz, J. Neural Transm., Suppl. 19:53, 1983), were used in these experiments. Cultures of rat cerebral cortex (CX), caudate nucleus (CA) or combination cortex-caudate (CXCA) cultures, all more than 21 days in vitro, were exposed to 100 nM QUIN in feeding medium for up to six weeks; control feeding medium contained 34 nM QUIN, a concentration which is similar to that seen in human CSF. QUIN concentrations were measured radioenzymatically by the method of Foster et al. (Anal. Biochem. 158:98, 1986). Sequential light microscopic evaluation of QUIN-exposed cultures showed no detectable alterations in the living state (compared to controls) until the fourth weeks of exposure. At that time, QUIN-exposed cultures exhibited some increased density; by six weeks exposure, some cultures showed scattered small vacuoles like those seen acutely after incubation with 1 mM QUIN. In several cases, cultures continued to deteriorate so that they detached from their carrying coverslips by six weeks exposure to QUIN. Ultrastructural evaluation demonstrated that there was no alteration in cultures exposed to control feeding medium for up to six weeks. CA cultures exposed to 100 nM QUIN for six weeks also showed no ultrastructural change. CX and CXCA cultures exposed to 100 nM QUIN exhibited scattered isolated post-synaptic swelling of apparently asymmetrical synapses or occasional swelling of dendrites at 15 days of QUIN incubation. By six weeks of QUIN treatment, such cultures had undergone severe degenerative changes indistinguishable from changes observed after 24 to 36 hours exposure to 1 mM QUIN. Results of this study indicate that in this tissue culture model chronic low-level QUIN can induce a slowly evolving neurodegenerative change; the change appears to be dependent upon normal corticostriatal synaptogenesis.

(Supported by USPHS grant NS20509.)

287.10 **N-METHYL-D-ASPARTATE RECEPTOR ANTAGONIST MK-801 IMPROVES OUTCOME FOLLOWING EXPERIMENTAL SPINAL CORD INJURY IN RATS.** A. I. Faden and R. P. Simon. Department of Neurology, Univ. of California, San Francisco, and Center for Neural Injury, Veterans Administration Medical Center, San Francisco, CA 94121.

It has recently been shown that a selective N-methyl-D-aspartate (NMDA) receptor blocker — 2-amino-7-phosphonoheptanoic acid (2-APH) — can protect central nervous system neurons from ischemia or hypoglycemia. However, 2-APH does not readily cross the blood-brain-barrier and therefore it has been administered by direct parenchymal injection. MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate] is a selective, non-competitive, centrally active NMDA receptor antagonist, which may require activation of NMDA receptors for its action. We examined the possibility that NMDA receptors, which are present in the spinal cord, may mediate certain of the secondary pathophysiological consequences of traumatic spinal cord injury by evaluating the effects of MK-801 on outcome following traumatic spinal cord injury in rats. Animals were anesthetized with pentobarbital (60 mg/kg, i.p.). Following laminectomy, trauma was produced at the T-10 spinal segment utilizing the Allen method, in which a 10 g weight was dropped 5 cm through a guide tube onto an impounder plate resting on exposed dura mater. Animals (n = 30) were randomly assigned to treatment with MK-801 (1 mg/kg) or equal volume saline, administered as a slow intravenous injection at 15 min posttrauma. Animals were evaluated neurologically utilizing both behavioral measures (Tarlov scale) and angle board performance by an individual unaware of treatment group. Rats treated with MK-801 showed significantly improved neurological recovery at 4 weeks posttrauma as evidenced by both motor scores ($p < 0.05$) and angle board scores ($p < 0.01$). Eight of fifteen MK-801-treated animals regained walking ability as compared to three of fifteen controls. These results are consistent with the hypothesis that excitatory amino acids, through actions at NMDA receptors, contribute to the pathophysiology of traumatic spinal cord injury. The data suggest that selective NMDA receptor antagonists may be beneficial in the treatment of spinal cord injury.

287.11 EFFECTS OF MK801, AN NMDA RECEPTOR ANTAGONIST, ON KAINATE INDUCED SEIZURES IN RATS. G.G. Smith*, G.T. Golden, P.F. Reyes*, R.G. Fariello (SPON: C.P. Bianchi). Research & Neurology, VAMC, Coatesville, PA and Neurology & Pharmacology, Thomas Jefferson University, Philadelphia, PA

The systemic administration of kainic acid (KA), a rigid glutamate analog, induces seizures and neuronal damage through several mechanisms some of which involve activation of the NMDA receptor. Therefore we have studied the effects of MK801 pretreatment on the limbic status epilepticus induced by KA in rats. Male Wistar/Furth albino rats had electrodes implanted under anesthesia in the hippocampus and cortical leads for chronic EEG recordings. Ten days after recovery various groups (n=10) received MK801, up to 10 mg/kg alone, KA (10 mg/kg ip) alone or 30 min after 0.1, 1 and 10 mg/kg MK801. The behavior and EEG were monitored for the subsequent 250 min. The number of subclinical (only EEG) and electroclinical seizures, number of wet dog shake (WDS) episodes, the time of onset of seizures and the total percent of time spent in ictal episodes were recorded and compared in the various groups. MK801 alone did not cause seizures but at 1 and 10 mg/kg reduced and abolished, respectively, all spontaneous motor activity. MK801 potentiated, in a dose dependent manner, KA EEG epileptogenicity. Behavioral seizures were increased only in the 0.1 mg/kg group but were abolished in the 1 and 10 mg groups. WDS were reduced in a dose dependent fashion by MK801. Rats in the MK801 groups spent more time and entered earlier in EEG seizure activity. The electrophysiological results will be compared to histological data assessing presence and severity of neuronal damage in the various experimental groups. (Supported by Veterans Administration.)

287.12 THE EFFECTS OF N-METHYL-D-ASPARTATE (NMDA) RECEPTOR BLOCKADE UPON LOCAL CEREBRAL GLUCOSE UTILISATION AND LOCAL CEREBRAL BLOOD FLOW. D.G. Nehls*, C. Park*, and J. McCulloch. (SPON: S.E. Blackshaw). Wellcome Surgical Institute, University of Glasgow, Glasgow, G61 1QH, Scotland.

N-methyl-D-aspartate (NMDA) receptors are involved in excitatory (glutamate) neurotransmission and have been implicated in a wide range of neurological diseases including cerebral ischemic damage and chronic degenerative disorders (Meldrum, B., Clinical Science, 68:113, 1985). MK-801 is a potent NMDA antagonist which readily crosses the blood-brain barrier (Wong, E.H.F. , et al., Proc. Natl. Acad. Sci. USA, 83:7104, 1986). We have examined the functional consequences of NMDA receptor blockade using deoxyglucose autoradiography, and have also studied the concurrent alterations of local cerebral blood flow.

The effects of NMDA blockade upon local cerebral glucose utilisation (LCGU) and local cerebral blood flow (LCBF) were examined in 41 discrete neuroanatomic loci. After insertion of femoral vessel cannulae, Sprague-Dawley rats were allowed to recover from anesthesia for two hours, and received either saline or MK-801 (0.5 mg/kg i.v.) prior to determination of LCBF of LCGU by quantitative autoradiography.

Glucose Use. MK-801 administration produced significant increases in LCGU in limbic circuits. This was especially marked in the posterior entorhinal cortex (+137%) and posterior cingulate cortex (+62%), and in the hippocampus. There was a significant depression of cortical metabolism in the auditory, sensory-motor and frontal cortices (-34%). LCGU remained relatively constant in the thalamus, with the exception of the anterior thalamic nucleus (+63%). LCGU in the cerebellum and brainstem was minimally altered with MK-801.

Cerebral blood flow. In the majority of regions, LCBF was not significantly altered. However, it was markedly and significantly increased in the limbic system and in the visual cortex, mediodorsal thalamus, caudate nucleus, dorsal septal nucleus and nucleus accumbens. LCBF was significantly lowered in the cerebellum.

There was evidence of LCBF:LCGU uncoupling as increases in LCGU occurrerd in specific areas (superficial layers of the cortex and the molecular layer of the hippocampus), whereas LCBF was increased diffusely throughout the posterior cortex and hippocampus.

NMDA receptor blockade has a profound effect upon LCBF and LCGU throughout the brain, but especially in the limbic system where NMDA receptors are plentiful. Such large and widespread effects on metabolism and blood flow will be of importance as NMDA antagonists are used with increasing frequency for the treatment of neurologic disorders.

288.1 **STUDIES ON DESCENDING PATHWAYS MEDIATING PRESSOR RESPONSES PRODUCED BY CENTRAL ADMINISTRATION OF ANGIOTENSIN II (ANG II) HYPERTONIC SODIUM CHLORIDE (HS) AND CARBACHOL (CARB).** H. Ohta and M. J. Brody. (SPON. R.L. Dundore). Dept. of Pharmacol., Cardiovasc. Ctr., Univ. of Iowa, College of Medicine, Iowa City, IA 52242.

The pressor response produced by cerebroventricular (i.c.v.) administration of ANG II involves midline structures along the lamina terminalis. Receptor regions in the circumventricular organs send projections to the median preoptic nucleus which in turn is the source of two descending pathways. Our previous studies demonstrated that the pathway descending medially through anterior hypothalamus, ventromedial hypothalamus and periaqueductal gray is necessary for the central pressor actions produced by ANG II. The descending pathways necessary for the pressor actions of HS and CARB have not been determined. The purpose of the present study was to determine whether the medial projections or lateral projections descending through medial forebrain bundle (MFB) region are involved differentially in the pressor responses to the 3 agents. Studies were conducted on conscious unrestrained rats instrumented for continuous recording of arterial pressure (AP) and heart rate (HR). The agents were administered through an i.c.v. cannula. Microinjections of lidocaine (LIDO) (200 nl, 2%) were used to reversibly interrupt neuronal transmission. The pressor responses produced by ANG II (100 ng) 1 M HS (5 µl) and CARB (50 ng) were reduced by the injection of LIDO bilaterally in the region of paraventricular nucleus (PVN). Responses to ANG II and HS were affected to a greater extent than those produced by CARB. Little effect was produced by single injection of LIDO into the midline region just above the PVN. The responses to the 3 pressor agents were not affected on the average by LIDO injected bilaterally into lateral aspects of MFB, however of 8 rats tested, no effect was produced by MFB injection of LIDO in 5 rats whereas the responses to HS were reversibly attenuated in 3 rats without any change in response to ANG II or CARB. LIDO injected into the medial structures had no effect on baseline HR or AP however in contrast the bilateral injections into lateral MFB produced a marked increase in HR (86 bpm) and a lesser increase in AP (14 mmHg). These results suggest: 1) pressor responses produced by 3 different agents appear to depend upon pathways descending medially in the region of the third ventricle; 2) the effects of interrupting these midline descending projections appear to be partially differential in that responses to CARB are affected less than those of ANG II and HS; 3) lateral projections through MFB play a less significant role in mediating the responses to the pressor agents; 4) projections through lateral MFB appear to be involved in tonic inhibition of primarily HR and secondarily AP since their neuronal interruption in the conscious state produces marked changes in these cardiovascular parameters. (Supp. by HLB-14388.)

288.2 ALTERNATIVE MECHANISM FOR ATTENUATED ANGIOTENSIN II AND NOREPINEPHRINE PRESSOR RESPONSES IN AV3V LESIONED DOGS. A.F. Tramposch, O.U. Lopes,* C.M. Ferrario, C.L. Chernicky. The Research Institute, The Cleveland Clinic Foundation, Cleveland Ohio 44106.

To determine the role of preoptic hypothalamic structures in the pressor response to intravenous (IV) infusion of angiotensin II (Ang II), the anteroventral third ventricle (AV3V) region was lesioned in 11 dogs using a bayonet shaped microknife (Marson et al. Hypertension 7: I-80, 1985). The lesion denervated the organum vasculosum of the lamina terminalis, nucleus medianus and the medial preoptic nucleus. 2-3 days after denervation dogs displayed adipsia associated with hypernatremia (175 ± 2 mEq/L) and increases in osmolality (352 ± 5 mOsm/kg) and angiotensin II immunoreactivity (Ang II-ir) (124 ± 26 pg/ml). In contrast, plasma levels of arginine vasopressin (AVP) were reduced from (4 ± 2 pg/ml to 0.1 ± 0.1 pg/ml). AV3V lesion did not produce any significant change in baseline mean arterial pressure (MAP) (99 ± 4 mmH Hg) whereas heart rate increased (131 ± 8 beats/min). These changes were accompanied by a significant ($p < 0.05$) decrease in the peak change in MAP produced by IV Ang II infusions at rates of 4 and 20 ng/kg/min. The slope of the Ang II dose response curve decreased from 1.4 to 0.6 (mm Hg/(ng/kg/min)). Four lesioned dogs in which vascular reactivity was tested showed significant ($p < 0.05$) decreases in the pressor responses to IV norepinephrine (NE) at doses of 5, 10, and 20 µg. The slope of the NE dose response curve decreased from 3.1 to 1.8 (mm Hg/µg). Nine AV3V lesioned dogs treated with desmopressin acetate (DDAVP) for 1-3 days, normalized plasma sodium levels and Ang II-ir in conjunction with a restoration of the pressor responsiveness to IV Ang II. Pressor responsiveness to NE remained depressed after teatment with DDAVP. These data suggest that a decrease in vascular reactivity associated with increases in plasma sodium and Ang II-ir may be a cause of the attenuated pressor response to IV infusion of Ang II and NE in AV3V lesioned dogs. These data raises a question about the interpretation of previous studies which suggested an exclusive, direct, central effect of Ang II on AV3V structures. (Supported in part by NIH Grant HL-6835).

288.3 GLUTAMATE-SENSITIVE NEURONS IN CIRCUMVENTRICULAR ORGANS SUPPORT HYPERTENSION IN SPONTANEOUSLY HYPERTENSIVE RAT (SHR). D.K. Hartle. Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322

High concentrations of L-glutamate (G) produce receptor-mediated neurotoxicity thought to be subsequent to unregulated influx of Ca++ during sustained excitation of ion-channel linked G receptors. These exist within the CNS, but not in the peripheral autonomic nervous system. G can be administered s.c. to achieve plasma levels high enough to kill G-sensitive neurons in regions of the brain not protected by the blood-brain-barrier (the circumventricular organs, CVOs). Of these, only area postrema and the subfornical organ sustain a significant number of cell deaths after 9mg/g G, s.c., but not with control injections of hypertonic saline. Parenteral G treatment (9mg/g, s.c.) produced a permanent lowering of BP in the SHR, but did not lower BP in normotensive rats. The depressor effect was accounted for by a reduction in total peripheral resistance. G treatment in the adolescent SHR prevented the expression of hypertension in the adult period. A non-specific G antagonist, GDEE (glutamate diethyl ester), was found to protect the SHR from the BP lowering effect of G toxcity when it was infused at the rate of 3.3 mg/min for 30 min, starting 10 min prior to the s.c. G injection. GDEE infused alone produced significant bradycardia (-65 BPM) in normotensive rats that was rapidly reversible by muscarinic blockade with atropine. Bradycardia was reversed totally within 1 hr after discontinuation of the GDEE infusion. Bilateral cervical vagotomy blocked bradycardia during GDEE infusions, indicating a probable central site of GDEE action. Because GDEE produces tachycardia when injected behind blood brain barrier in certain brainstem cardiovascular regions, these negative chronotropic effects of GDEE may be via modulation of neurons outside the blood-brain barrier. Both CVOs damaged by G treatments obviously contain G receptors. GDEE may protect blood pressure in the SHR and produce reversible bradycardia in the normotensive rat by occupying some of these CVO receptors. These experiments also suggest that G receptors exist on CVO neurons that are required to support SHR hypertension. EURF,AHA-GA Affil, and NIH-HLBI 1 RO1 HL37705-01.

288.4 CARDIOVASCULAR EFFECTS OF ELECTRICAL AND CHEMICAL STIMULATION OF THE AMYGDALA OF ANESTHETIZED AND CONSCIOUS RATS. A.J. Gelsema*, D.J. McKitrick*, and F.R. Calaresu. Dept. of Physiology, University of Western Ontario, London (Ont), Canada N6A 5C1.

It has been shown in anesthetized rats that electrical stimulation of sites located in the central, medial and basolateral nuclei of the amygdala results in changes in arterial pressure (AP) and heart rate (HR). To dissociate the effects of electrical stimulation on neuronal cell bodies and fibers, in 18 artificially ventilated, paralyzed, urethane anesthetized rats we compared the effect on AP and HR of electrical stimulation (90-150 µA) of 92 histologically verified sites in and around these 3 nuclei with the effect of microinjections of DL-homocysteate (DLH, 0.15 M, 50-100 nl) into the same sites. Electrical stimulation resulted in depressor responses in most sites (89%), whereas chemical stimulation produced significantly fewer (25%) depressor responses. Changes in AP were accompanied by variable changes in HR. In an additional series of 17 rats comparison of the responses to electrical stimulation and injections of a more concentrated solution of DLH (1.0 M) into 70 amygdaloid sites showed that electrical stimulation elicited depressor responses in 83% of the sites and chemical stimulation resulted in depressor responses in only 16% of the sites. Finally, to study the effect of chemical and electrical stimulation without the influence of anesthetics, 9 rats were instrumented for electrical and chemical stimulation and recording of AP and HR in the conscious state. Electrical stimulation (50-250 µA) in the area of the central and basolateral nuclei in these rats resulted in an increase in AP in 6, in a decrease in 2 and in a biphasic response in one rat. Restlessness and exploratory behavior accompanied these cardiovascular responses. Injections of 50 to 200 nl amounts of a 1.0 M solution of DLH into the same sites never elicited behavioral or cardiovascular changes. These results suggest that either the cardiovascular effects of electrical stimulation in the amygdala are mainly due to activation of fibers or that chemical and electrical stimulation activate different neuronal elements in the same anatomical site.

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288.5 CHANGES IN REGIONAL HEXOKINASE ACTIVITY IN THE BRAIN OF DOCA-SALT HYPERTENSIVE RATS. W.E. Turton* and J. Ciriello. (SPON: J.A. Kiernan). Department of Physiology, University of Western Ontario, London, Ontario, Canada N6A 5C1.

It has been demonstrated that treatment of unilaterally nephrectomized (Nx) rats with deoxycorticosterone acetate (DOCA) results in elevated arterial pressure (AP). While the role of the kidney in this experimental model of hypertension has been much discussed, little is known about the associations of the brain with the hypertensive process. In the present study, those regions of the brain whose metabolic activity was altered after DOCA-salt treatment were functionally mapped using hexokinase (HK) histochemistry, which reflects the rate of glucose utilization in the brain. After recording AP (tail cuff) and heart rate (HR) for a control period of 4 days, rats were either Nx unilaterally or sham-Nx. After recording AP and HR for 10 days, the Nx rats were then subjected to daily injections of either 2.5 mg DOCA suspended in 0.2 ml peanut oil or 0.2 ml peanut oil. All animals were allowed to drink 1% saline ad libitum. AP and HR were recorded for an additional 18 days, at which point the rats were anesthetized with sodium pentobarbital and perfused transcardially with saline. The brains were removed and quickly frozen, sectioned (20μm) on a cryostat and mounted on glass slides. Transverse sections extending from the rostral aspect of the septum to the spinomedullary junction were processed for HK histochemistry as described previously (Acta.Histochem.28:286,1967). Changes in HK activity were assessed by densitometric measurements of areas containing HK reaction product. The AP of DOCA-salt treated rats was significantly elevated compared to the oil injected and sham-Nx control animals (146 ± 2 mmHg, 119 ± 4 mmHg and 114 ± 3 mmHg, respectively), while HR was significantly reduced in these animals compared to the oil injected and sham-Nx control groups (375 ± 11 bpm, 432 ± 8 bpm and 419 ± 7 bpm, respectively). Significant increases in HK activity were observed in the median preoptic nucleus, bed nucleus of the stria terminalis, subfornical organ, magnocellular and parvocellular components of the paraventricular nucleus of the hypothalamus, supraoptic nucleus, nucleus ambiguus, rostral (C_1 region) and caudal (A_1 region) of the ventrolateral medulla, intermediate portion of the nucleus of the solitary tract and dorsal motor nucleus of the vagus, and caudal dorsal vagal complex (A_2 region) of the DOCA- salt treated animals compared to both groups of control animals. These data have shown that DOCA-salt treatment alters the activity of central structures previously implicated in the regulation of AP and body fluid balance and suggest that these structures are associated with the hypertensive process.

(Supported by the Heart and Stroke Foundation of Ontario)

288.6 INVOLVEMENT OF CENTRAL NERVOUS SYSTEM OXYTOCIN (OX), VASOPRESSIN (VP), OR ANGIOTENSION II (AII) SYSTEMS IN THE CARDIOVASCULAR RESPONSES TO ACUTE STRESS IN RATS. M.F. Callahan, S.L. Eskridge*, and K.A. Gruber*. Wake Forest University Medical Center, Winston-Salem, NC 27103.

Lesions of the anteroventral third ventricle (AV3V) region of the forebrain disrupt the tachycardia response to footshock stress. We have shown that a similar effect is produced when the V_1 vasopressin receptor antagonist ($(CH_2)_5$Tyr(ME)AVP) is administered into the fourth ventricle of the hindbrain, suggesting that central Vp systems participate in the sympathetic nervous system response to stress. The current studies explored the role of central peptidergic control of the cardiovascular responses by utilizing animals with lesions of the paraventricular nucleus (PVN), animals genetically deficient in Vp synthesis, i.e., Brattleboro rats, and antagonism of central AII receptors.

Male Sprague-Dawley (S-D) rats received anodal electrolytic lesions (1.0 mA, 20 sec) of the PVN. Two weeks later, the rats received a right carotid artery catheter. One day later, mean arterial prsesure (MAP) and heart rate (HR) were determined in the home cage and upon transfer, immediately and 5 min post-footshock. The stress consisted of 5 min of intermittent footshock (1.0 mA, 0.5 sec every 5 sec). Brattleboro and Long-Evans control rats received chronic arterial and venous catheters and were maintained in metabolic cages. Approximately 2 weeks after surgery, these animals were also subjected to the stress protocol. An additional group of male S-D rats received a lateral cerebral ventricle cannulae followed one week later by carotid artery catheterization. Approximately 2 weeks later, these rats were subjected to 2 stress sessions, one following an i.c.v. infusion of balanced Earle's solution, and one following i.c.v. [Sar^{1}Ile^8]-Angiotensin II infusion (SarIle, 20 ug/18 ul/10 min).

Lesion of the PVN had no effect on baseline CV values, but attenuated the immediate post-footshock tachycardia (HR +74±25 in controls and +20±12 bpm in rats with the PVN destroyed bilaterally). A group of animals with less than 50% of the PVN destroyed showed a tachycardic response (93±6bpm). In the second study, Brattleboro rats and Long-Evans controls showed similar increases in HR (130±13 vs 147±12 bpm, respectively). Finally, infusion of SarIle had no effect on either the resting CV parameters or the stress response. These results suggest that while the PVN plays a role in CV stress responses, neither central Vp nor AII systems are involved. Since previous studies showed that lesions of the AV3V region interfere not only with VP but also Ox secretion, that the Vp antagonist also blocks Ox receptors, and that Ox cell bodies are also located in the PVN, the current studies would suggest that the central Ox system may be prepotent in mediating the CV responses to stress. (Supported by NIH HL35112.)

288.7 MU-OPIOID RECEPTORS, THYROTROPIN RELEASING HORMONE AND GLUTAMATE IN THE PREOPTIC AREA MODULATE DISTINCT HEMODYNAMIC FUNCTIONS IN THE RAT.

A-L Sirén and G. Feuerstein, Department of Neurology, USUHS, Bethesda, Maryland

Electrical stimulation of the median preoptic nucleus (POM) of the rat is known to induce a distinct cardiovascular response ("defense reaction") which features increases in mean arterial pressure, cardiac output and heart rate as well as hindquarter skeletal muscle vasodilation and renal and gastrointestinal vasoconstriction. The present studies aimed to investigate the role of thyrotropin releasing hormone (TRH) and the mu-opioid agonist [D-Ala^2,Me-Phe^4, Gly-ol^5] Enkephalin (DAGO) in mediating these responses. TRH and DAGO were microinjected into the POM of both conscious and anesthetized rats while monitoring selected organ blood flow and efferent sympathetic nerve activity. In conscious rats (n = 20) prepared chronically with Doppler flow probes around the lower abdominal aorta (mainly hindquarter skeletal muscle blood flow), renal (R) and mesenteric (M) arteries TRH microinjected into the POM (2.4-24 nmol/500nl) increased blood pressure, heart rate and hindquarter (HQ) vascular resistance (VR). The RBF and MBF significantly decreased (M > R) due to an increase in RVR and MVR respectively. The maximum changes in hemodynamic variables were reached within 1 min after the TRH injections and the effects subsided within 5 min. DAGO (0.1 -10nmol/500nl) in the POM induced pressor and bradycardiac/tachycardiac effects which were accompanied with HQ vasodilation and M and R vasoconstriction. The effects of DAGO, however, became apparent 2-5 min after the injection and subsided within 30 - 60 min. Injections of glutamate (0.1 - 100nmol/500nl) induced variable effects on the hemodynamic variables. In some rats a slight hypotensive effect with no significant changes in regional BF were observed, while in some rats pressor, HQ vasodilator and M and R vasoconstrictor responses followed glutamate injections. However, high doses of glutamate (100nmol/500nl) were always required to induce the similar pattern of hemodynamic changes than with picomole doses of TRH. In anesthetized rats (n = 12) TRH and DAGO also markedly increased renal and splanchnic sympathetic nerve activity. In summary, microinjections of TRH or DAGO into the POM increased blood pressure, induced HQ vasodilation and R and M vasoconstriction in the conscious rat. In anesthetized rats an increase in efferent sympathetic nerve activity was found after placement of these peptides into POM. Though the TRH and mu-opioid connections from the POM to other cardiovascular brain nuclei and to spinal cord remain to be evaluated, the present results might suggest that both TRH and mu-opioid receptors have a role in the hypothalamic regulation of peripheral organ flow by modulating the sympathetic outflow.

288.8 LESIONS OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS ALTERS THE DEVELOPMENT OF TWO-KIDNEY, ONE-CLIP RENAL HYPERTENSION IN THE RAT. T.X. Zhang* and J. Ciriello. (SPON: D.L. Jones). Dept. of Physiology, University of Western Ontario, London, Canada N6A 5C1.

We have previously shown that the paraventricular nucleus of the hypothalamus (PVH) is involved in the development and maintenance of neurogenic hypertension resulting from aortic baroreceptor denervation and in the development of spontaneous hypertension in the rat. In the present study the contribution of PVH neurons to the development of the elevated arterial pressure (AP) in the renin-dependent two-kidney, one-clip (2K1C) model of renal hypertension was investigated in the rat. After recording AP (tail cuff) for a control period of one week the rats were randomly assigned to two groups: one group was subjected to bilateral electrolytic lesions of the PVH region and the other to sham-PVH lesions. Animals in these two groups were later randomly assigned to two additional groups: in one group a 0.2 mm diameter clip was placed on the left renal artery and in the other group the artery was exposed but a clip was not placed on it. PVH lesions did not alter significantly AP from control levels. On the other hand, AP in the sham-PVH lesioned-2K1C rats was significantly elevated (154 ± 5 and 166 ± 6 mmHg at 7 and 14 days after placement of the renal artery clip, respectively) compared to the other three groups. In addition, AP was significantly elevated (136 ± 4 mmHg) in the PVH lesioned-2K1C animals on day 14 compared to sham-PVH lesioned sham-clip (125 ± 3 mmHg) and PVH lesioned-sham-clip (118 ± 5 mmHg) animals, but not on day 7 after the placement of the renal artery clip. These data demonstrate that PVH neurons are involved in the development and full expression of the elevated AP in the 2K1C model of renal hypertension and that these changes in AP are dependent on the integrity of this hypothalamic structure.

(Supported by the Heart and Stroke Foundation of Ontario)

288.9 CONTRIBUTION OF NEURONS IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS TO THE PRESSOR RESPONSE ELICITED BY ACTIVATION OF AFFERENT RENAL NERVES. J. Ciriello and M.M. Caverson. Department of Physiology, University of Western Ontario, London, Canada N6A 5C1.

Afferent renal nerves (ARN) are known to contain sensory fibers that carry information from mechanoreceptors and chemoreceptors located in the kidney to the central nervous system. Recently, activation of ARN has been shown to: 1) alter the firing frequency of neurons in the paraventricular nucleus (PVH), 2) elicit a pressor response which is abolished by ganglionic blockade and administration of a vasopressin (AVP) antagonist, and 3) result in an increase in circulating levels of plasma AVP. These data suggest that ARN may influence arterial pressure (AP) by altering the discharge of AVP magnocellular neurosecretory neurons in the PVH. Therefore, two series of experiments were done to determine the contribution of PVH magnocellular neurons to the pressor response elicited by stimulation of ARN. In the first series, electrical stimulation (10 s train, 2 ms pulse duration, 40 Hz, 500 μA) of ARN in chloralose anesthetized, paralyzed and artificially ventilated, sinoaortic and vagal denervated cats elicited a pressor response that consisted of two components: a primary (1°) component (+45.6 ± 8.4 mmHg) that was time-locked to the stimulus and a secondary (2°) component (+43.4 ± 11 mmHg) that outlasted the 10 s stimulation period by 2-3 min. Reversible blockade of PVH neurons induced by bilateral injections of procaine hydrochloride or bilateral electrolytic lesions of the PVH resulted in a 43% and 68% attenuation of the 1° and 2° components, respectively, of the ARN pressor response. On the other hand, injections of 0.9% saline into the PVH or electrolytic lesions of hypothalamic regions anterior, dorsal or ventral to the PVH did not alter the ARN pressor response.

In the second series, following transpharyngeal exposure of the ventral surface of the hypothalamus and hypophysis, extracellular recordings were obtained from 86 single units in the PVH identified by antidromic invasion from the neurohypophysis in chloralose anesthetized, paralyzed and artificially ventilated cats. Of the 86 antidromic units, 17 (20%) were excited by stimulation of ARN. The latencies of the orthodromic response to stimulation of ARN ranged from 70 to 325 ms. The anatomical location of these responsive cells overlapped with that of AVP immunoreactive cells in the PVH. These data suggest that PVH neurons are components of long-loop reflex pathways involved in the integration of afferent inputs from the kidney and which function in the control of AP and AVP release.

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288.10 CARDIOVASCULAR REGULATION IN THE EARLY PHASE OF STRESS-INDUCED HYPERTENSION IN THE BORDERLINE HYPERTENSIVE RAT (BHR). B.J. Sanders, S. Knardahl*, and A.K. Johnson. Departments of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

The BHR is the first generation offspring of WKY x SHR and has been shown to become hypertensive when chronically exposed to a shock-shock conflict paradigm (Lawler et al. Hypertension, 3:406-505, 1981). Analysis of the time course of development of this hypertension reveals at least three distinct stages. During the early phase blood pressure has not begun to rise, the middle phase is characterized by a significant increase in pressure, and the full expression of permanent hypertension is observed in the late phase. The underlying mechanisms involved in each of these stages remain unclear. The purpose of the present study, therefore, was to compare cardiovascular responses to ganglionic blockade and central administration of hypertonic cerebrospinal fluid (CSF) in BHR during the early phase of stress-induced hypertension with age matched control animals. After 3 weeks of exposure to the conflict paradigm animals were instrumented with femoral arterial catheters and lateral ventricular cannulae. Following recovery, mean arterial pressure (MAP) and heart rate (HR) were measured in their home cages and plasma samples were taken for norepinephrine (NE) and epinephrine (E) analysis. Also, depressor responses to hexemethonium (30 mg/kg, i.a.) and pressor responses to 4 ul injections of hypertonic CSF (.64, 1.0, 1.9 uEq/L) were determined. The data reveal that after 3 weeks of conflict stress there were no differences between stressed and control animals with respect to MAP (133 vs 134 mmHg), HR (327 vs 350 bpm), NE (253 vs 215 pg/ml), and E (244 vs 201 pg/ml). However, there was a significantly ($p < .05$) greater fall in MAP to ganglionic blockade in stressed animals compared with controls (-56 vs -39 mmHg). Finally, although there were significant elevations in MAP to all doses of hypertonic CSF, stressed and control BHR responded similarly to each dose. These data suggest that during the early phase of stress-induced hypertension, when blood pressure has not yet increased, the sympathetic nervous system contribution to vascular tone is exaggerated in stressed BHR compared to controls.

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288.11 BETA-2 ADRENOCEPTORS INFLUENCE THE CARDIOVASCULAR RESPONSES TO AN ACUTE STRESSOR IN THE BORDERLINE HYPERTENSIVE RAT. R. F. Kirby, R. Fisher, C. H. Woodworth, and A. K. Johnson, Depts. of Psychology and Pharmacology and the Cardiovascular Center, University of Iowa.

We have previously demonstrated that rats of varying genetic predispositions to develop hypertension have minimal increases in blood pressure immediately following the cessation of an acute footshock episode that produces large increases in heart rate and plasma catecholamines. The ability to maintain pressure near resting levels following footshock stress appears to depend upon specific changes in regional blood flow; including a vasodilation in the hindlimb vasculature. Evidence from many studies indicates that this vasodilation may be mediated by adrenal epinephrine acting on beta-2 adrenoceptors located in the hindlinb vasculature. In the present study, the cardiovascular responses to transfer to a novel environment and intermittent footshock stress were compared following beta-2 antagonism or vehicle treatment in Wistar-Kyoto (WKY) or borderline hypertensive rats (BHR). All animals were raised in our laboratory and BHR were the F1 generation offspring of WKY females and spontaneously hypertensive males. At 20 weeks of age, catheters were implanted into the right common carotid artery for determination of mean arterial pressure (MAP, mmHg) and heart rate (HR, beats/min) and the administration of drugs. Resting home cage HR was comparable between WKY and BHR, while MAP was elevated in BHR. The increase above resting levels following transfer and footshock for WKY and BHR animals are presented below.

	Transfer		0' Post FS		5' Post FS	
	HR	MAP	HR	MAP	HR	MAP
WKY-Veh	56±17	8±4	99±21	13±3	43±11	3±3
WKY-ICI	34±17	17±4	91±12	15±3	51±8	7±4
BHR-Veh	120±10	12±4	217±7	15±6	157±12	13±4
BHR-ICI	6±11	37±5	78±13	26±2	56±13	24±3

data are means ± SEM; n= 6 to 12 animals per group.

The HR increase in vehicle-pretreated BHR was greater than that of the WKY following stress. However, MAP responses were fairly comparable. Pretreatment with the selective beta-2 antagonist ICI118.551 (1mg/kg i.a.) attenuated the HR increase and exaggerated the MAP response to the stressors in the BHR but did not affect the WKY response. In the present findings, it is unknown to what extent the greater MAP response of the BHR-ICI animals influenced the HR response. These data indicate that beta-2 adrenoceptors may help to offset a large pressor response to stress in BHR and point to the need for examining regional blood flow changes of animals susceptible to the development of hypertension in response to environmental challenges.

288.12 EFFECTS OF BASAL FOREBRAIN LESIONS AND CHOLINOMIMETICS ON CEREBRAL CORTICAL MICROVASCULAR PERFUSION (CCMP) IN RAT: CONTINUOUS MEASUREMENT BY LASER-DOPPLER FLOWMETRY. A.M. May* and S.P. Arnerić (SPON: Wm. H. Cline, Jr.), Department of Pharmacology, Southern IL University School of Medicine, Springfield, IL 62708

Alzheimer's disease (AD) is characterized by decreased cholinergic innervation of the cerebral cortex and decreased cerebral blood flow (CBF) (J. Cereb. Blood Flow Metab. 5:S131-S144, 1985). Since acetylcholine dilates blood vessels in brain and, thus, would increase CBF, it is possible that the decrease in CBF occurring with AD is the result of a decreased cortical cholinergic innervation arising from the basal forebrain (BF). This study sought to determine in rat whether: 1) ibotenic acid (IBO) lesions of the BF, a procedure that decreases the cortical cholinergic innervation, mimics the reductions in CCMP found in AD; and 2) physostigmine (PHYSO), a cholinomimetic which effectively improves the memory defects of AD, may act to locally increase CCMP. IBO (5 μg/0.5 μl) was microinjected unilaterally into the BF of male rats under halothane anesthesia and vehicle contralaterally. Twenty-one days later rats were evaluated for concurrent changes in CCMP, biochemical alteration in cortical enzymes and histological confirmation of the lesion site. Rats were anesthetized (chloralose), paralyzed, artificially ventilated and arterial blood gasses controlled. A craniotomy was performed over the parietal cortex (PCx) and occipital cortex (OCx). CCMP was measured continuously with laser-doppler flowmetry. Drugs were delivered intravenously (i.v.) or microinjected intracortically (ict.) through a glass micropipette (70 μm, o.d.; 100-400 nl) positioned 0.7 mm beneath the surface probe (0.8 mm, o.d.). In unoperated rats resting CCMP did not differ between right and left sides ($p > 0.05$; N=7). In contrast, IBO lesions of the BF decreased CCMP in the PCx by 26±7% ($p < 0.05$; N =6) without affecting CCMP in contralateral or adjacent OCx. Choline acetyltransferase activity was correspondingly decreased within the lesioned PCx by 55±5%; ($p < 0.05$, N=6), while intrinsic cortical neurons containing glutamic acid decarboxylase were unaffected. Dose-dependent (0.2-0.8 nmol, ict.; N=3-8; $p < 0.05$) increases in CCMP occurred with PHYSO (up to +148%), carbachol (+232%) and papaverine (+325%); vehicle was without effect (+12%); arterial pressure was unchanged. Similarly, CCMP was maximally increased (+181%) 3 min after i.v. PHYSO (0.154 μmol/ kg), remaining elevated 10-15 min. Atropine (0.8 nmol, ict.) prevented the increase in CCMP by i.v. PHYSO without affecting the accompanying hypertension. Conclusions: 1) IBO lesions of the BF selectively decrease CCMP and cortical cholinergic innervation similar to that reported in patients with AD; 2) Local and systemic administration of cholinomimetics increase CCMP via a local muscarinic cholinergic mechanism which operates independent of changes in arterial pressure or dilation of large resistance vessels (Supported by SIU Sch. of Med.).

289.1 EFFECTS OF IRON DEFICIENCY AND BRAIN LESIONS ON THE MOTOR ACTIVITY AND BODY T° CIRCADIAN CYCLES IN RATS. S. Yehuda and M. B. H. Youdim*. Dept. of Psychol., Bar-Ilan Univ., Ramat-Gan, and Rappaport Fam. Inst. Med. Sci., Technion, Haifa, Israel.

Iron deficiency is the most prevalent nutritional disorder in man. Rats made nutritionally iron-deficient (ID) have significantly lower brain iron (40-60%) and dopamine D2 receptor number (Bmax) (50%) (Ben Shachar et al., J. Neurochem., 45:999, 1975). On the behavioral level, the ID rats exhibit a clear deficit in learning (Yehuda et al., Pharmacol. Biochem. Behav., 25:141-144, 1986), and reversal of motor activity and body temperature circadian cycles (Youdim et al., Eur. J. Pharmacol., 74:295-301, 1981).

The effects of selective brain lesions on the circadian cycles of motor activity and body T° were studied in intact, pinealectomized, hypophysectomized, and area postrema-lesioned rats.

	No lesion		Pinealec-tomized		Hypophysec-tomized		Area Postrema X	
Diet	Control	ID	Control	ID	Control	ID	Control	ID
Colonic T°								
Peak	24:00	09:00	03:00	09:00	24:00	24:00	24:00	12:00
Trough	12:00	03:00	12:00	24:00	12:00	12:00	09:00	03:00
Motor Activity								
Peak	03:00	09:00	03:00	03:00	03:00	09:00	24:00	09:00
Trough	09:00	03:00	09:00	09:00	09:00	03:00	06:00	24:00

The results showed that among ID intact rats both circadian cycles were reversed. ID pinealectomized rats showed body T° reversed cycle but not motor activity, while ID hypophysectomized rats behaved in an opposite manner; their motor activity cycle (but not the body T° cycle) was reversed. Area postrema-lesioned rats exhibited shifted phase cycles, and ID AP-lesioned rats also exhibited shifted phase reversal cycles. These results may explain the neuropharmacological basis of ID effects.

289.2 **OLFACTORY BULBECTOMY MODEL OF DEPRESSION: EJACULATORY AND CIRCADIAN DYSFUNCTION AND THEIR RESPONSE TO ANTIDEPRESSANT TREATMENT** A.R. LUMIA*[1], F.J. SALCHLI*[1], E.L. AYERS*[1], M.Y. McGINNIS*[2], B.S. McEWEN[3], M.H. TEICHER*[4] (SPON: J.W. GERST)

1: Dept. of Psychology, Skidmore College, Saratoga Springs, NY, 12866. 2:Mount Sinai Medical School, CUNY, New York, NY, 10021. 3: The Rockefeller University, New York, NY, 10021. 4:Mailman Research Center, McLean Hospital, Belmont, MA, 02178.

The bilateral olfactory bulbectomized rat (**OBX**)has been proposed as an animal model of depression and as a screen for antidepressant drugs(Jesberger,et. al., Behav. Neurosci. 100:2,1986).OBX abolishes sexual behavior (Lumia et.al, Brain Research, 404,1987) and disrupts activity (Jesberger,et. al., Behav. Neurosci. 100:2,1986); symptoms indicative of depression. .We hypothesized that OBX would (1) eliminate ejaculation and disrupt normal daily activity patterns, (2) that treatment with a known anti-depressant drug would normalize the **OBX** induced ejacualtory and circadian activity deficits. This study examined the effect of amitriptylene (**AMI**) (5mg./kg/BW) effectiveness in normalizing the **OBX** induced copulatory deficit and daily running wheel activity rhythms. Male Sprauge-Dawley rats were maintained individually under a 12:12 reverse light/dark cycle in standard running wheels interfaced with a real time computerized data collection system. Sexually experienced animals were either **OBX** or sham operated and tested for copulation prior to and following surgery and following either 14 days of **AMI** or control vehicle administration. All animal activity was recorded for four consecutive days from a subset of 13 animals prior to and following surgery and after treatment with **AMI** or saline. For each animal the total number of revolutions in the dark and light phase was recorded and a D/L ratio computed.

Results show that significantly fewer OBX ejaculated (**p=.02**). **AMI** increased the number of OBX males that achieved ejaculation (**OBX/saline = 40%:OBX/AMI = 77%**).In contrast, sham operates treated with **AMI** showed a temporary decreased ejaculatory potential (**p=.02**). For running wheel activity **OBX** significantly increased activity in the light phase and **AMI** normalized this abberant circadian pattern. An ANOVA indicated that surgery was significant at **p<.02** and the surgery/drug interaction at **p<.005**. Chronobiological analysis' are currently underway to further delineate the mechanisms underlying these changes in activity patterns The results of this study indicate that **OBX** may serve as a model for the vegetative symptoms of unipolar major depression. (This work was supported by grants from the FREED FOUNDATION and SKIDMORE COLLEGE to A.R.L.)

289.3 IN VIVO ELECTROPHYSIOLOGICAL EVIDENCE IN THE RAT BRAIN SUGGESTING AN INTERACTION BETWEEN IMIPRAMINE BINDING SITES AND TERMINAL 5-HT AUTORECEPTORS. P. Blier, Y. Chaput* and C. de Montigny. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Canada H3C 3J7.

Autoreceptors on 5-HT terminals modulate the release of 5-HT into the synaptic cleft. [^{3}H]Imipramine, and more specifically [^{3}H]citalopram and [^{3}H]paroxetine, bind to a site associated with the 5-HT reuptake pump on the same terminals. We have shown that long-term treatment with the antidepressant 5-HT reuptake blocker citalopram decreases the function of the terminal 5-HT autoreceptor (Naunyn-Schmiedeberg's Arch. Pharmacol., 333: 342, 1986). The present study was undertaken to determine whether increased synaptic availability of 5-HT or activation of the [^{3}H]imipramine binding sites is responsible for this modification.

Male Sprague-Dawley rats were treated with the 5-HT reuptake blocker fluoxetine (10 mg/kg/day, i.p.) for 14 days, the MAO-A inhibitor clorgyline (1 mg/kg/day, s.c.) for 21 days, or saline. Extracellular recordings were obtained from CA_3 dorsal hippocampus pyramidal neurons under chloral hydrate anesthesia. The effectiveness of the electrical stimulation of the ascending 5-HT pathway at the level of the ventromedial tegmentum in depressing the firing activity of these neurons was determined from computer-generated peristimulus time histograms. The function of the terminal 5-HT autoreceptors was assessed by comparing the effectiveness of the stimulation prior to, and immediately following, methiothepin (1 mg/kg, i.v.), a terminal 5-HT autoreceptor antagonist, and by determining the ratio (S2/S1) of effectiveness 0.8 Hz (S1) and 5 Hz (S2) stimulations.

Long-term administration of both fluoxetine and clorgyline increased the efficacy of the stimulation of the 5-HT pathway. However, the enhancing effect of the terminal 5-HT autoreceptor antagonist methiothepin was reduced in the fluoxetine but not in the clorgyline group. The reduction of the function of the terminal 5-HT autoreceptor by fluoxetine was further evidenced by the decreased ratio of effectiveness of the 0.8 and 5 Hz stimulations. To verify that the reuptake blockade per se could not account for the increased synaptic efficacy of 5-HT projections following long-term fluoxetine, the drug was administered acutely to naive rats (5 mg/kg, i.v.). It did not increase the efficacy of the stimulation of the 5-HT pathway.

Two conclusions are drawn from these results: 1) the increased efficacy of 5-HT synaptic transmission by long-term treatment with antidepressant 5-HT reuptake blockers is not directly due to 5-HT reuptake blockade, but rather to a reduced function of the terminal 5-HT autoreceptor; 2) the latter phenomenon cannot be ascribed to an increased availability of 5-HT in the synaptic cleft as it was not produced by long-term clorgyline treatment. Hence, these results suggest that the desensitization of terminal 5-HT autoreceptors might result from the long-term activation of the [^{3}H] imipramine binding sites.

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289.4 TRACAZOLATE INCREASES PUNISHED RESPONDING IN A GELLER-SEIFTER CONFLICT TEST WITH INCREMENTAL SHOCK. J.L. Howard and G.T. Pollard. Dept. of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

Standard anxiolytics such as benzodiazepines increase lever-pressing that has been suppressed by electric shock (Geller, I. and Seifter, J., Psychopharmacologia, 1:482, 1960). The atypical putative anxiolytic tracazolate has been reported not to increase punished lever-pressing significantly in rat at doses up to 40 mg/kg p.o., although it did significantly increase punished drinking in the Vogel test at 10 and 20 mg/kg p.o. (Patel, J.B. and Malick, J.B., Eur. J. Pharm., 78:323, 1982). We tested chlordiazepoxide 5-40 mg/kg p.o. and tracazolate 20-320 mg/kg p.o. in a modified Geller-Seifter procedure with incremental shock (Pollard, G.T. and Howard, J.L., Psychopharmacology, 62:117, 1979). Both compounds significantly increased punished lever-pressing; tracazolate's effect occurred clearly only at doses higher than those previously tested. We also explored tracazolate's possible mechanism of action at the benzodiazepine-GABA-chloride ionophore complex. We attempted to replicate in our procedure the finding that the benzodiazepine receptor blocker RO15-1788 had no effect on tracazolate's anti-conflict action in the punished drinking test (Patel, J.B. et al., Eur. J. Pharm., 86:295, 1983); and we tested the interactive effect of a GABA transaminase inhibitor to determine whether it would potentiate tracazolate's anti-conflict action as aminooxyacetic acid (AOAA) did in the case of diazepam (McCloskey, T.C., et al., Fed. Proc., 46:1302, 1987).

289.5 DISCRIMINATION BETWEEN NALTREXONE AND SALINE IN SQUIRREL MONKEYS RESPONDING UNDER A FIXED-RATIO SCHEDULE OF STIMULUS-SHOCK TERMINATION. C.P. France* and W.H. Morse* (SPON: J. Maggio). Laboratory of Psychobiology, Harvard Medical School, Boston, MA 02115.

The discriminative stimulus effects of naltrexone were studied in squirrel monkeys discriminating between saline and 3.2 mg/kg of naltrexone while responding under a fixed-ratio (FR) 5 schedule of stimulus-shock termination. Monkeys received an i.m. injection (saline or drug) during the first min of a 10-min timeout (TO) that preceded the session. During the session electric shocks were scheduled to be delivered every 5 sec in the presence of a stimulus light. Five consecutive lever presses on the injection-appropriate lever terminated the shock-associated stimulus for 50 sec. Failure to complete the response requirement within 5 sec resulted in the delivery of brief electric shock to the tail. Responses on the injection-inappropriate lever reset the FR requirement on the injection-appropriate lever. Test sessions were identical to training sessions except that 5 consecutive responses on either lever terminated the shock schedule. Squirrel monkeys discriminated reliably between injections of naltrexone and saline after approximately 200 training sessions. Naltrexone occasioned responding on the drug lever in a dose-related manner and monkeys generalized completely to naltrexone (i.e., >90% responding on the naltrexone lever) at doses of 1.0-3.2 mg/kg. Smaller doses of naltrexone occasioned responding predominantly on the saline lever and larger doses occasioned responding predominantly on the drug lever. Up to a dose of 10.0 mg/kg of naltrexone the latency to terminate the shock-associated stimulus was not different between drug and saline sessions. Among other drugs that were studied, only those with opioid antagonistic actions (e.g., nalorphine) substituted completely for the naltrexone discriminative stimulus. Drug discrimination with naltrexone in squirrel monkeys is of special interest because this species shows a pronounced super-sensitivity to some effects of opioid antagonists.
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289.6 MOTIVATIONAL PROPERTIES OF OPIOIDS: EVIDENCE THAT TOLERANCE DEVELOPS TO THEIR REINFORCING AND AVERSIVE EFFECTS.
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The motivational properties of opioids are well documented. μ-Receptor agonists function as positive reinforcers, producing euphoria whereas κ-agonists induce aversive motivational states. It is not at all clear, however, whether tolerance develops to either of these effects. This study employed a place preference conditioning procedure to characterize the motivational properties of μ- and κ-agonists and determine whether prior, non-contingent exposure to an opioid modifies their reinforcing and/or aversive effects. Male S/D rats received sc injections of the μ-agonist, morphine (10.0-20.0 mg/kg/day), the κ-agonist, U-69593 (0.64 mg/kg/day) or vehicle for 4 days prior to place conditioning. They were then exposed to 1 distinct environment following injections of the conditioning drug and to a different environment following vehicle injections. After 4 such sessions, place preference was assessed by allowing uninjected rats access to both places and measuring time spent in each. Morphine at doses of 1.0 mg/kg and higher produced a marked preference for the drug-paired place in vehicle treated rats. In contrast, U-69593 (0.16-0.64 mg/kg) produced dose-related place aversions. Administration of morphine prior to conditioning resulted in a 3-fold shift to the right of the morphine dose-response curve. Significant preferences were only observed at doses of 10.0 mg/kg and higher. Chronic morphine treatment did not modify the reinforcing effects of another drug of abuse, amphetamine or the aversive properties produced by a κ-agonist. Chronic U-69593 treatment eliminated the aversive effect associated with its acute administration. This treatment did not, however, attenuate the reinforcing effects of morphine. These data demonstrate that tolerance develops to the reinforcing and aversive properties of opioids and that this effect occurs in non-opioid dependent subjects. Furthermore, they suggest that tolerance to the euphorigenic or positive reinforcing effects of opioids may be a critical factor underlying chronic opioid abuse.

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289.7 ETHANOL ENHANCES THE NICOTINE-INDUCED BEHAVIORAL DESENSITIZATION TO NICOTINE IN LONG-SLEEP AND SHORT-SLEEP MICE. C.M. de Fiebre* and A.C. Collins Inst. for Behav. Genetics, University of Colorado, Boulder, CO 80309.

Long-Sleep (LS) and Short-Sleep (SS) mice, which have been selectively bred for differential sensitivity to ethanol (EtOH) anesthesia, also differ in sensitivity to nicotine. The greater sensitivity of the LS mouse line is not due to differential metabolism or distribution of the drug. Therefore these mice differ in sensitivity to nicotine because of differential CNS sensitivity. However, these two mouse lines do not differ in receptor numbers as measured by the binding of nicotine and α-bungarotoxin. Pretreatment with nicotine (2mg/kg) causes a decreased sensitivity to nicotine-induced seizures in these mice. This decreased sensitivity, a behavioral desensitization, could be due to desensitization of nicotinic receptors. Alcohols have been found to stabilize the nicotinic receptors from electroplaques in the high affinity (desensitized) state (Young, A.P. & Sigman, D.S., Mol. Pharmacol., 20:498, 1981). If the behavioral desensitization displayed by LS and SS mice is due to desensitization of nicotinic receptors, co-pretreatment with nicotine and alcohols should result in an enhancement of this phenomenon. LS and SS mice were pretreated with nicotine (2 mg/kg) and EtOH (0.25 and 1.5 g/kg for the LS and SS, respectively) 30 min prior to challenge with seizure-producing doses of nicotine. EtOH doses which did not display anticonvulsant properties were used in this experiment. In SS mice, EtOH co-pretreatment greatly enhanced the behavioral desensitization produced by nicotine pretreatment alone. No enhancement was seen in the LS mice probably due to the low dose of EtOH given to the LS mice and the relatively long pretreatment time. Therefore, animals of both selected lines were pretreated with nicotine (2 mg/kg) and EtOH (0.75 g/kg) 15 min prior to nicotine challenge. In LS mice, this pretreatment greatly enhanced the behavioral desensitization seen with nicotine alone. Furthermore, pretreatment with this dose of EtOH alone did not produce a significant change in nicotine-induced seizure-sensitivity in LS mice. SS mice co-pretreated with this dose of EtOH showed an enhanced behavioral desensitization, although this enhancement was not as great as that seen at the higher EtOH dose. This enhancement produced by EtOH is consistent with a stabilization of nicotinic receptors in a desensitized state. Perhaps the LS and SS mice differ in sensitivity to nicotine due to differences in the ratio of desensitized to sensitized receptors in non-pretreated animals.
Supported by AA-06391 and NIMH training grant MH-16880.

289.8 THE CONDITIONED SUPPRESSION OF DRINKING (CSD) PARADIGM: A POTENTIAL "ANIMAL MODEL" FOR THE STUDY OF PANIC DISORDER? David J. Fontana and Randall L. Commissaris (SPON: Ernest L. Abel). Department of Pharmaceutical Sciences, College of Pharmacy & AHP, Wayne State Univ., Detroit, MI 48202.

Although numerous animal procedures have been employed as "model" behaviors for the study of generalized anxiety and agents effective in the treatment of generalized anxiety, an analogous "behavioral model" for the study of Panic Disorder (PD) does not exist. In the present study, the effects of imipramine and desipramine were examined in a potential "animal model" for the study of PD, the Conditioned Suppression of Drinking (CSD) paradigm. In daily ten-minute sessions, water-deprived rats were trained to drink from a tube which was occasionally electrified (0.5mA). Electrification was signalled by a tone. Within 2-3 weeks, control (i.e., non-drug) CSD behavior had stabilized (approximately 20 shocks/session and 10-15 ml water/session) and drug testing was begun. Imipramine was administered both in an acute (3.5 - 20 mg/kg; 10-minute pre-treatment) and a chronic (2.5 mg/kg, i.p., twice daily for 5 weeks) regimen; desipramine was also administered in a chronic (5.0 mg/kg, i.p., twice daily for 5 weeks) regimen. Acute administration of imipramine failed to produce a selective effect on this conflict behavior, decreasing both the number of shocks received and water intake (unpunished responding) in a dose-dependent manner relative to saline controls. In contrast, chronic administration of either imipramine or desipramine resulted in a gradual and selective increase in the number of shocks received in CSD sessions over the course of several weeks of testing. This time-dependent increase in punished responding in the CSD observed during chronic imipramine or desipramine treatments parallels the time-dependent reduction in the frequency and severity of panic attacks in PD patients receiving chronic tricyclic anti-depressants. Thus, the CSD paradigm might serve as an "animal model" for the study of PD and potential anti-panic agents.
(Supported in part by MH42501-01).

289.9 ASYMMETRIC PURINERGIC RESPONSES TO DOPAMINE RECEPTOR ACTIVATION IN THE LESIONED RAT: NEUROCHEMICAL AND BEHAVIORAL CORRELATIONS. D.M.Togasaki[1,2], T.S.Brannan*[3], L.K.H.Leung*[2], J.G.Young[2,4] and P.J.Knott[1,2,5]. Departments of Neurobiology[1], Psychiatry[2], Neurology[3], Pediatrics[4] and Pharmacology[5]. The Mount Sinai School of Medicine, New York, N.Y. 10029, U.S.A.

Striatal extracellular fluid (ECF) concentrations of uric acid (UA) can be monitored with linear sweep voltammetry at carbon paste electrodes (Mueller,K.J. et al Brain Res. 335: 231, 1985). We also find that L-DOPA induced turning behavior in the unilaterally lesioned rat correlates with increased striatal UA production.

Male Sprague-Dawley rats (200-225g), unilaterally lesioned with 6-OHDA were tested 6-8 days later with apomorphine (0.25mg/kg). Rats exhibiting clear circling were implanted bilaterally with carbon paste working electrodes for chronic voltammetric recording 3-4 days after the apomorphine screen. 3 days following implantation they were injected with L-DOPA (25mg/kg i.p.). Semi-differentiated linear sweep voltammograms (from -430 to +900mV at 10mV/s using a BAS DCV5 voltammetry controller) were obtained at 10 minute intervals concurrently with videorecordings of circling behavior. In other studies, bilateral striatal microdialysis probes similar to those previously described (Clemens,J.A. and Phebus,L.A. Life Sci., 35: 671, 1984) were used to monitor ECF L-DOPA and dopamine metabolite concentrations in response to L-DOPA (50mg/kg i.p.) in similarly lesioned apomorphine-screened anesthetized rats.

Voltammetric Peak 2 (uric acid) also increased in both lesioned and intact striata but the change was larger on the lesioned side. Moreover, the difference in the increase of UA signal (lesioned minus unlesioned) correlated strikingly with the net turning behavior away from the lesioned side which had occurred 10min previously. In separate studies uricase microinfusion close to the voltammetric electrode abolished the L-DOPA induced increase of Peak 2 and also this peak was increased following infusion of the dopaminergic agonist, apomorphine. Using microdialysis, ECF L-DOPA changes were similar in both lesioned and intact striata and that DOPAC and HVA changes were much larger in the unlesioned striata. Changes of L-DOPA, the dopamine metabolites or 5-HIAA measured in striatal dialysates by HPLC-EC could not account for the differential response of peak 2 in the lesioned and unlesioned striata measured by voltammetry. This coupled with other studies from our laboratory continue to confirm that peak 2 is largely due to UA. The correlation of striatal UA production with a behavioral change suggests that UA may reflect functional neuronal events related to dopamine receptor interaction rather than tissue damage or other consequences of the invasive technique.

289.10 CAFFEINE ELEVATES THRESHOLD FOR INTRACRANIAL ELECTRICAL BRAIN STIMULATION IN THE RAT: TOLERANCE AND WITHDRAWAL. G.K. Mumford*[1], D.B. Neill*[2] and S.G. Holtzman*[1] (SPON: W.T. Frazier). Depts. of Pharmacology[1] and Psychology[2], Emory University, Atlanta, GA 30322.

Many behavioral stimulants, such as d-amphetamine, decrease the reinforcement threshold for intracranial self-stimulation (ICSS). Caffeine is a behavioral stimulant and the most widely consumed behaviorally-active compound in the world. Its effect on ICSS has not been well characterized. In this study we used an autotitration procedure to examine the effects of both acute and chronic caffeine administration on rate of responding and threshold for ICSS.

Adult male rats were trained to press one of two levers for an electrical stimulus delivered via electrodes stereotaxically implanted in the medial forebrain bundle of the lateral hypothalamus. Pressing the first lever delivered a 150 msec train of current and was recorded as a response. Every fifth response decreased the current intensity by a "step" of 3 μA. Pressing the second lever reset the current intensity to a preset maximum starting value, which was set individually such that each rat typically allowed the current to drop 10-12 steps before resetting In this way a characteristic reinforcement threshold (in μA) was established for each animal. Rats were overtrained in this procedure in daily 15 minute sessions before drug testing began.

Caffeine (1.0-56 mg/kg IP) administered 30 min prior to a session produced a significant dose-dependent elevation of reinforcement threshold (ie, earlier resetting), with response rate affected only at the highest dose (40% decrease; n=13). In contrast, d-amphetamine (0.75 mg/kg IP) significantly lowered reinforcement threshold in these subjects, with no effect upon response rate. Other rats (n=9) were allowed 10-min of access every 6 hr to drinking bottles containing either water (baseline condition) or a caffeine solution (1.0 mg/ml) as their sole fluid source for 10 consecutive days. Caffeine intake averaged 72 mg/kg/day. Animals were tested in the autotitration procedure at the same time each day, 5 hr after a period of access to their drinking bottles. After one day of caffeine intake, mean reinforcement thresholds were elevated significantly, with no corresponding change in response rate. Tolerance developed rapidly to this effect: thresholds returned to baseline (precaffeine) on day 2 and remained stable until the caffeine solution was replaced with drug-free tap water on day 10. Both reinforcement thresholds and response rates were decreased 24 hr later, and returned to baseline levels gradually, over 3-5 days. These changes are consistent with a drug withdrawal phenomenon. (Supported in part by Grants DA03413 and MH37340 and by RSA DA00008.)

289.11 THE SENSITIVITY OF CONDITIONED TASTE AVERSIONS AS A BEHAVIORAL BASELINE TO ASSESS THE DISCRIMINATIVE STIMULUS PROPERTIES OF NALOXONE. M. Kautz*, R. Jeffreys*, S. McBride*, S. Pournaghash*, M. Schwartz*, T. Titley*, A. Wachsman*, J.P. Mastropaolo and A.L. Riley (SPON: G. Gelanutsos). Psychopharmacology Lab, The American University, Washington, D.C. 20016.

Drug discrimination learning is a powerful tool for the in vivo assessment of the mechanism of drug action and has served as a technique for the classification of drugs on the basis of the "subjective state" they produce. Although discriminative control of behavior has been produced by a wide range of drug stimuli, there are differences in the rate of acquisition and degree of control obtained with various drugs. Naloxone hydrochloride, for example, produces only weak discriminative control, and even here, only at extremely high doses (e.g., 25 mg/kg).

Recently, the conditioned taste aversion procedure has been demonstrated to be a viable behavioral baseline to examine control by drug states (Mastropaolo, J.P., Moskowitz, K.H., Dacanay, R.J., & Riley, A.L., Neurosci. Abstr., 12:912, 1986). Specifically, following the injection of phencyclidine (PCP) rats were given a pairing of a saccharin taste and the emetic LiCl. Following an injection of distilled water (i.e., the absence of PCP), these same rats were given saccharin alone. After only three conditioning trials, the presence or absence of PCP controlled the avoidance or acceptance of saccharin. Since such rapid control by PCP is in contrast to that seen in more traditional operant assessments of discriminative control by drugs, it may be that the conditioned taste aversion design is more sensitive to control by drug states.

To test further the sensitivity of this paradigm, the present study examined the ability of naloxone to serve as a discriminative cue within the conditioned taste aversion design. Specifically, groups of water-deprived rats were injected with either .1, .3, 1 or 3 mg/kg naloxone 10 min prior to receiving 20-min access to saccharin. Immediately following saccharin consumption, all rats were injected with the toxin LiCl. This procedure was repeated every fourth day for 10 conditioning trials. On intervening days, subjects were injected with distilled water prior to receiving a non-poisoned presentation of saccharin.

Animals learned to avoid saccharin after six conditioning trials, with the degree of the aversion directly related to the dose of naloxone. Only at the lowest dose administered (i.e., .1 mg/kg) was there no evidence of stimulus control. These data suggest that the conditioned taste aversion paradigm may be useful as a sensitive behavioral baseline for the assessment of drug discrimination.

289.12 MOTOR ABNORMALITIES EVOKED BY CO-TRANSMITTERS: ANALYSIS OF TRH AND 5-HT SYNDROMES. A. Dailey* and M.R. Pranzatelli. (SPON: T.A. Pedley). Dept. of Neurology, Div. of Pediatric Neurology, College of Physicians and Surgeons of Columbia Univ., N.Y., NY 10032.

The motor abnormalities evoked by the co-transmitters thyrotrophin-releasing hormone (TRH) and serotonin (5-HT) offer a unique opportunity to study the functional significance of anatomic co-localization to the pharmacology of motor systems. The relationship of the two behavioral syndromes has not been previously studied using putative selective ligands at 5-HT receptor subtypes. Both the degradation-stabilized TRH analog MK-771 (in naive rats) and 5-hydroxytryptophan (5-HTP) (in rats with 5,7-dihydroxytryptamine DHT lesions) evoked forepaw tapping, Straub tail, hindlimb abduction, lateral head weaving, and shaking behavior. Sniffing and rearing were features of the MK-771 but not the 5-HT syndrome. Axial myoclonic jerks were evident in the 5-HT- but not the MK-771-evoked syndrome. MK-771 induced two types of shaking behavior: head shakes and wet-dog shakes (WDS). Neither independently was dose-related, unlike total shaking behaviors. MK-771-induced shaking behavior was pharmacologically dissociated from other MK-771-evoked motor behaviors. Both a 5-HT-1A agonist (8-OH-DPAT) and antagonist (isapirone) blocked WDS, but these drugs and putative 5-HT-1B (RU 24969) and 5-HT-2 (DOI) agonists and the 5-HT antagonists methysergide (nonselective), ritanserin (5-HT-2 selective), and l-propranolol (5-HT-1 selective) did not block other behavioral effects of MK-771. Each 5-HT agonist added its characteristic features to the MK-771 syndrome, such as hyperactivity (RU 24969) and lateral head weaving with reduction of rearing (8-OH-DPAT). 5-HTP reduced the threshold dose for MK-771-induced hyperthermia. Except for an antitremor effect, the putative 5-HT-1 antagonists isapirone and l-propranolol did not have similar effects, suggesting different sites of action. DHT-treated rats were behaviorally supersensitive to 10 mg/kg MK-771 as indicated by a significantly shortened latency of onset of WDS and greater frequency of abnormal forepaw movements. The same rats were also supersensitive to 50 mg/kg 5-HTP to a significantly greater degree. MK-771 did not evoke new behaviors in rats with DHT lesions. These data suggest behavioral relatedness of the TRH and 5-HT motor syndromes, but distinctive pharmacologic features and presumed mechanisms of action, despite the relation of TRH and 5-HT as co-transmitters. The pharmacologic dissociation of WDS and other behaviors suggests different regional loci or effector pathways. Further studies of the relation of the two syndromes await the availability of TRH antagonists.

290.1 ENVIRONMENTAL AND ENDOGENOUS CONTROL OF FEEDING AND PERCH HOPPING RHYTHMICITY IN THE HOUSE SPARROW. C.C. Chabot* and M.Menaker (SPON: D.J.Hudson). Institute of Neuroscience, University of Oregon, Eugene, Oregon. 97403.

The perch hopping activity of several avian passerine species can be rendered arrhythmic by removal of the pineal gland (P-X) or by exposure to constant light (LL). The pineal hormone melatonin also has effects on perch hopping rhythms of birds. While P-X always abolishes perch hopping rhythmicity in house sparrows, it often leaves the perch hopping activity of starlings rhythmic. In those individual starlings whose perch hopping rhythmicity is abolished by P-X or LL, feeding activity measured concurrently from the same bird remains rhythmic (Gwinner, unpublished; Ganshirt et al., 1983). One of several interpretations of this result is that the two behaviors are controlled by different clock systems. To test this idea we asked whether rhythmic feeding activity of the house sparrow persists under conditions which abolish locomotor rhythmicity.

Records of locomotor activity and feeding behavior were obtained concurrently from individual sparrows treated in several different ways: intact sparrows exposed to light/dark cycles, constant darkness and constant light of several intensities; P-X sparrows in constant darkness; and sparrows implanted with melatonin filled silastic capsules held in constant darkness.

The results are clear: Rhythms of feeding behavior are present in a light/dark cycle and persist in constant darkness with the same period as perch hopping rhythms; P-X consistently abolishes both feeding and perch hopping rhythms; implantation of melatonin filled silastic capsules leads to arrhythmicity in both behaviors; and exposure to LL affects both the feeding and perch hopping activity patterns in near identical fashion.

These results suggest that, at least in the house sparrow, feeding and perch hopping rhythms are under the influence of the same circadian control system of which the pineal gland is an integral part.

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290.2 MATERNAL-FETAL COMMUNICATION OF DAYLENGTH: DEFINITION OF THE CRITICAL PERIOD FOR RECEPTION OF THE PRENATAL SIGNAL. D.R. Weaver, J.T. Keohan*, and S.M. Reppert. Children's Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114.

Recent studies show that an animals's prior photoperiodic history influences its response to a subsequent photoperiodic treatment. Photoperiodic history begins during the prenatal period in montane voles and Djungarian hamsters, as the prenatal light-dark cycle is perceived by the fetus and influences reproductive development in these species (Biol Reprod 1984, 31: 499; Biol Reprod 1986, 34:664). Production of melatonin from the maternal pineal gland is involved in communication of daylength to the fetus in Djungarian hamsters. By varying the nightly duration of melatonin infusion into pinealectomized (PNX) dams during pregnancy, we showed that the duration of melatonin secretion is the signal communicating daylength to the fetus (Endocrinology 1986, 119: 2861). In the present study, we administered melatonin to PNX Djungarian hamsters (Phodopus sungorus) during restricted periods of gestation to delineate when maternal-fetal communication of daylength occurs.

First, we established that infusions of melatonin (50 ng delivered over 10 hr per night) to PNX dams for the last 4 to 7 nights of gestation consistently stimulate reproductive development in pups reared in 14L:10D (14L) postnatally. The offspring of melatonin-infused PNX dams reared in 14L had paired testes weights on day 34 approximately seven times greater than pups of uninfused PNX dams reared in 14L (420 vs. 60 mg, respectively).

To determine when during gestation this effect of melatonin is maximal, we restricted the period of infusion to a single night. Regardless of when the infusion was delivered, one night of infusion was ineffective in stimulating postnatal reproductive maturation. Two consecutive nights of melatonin infusion did stimulate testicular weight of the male offspring, however. By varying the timing of infusion relative to the time of birth, we have identified an apparent critical period 2 to 5 days before birth during which melatonin infusions are most effective in stimulating postnatal testicular growth of the male offspring. Even the peak response to two infusions was less than the response to 4 or more consecutive infusions, e.g., the response appears to be graded. This, along with the ineffectiveness of the single-night infusions, suggests that the prenatal daylength signal must be repeated, reinforcing itself over several days, in order to be effective. Interestingly, infusions delivered the last 2 nights of gestation were relatively ineffective in stimulating postnatal reproductive development. The decline in response to infusions delivered at the end of pregnancy may indicate that there is a programmed neural insensitivity to melatonin during the late prenatal and early postnatal periods. Supported by HD14427.

290.3 EFFECTS OF MELATONIN ON SUPRACHIASMATIC NUCLEUS METABOLISM IN RATS. M.H. Roberts, V.M. Cassone and R.Y. Moore, Depts. of Neurology and Neurobiology and Behavior, SUNY-Stony Brook, Stony Brook, NY 11794

The suprachiasmatic nucleus (SCN) is a central pacemaker in the rodent circadian system. In non-mammalian vertebrates the pineal gland also plays a prominent role in circadian organization. While pinealectomy in some birds results in arrhythmicity, pinealectomy in rodents has little effect on behavioral rhythms. Despite the lack of effect of pinealectomy, recent studies indicate that daily injection of the pineal hormone, melatonin, can entrain circadian rhythms of locomotor activity in rats if administered at CT 12. This effect depends upon the SCN. In an attempt to determine the physiology of melatonin's effects, we addressed two issues in the current study; the effect of melatonin on metabolic activity and protein synthesis in the SCN.

In the first study we measured the effect of melatonin injection at CT10 and CT14 on 2-deoxyglucose (2DG) uptake in the SCN of blind and intact rats. The effect of melatonin (1mg/kg in 1% ethanol: saline) was compared to the effect of ethanol:saline alone. Fifteen minutes following drug injection animals were injected with 150uCi/kg of 14C-2DG. Forty-five minutes later, animals were sacrificed, brains removed and processed for autoradiography. Densitometric analysis indicates that deoxyglucose uptake is inhibited at CT10 by melatonin treatment ($p<0.01$). The results, summarized below, are expressed as deoxyglucose uptake in pM/g/hr $\pm$ SD (n=3 in each group).

		DEOXYGLUCOSE UPTAKE	
TIME	ANIMAL	SALINE	MELATONIN
CT10	intact	395.0 $\pm$ 98.7 *	290.1 $\pm$ 21.5
CT10	blind	386.3 $\pm$ 43.1 *	279.5 $\pm$ 18.0
CT14	intact	293.8 $\pm$ 5.7	288.4 $\pm$ 24.6
CT14	blind	289.6 $\pm$ 7.6	271.4 $\pm$ 17.8

To determine the effect of melatonin on protein synthesis, 6 rats were cannulated in the lateral ventricle, five days prior to being injected with melatonin (n=3) or ethanol:saline at CT 10. Fifteen minutes later animals were intraventricularly injected with 1.4mCi of 35S-methionine. Two hours later animals were sacrificed, brains removed, and the SCN was dissected and homogenized in SDS buffer. Ethanol:saline treated rats showed an SCN specific activity of 18598 $\pm$ 1666 DPM/ug protein, which was decreased to 8095 $\pm$ 3580 DPM/ug by melatonin. These values are equivalent to values obtained mid-day and mid-night in an initial pilot study (17686 D; 7996 N).

These results indicate that the SCN may be a target for melatonin's effects on the rodent circadian system. Furthermore, the effect of melatonin on protein synthesis, although preliminary, may provide a basis for using this hormone as a probe to dissect the biochemical mechanism of circadian rhythm generation in the SCN. Supported by NIH NS16304 to RYM and NSF BNS19660 to VMC.

290.4 LUZINDOLE (N-0774) ANTAGONIZED THE MELATONIN-INDUCED INHIBITION OF ^{3}H-DOPAMINE RELEASE FROM RABBIT RETINA. M. L. Dubocovich. Dept., Pharmacol., Northwestern Univ. Med. Sch. Chicago, IL 60611.

Melatonin, at picomolar concentrations, inhibits the calcium-dependent release of ^{3}H-dopamine from the rabbit retina, through activation of a site possessing pharmacological characteristics of a receptor (Dubocovich, M.L., J. Pharmacol. Exp. Ther., 234: 395, 1985). We demonstrate here that the indole compound luzindole (N-0774) antagonizes the melatonin-induced inhibition of ^{3}H-dopamine release from rabbit retina. Albino rabbits maintained on a 10/14 hour dark/light cycle were killed in the middle of the dark cycle. Retinas were labeled in vitro with ^{3}H-dopamine. Calcium-dependent release of ^{3}H-dopamine was elicited by field stimulation at 3 Hz (2 min, 2 msec., 20 mA) twice in each experiment. The percent of total tissue radioactivity release above basal during the first period of stimulation (S_1) was 2.31 $\pm$ 0.13 % (n=14), and the ratio S_2/S_1 was 0.98 $\pm$ 0.05 (n=14). Melatonin (IC_{50}=40 pM), and the melatonin receptor agonists 6-chloromelatonin (IC_{50}=40 pM) and 6,7-dichloro-2-methylmelatonin (IC_{50}=10 pM) inhibited in a concentration-dependent manner the calcium-dependent release of ^{3}H-dopamine when added alone before the second period of stimulation (S_2). Maximal inhibitory effects (75-80 %) by the melatonin receptor agonists were obtained at a concentration of 1 nM. Luzindole did not modify the calcium-dependent release of ^{3}H-dopamine when added alone before S_2 (S_2/S_1 ratio: 0.78 $\pm$ 0.08, n=4; 1.00 $\pm$ 0.01, n=3; and 0.93 $\pm$ 0.11, n=4 for 0.1, 1 and 10 uM luzindole, respectively). Luzindole (0.1-10 uM) shifted the concentration-effect curves of the melatonin receptor agonists to the right without changing their maximal inhibitory effects. The equilibrium dissociation constant (K_B) for luzindole calculated from the Schild regression using melatonin as agonist was 20 nM. The equilibrium dissociation constant for luzindole determined using 6-chloromelatonin was 22 nM and using 6,7-di-chloro-methylmelatonin was 16 nM. Taken together these results suggest a) that there is a competitive relationship between the melatonin antagonist luzindole and the agonist melatonin, and b) that the three melatonin receptor agonists used in these experiments activate the same presynaptic melatonin receptor to mediate inhibition of ^{3}H-dopamine release as the calculated K_B values for luzindole were identical. Luzindole, expected to mimic the effects of light, may help to understand the role of melatonin in photoperiodic regulation of behavioral and physiological processes in vertebrates. Supported by Nelson Research and NIH RR 05470.

290.5 EFFECT OF THE MELATONIN RECEPTOR ANTAGONIST LUZINDOLE (N-0774) IN THE MOUSE BEHAVIOURAL DESPAIR TEST. E. Mogilnicka* and M.L. Dubocovich. (SPON: D.N. Krause). Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL. 60611.

The pineal hormone melatonin has been implicated in the regulation of behavioral and physiological processes such as circadian rhythms in birds, reproduction in rodents, and as recently reported seasonal affective disorders in humans (Lewy et al., Science 235: 352, 1987). Understanding of the role of melatonin in these processes has been hampered by the lack of a melatonin receptor antagonists. The availability of luzindole, a selective melatonin receptor antagonist (Dubocovich, M.L., Neurosc. Abs., 1987) led us to investigate the role of endogenous melatonin in the mouse behavioral despair test which is used to screen for antidepressant activity (Porsolt et al., Arch. Int. Pharmacodyn. Ther., 229: 327, 1977). This test measures the reduction of immobilitity during forced swimming. Experiments were conducted at noon and at midnight in albino ND/4 and C3H/HeN mice kept on a 14/10 hour light/dark cycle (8 mice per group). In C3H/HeN mice pineal melatonin levels are higher during the dark period (Hotz, M.M., Behavior Genetics 15: 595, 1985). In C3H/HeN mice the duration of immobility at midnight (68.4 $\pm$ 2.29 sec) was significantly longer than at noon (47.7 $\pm$ 3.4 sec, $p < 0.001$) while in ND/4 mice the duration of immobility was identical at both times (midnight: 88.1 $\pm$ 5.9 sec). The tricyclic antidepressant desipramine (30 mg/kg, i.p.) significantly reduced the duration of immobility both in the C3H/HeN mice (noon: 12.5 $\pm$ 3.3 sec; midnight: 5.2 $\pm$ 2.1 sec) and in the ND/4 mice (noon: 38.2 $\pm$ 5.1 sec; midnight: 44.7 $\pm$ 12.4 sec). In the C3H/NeH mice luzindole (10 mg/kg) 60 min after i.p. administration, significantly reduced the duration of immobility at noon (28.2 $\pm$ 6.9 sec) and at midnight (16.1 $\pm$ 5.2 sec). This effect was more pronounced at midnight. Luzindole did not affect the duration of immobility in the ND/4 mice either at noon (57.4 $\pm$ 8.9) or midnight (72.4 $\pm$ 4.9). Melatonin (30 mg/kg, i.p.) did not affect the duration of immobility in C3H/NeH mice, but completly abolished the effect of luzindole (10 mg/kg). In C3H/NeH mice kept in constant light during 1 week, which pressumably abolished the night increase in pineal melatonin, the duration of immobility measured at midnight was reduced (38.3 $\pm$ 7.9 sec) to values obtained in controls at noon. The light treatment abolished the effect of luzindole (10 mg/kg) at midnight (49.9 $\pm$ 10 sec). Taken together these data suggest that endogenous melatonin plays a role in the C3H/NeH mice behavioral despair test, but not in the ND/4 mice. We suggest that the melatonin receptor antagonist luzindole may have a potential therapeutic value in the treatment of chronobiologic mood disorders. Supported by Nelson Research and NIH RR 05470.

290.6 CIRCADIAN RHYTHMS IN ALZHEIMER'S DISEASE. J.W. Renfrew*, C. May*, L. Tamarkin*, R.P. Friedland and S.I. Rapoport (SPON: Q. Smith). Lab. of Neuroscience, Nat. Inst. on Aging, National Institutes of Health, Bethesda, MD 20892.

Patients with Alzheimer's disease (AD) often develop sleep disturbances and nocturnal wandering over the course of their illness. Previous animal studies have indicated that the suprachiasmatic nucleus of the hypothalamus may regulate circadian rhythms. This nucleus may be affected in AD.

To further study wandering in AD, we sought to characterize the circadian rhythms of plasma melatonin and physical activity in a group of 6 patients with probable AD (mean age 71.5 yr, range 54 to 89 yr) and 6 healthy, age-matched control subjects (mean age 73 yr, range 55 to 91 yr). The AD patients were moderately to severely demented and they were all living at home. All subjects were in good physical health and were medication-free at the time of study. Pairs of an AD patient and a control subject were admitted to the NIH Clinical Center for 5 days, including a 2-day acclimation period and were maintained on a set daily routine and a caffeine-free diet. An indwelling intravenous catheter was placed in one arm of each subject to obtain blood samples hourly, for 48 consecutive hours. Each subject also wore a patient activity monitor (PAM) on the other arm to monitor activity continuously over the same period. The behaviors of each subject (awake, resting, or sleeping) for each hour of the study were observed and recorded. An average 24-hr day was constructed from the data accumulated on the third and fourth day for each subject.

The circadian patterns of plasma melatonin differed significantly between the AD patient and control groups (analysis of variance; $F=2.43$, $df=23$, $p<0.05$); the nocturnal rise in plasma melatonin appeared much less prominent in the AD patients. The oldest subjects were found to have the lowest levels of plasma melatonin, in agreement with previous studies. The 2 AD patients who exhibited the most night-time wandering during the study were found by PAM to have the greatest nocturnal activity, and only modest nocturnal elevations in plasma melatonin. All subjects demonstrated significant diurnal variations in activity; however, the mean activity of the AD patients exceeded those of the control subjects in 21 of 24 hours. Thus, regulation of circadian rhythms may be abnormal in AD, possibly due to hypothalamic dysfunction. We suggest that the PAM may be useful in determining abnormal patterns of activity, and in assessing the efficacy of therapies for ameliorating these behaviors.

290.7 ENTRAINMENT AND DEVELOPMENT OF TOLERANCE OF THE MAMMALIAN CIRCADIAN CLOCK TO DAILY INJECTIONS OF TRIAZOLAM. F.W. Turek, J.-J. Vanderhaeghen and O. Van Reeth*. Lab. of Neuropathology & Neuropeptides Research, Free Univ. of Brussels, Belgium and Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60201.

The recent discovery that a single injection of the short-acting benzodiazepine, triazolam (Tz), can induce pronounced phase shifts in a master circadian pacemaker in hamsters, raises the possibility that repeated daily injections of Tz might be able to entrain a central circadian clock. To test this hypothesis, the circadian rhythm of wheel-running behavior was monitored in blind hamsters injected intraperitoneally with 2.5 mg of Tz (N=8) or vehicle (V,N=10) every day for five days and then every other day for 20 days. The first injection occured 3-6 hrs before the onset of activity and subsequent injections occurred at the same real clock time. Injections of V had no effect on the rhythm. While the first 2-3 injections of Tz induced pronounced advances in the activity rhythm (mean advance= 252$\pm$53 min.), subsequent injections had no effect. In a second study, six blind hamsters were injected every day for 25 days with 2.5 mg of Tz. The first few injections induced a pronounced advance in the activity rhythm (mean advance= 193$\pm$52 min.), but after 3-5 days, the injections had no effect.

In a third study, blind hamsters were injected intraperitoneally with 0.1 mg of Tz (N=10) or V (N=9) every day for 49 days; thereafter, the animals were left undisturbed for an additional 15 days. The timing of the daily injections followed the same pattern as in the first two studies. While injections of V did not induce any clear change in the activity rhythm, the first few injections of Tz induced a pronounced advance of the rhythm in 9 of the 10 animals that averaged 262$\pm$41 minutes. In these 9 animals, daily injections of Tz entrained the activity rhythm for about the first 15 days, and then the activity rhythm of all the animals began to free-run with a period less than 24 hrs. During the last 10 days of injections, the mean period of the activity rhythm for the Tz-treated animals (23.91$\pm$0.02 hrs) was significantly less ($p< 0.001$) than that observed in V-injected animals (24.26$\pm$0.03 hrs). Importantly, this difference in period persisted throughout the 15-days following termination of the injections.

These results indicate that it is possible to entrain a circadian clock for at least a limited time period with daily injections of triazolam. However, the clock becomes tolerant to the phase shifting effects of triazolam and the time course for the development of this tolerance is dose dependent. Furthermore, it is possible to induce major changes in the period of the circadian clock with daily injections of triazolam, and these changes persist for at least 15 days even after the termination of drug treatment. Treatment with a short-acting benzodiazepine can have pronounced and long-term effects on the mammalian circadian clock system.

290.8 TRIAZOLAM FACILITATES REENTRAINEMNT OF CIRCADIAN CLOCK FOLLOWING AN 8-HOUR ADVANCE OR DELAY IN THE LIGHT DARK CYCLE. O. Van Reeth*, J.J. Vanderhaeghen and F.W. Turek (SPON: B. Menco). Lab. of Neuropathology and Neuropeptides Research, Free Univ. of Brussels, Belgium and Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60201.

A single injection of the short acting benzodiazepine triazolam (Tz) can induce permanent and pronounced phase shifts in the circadian rhythm of locomotor activity of hamsters free running either in constant darkness or in constant light. The magnitude and the direction of these phase shifts are dependent on the time when Tz is administrated relative to the onset of locomotor activity. These results suggest that it might be possible to alter the time it takes for the biological clock to resynchronize to a new light schedule after a shift in the light dark cycle.

To test this hypothesis, male golden hamsters housed in individual running wheel cages and maintained on a LD 14/10 cycle were submitted to an 8 hour advance in the LD cycle. On the first or the second day after the light shift, they received a single intraperitoneal injection of vehicle(V) or 2.5 mg Tz 6-7 hours prior to the expected onset of locomotor activity. In the vehicle treated animals, the transient period (i.e. until the onset of activity again occured near the time of lights off) lasted an average of 8.1 days (N=16). The mean transient period was significantly reduced ($p<0.01$) in animals injected with Tz on either the first (3.75 days, N=8) or the second (5.5 days, N=10) day after the shift. Animals injected with Tz on day one reentrained significantly faster ($p<0.05$) than those injected on day two.

In another study, hamsters on a LD 14/10 cycle were submitted to an 8 hour delay in the LD cycle and received an injection of Tz or V 5-6 hours after the expected onset of the activity rhythm on the first day following the shift in the light cycle. The animals received two supplementary injections of Tz or V 24 and 48 hours after the first injection. The transient period necessary before the onset of locomotor activity was again within 30 min of the new LD cycle was 16.9$\pm$1.5 days in the animals treated with V (N=10) and 8.4$\pm$0.7 days (N=10) in the animals treated with Tz ($P<0.001$).

These findings demonstrate that triazolam can markedly reduce the time it takes for a biological clock of the hamster to be resynchronized after a phase advance or a phase delay in the LD cycle. This raises the possibility that, if triazolam can also induce phase shifts in human circadian rhythms, it might be possible to designate appropriate drug therapies for situations in which human circadian rhythms are desynchronized from the external environement, such as occurs during jet lag and shift work.

290.9 TRIAZOLAM ACCELERATES THE ADAPTATION OF THE CIRCADIAN RHYTHM OF CORTISOL TO AN 8-HOUR DELAY OF THE SLEEP-WAKE CYCLE IN MAN. E. Van Cauter*, A. Van Onderbergen*, D. Bosson* and G. Copinschi* (SPON: J. Joy). School of Medicine, Free University of Brussels, B-1070 Belgium and Dept. of Medicine, The University of Chicago, Illinois 60637.

It has recently been shown that triazolam, a short-acting benzodiazepine, can induce phase shifts of the circadian clock of hamsters. The magnitude and direction of these phase shifts depend on the time of drug administration. These findings prompted us to determine whether triazolam, a widely used hypnotic, would accelerate in man the adaptation of the 24-h periodicity of cortisol secretion following an abrupt shift of the sleep-wake cycle.

Six normal male volunteers, aged 21-30 yrs, were studied once with triazolam and once with placebo, in random order. The two studies were spaced 2 months apart. In each study, the 24-h profile of plasma cortisol was obtained at 20-min intervals under basal conditions, as well as 1, 3 and 5 days after an 8-h delay of the usual sleep-wake cycle. This delay was obtained by sleep deprivation from 23:00 to 07:00 on the first day. These conditions mimicked in the laboratory the shift in the sleep-wake cycle experienced in the course of a westward transatlantic flight crossing 8 time zones. A bedtime schedule of 07:00 to 15:00 was enforced for 5 consecutive 24-h periods. The entire study was preceded by 3 nights of habituation to the study unit. Triazolam (0.5mg) or placebo was given at 04:00 on the first shifted night and at 07:00 on the following nights. Best-fit patterns, based on the periodogram method, were calculated for each individual 24-h cortisol profile. Melatonin levels will also be measured on each plasma sample collected in the course of this investigation. The assay is currently under progress and the results will be analyzed and reported in a similar fashion as described here for cortisol.

Adaptation of the 24-hour cortisol profile to the 8-hour delay in the sleep-wake cycle involved an overall alteration of the complex waveshape of the cortisol rhythm. Therefore, the timing of the acrophase and of the nadir, the cortisol level at the acrophase, the total duration of the quiescent period (QP), the fragmentation of the QP and the time corresponding to the end of the QP were determined for each profile and these 6 parameters were combined to calculate a global adaptation index (GAI) to the time shift. On days 1 and 3, the GAI was significantly higher ($p<0.05$) following treatment with triazolam than after treatment with placebo indicating that the administration of triazolam had facilitated the rate of reentrainment of the cortisol rhythm to the 8-hour delay in the sleep-wake cycle. These results suggest that short-acting benzodiazepines may be useful in the treatment of conditions associated with abnormal circadian synchronization, such as occurs during "jet lag" and in shift-workers.

290.10 PHASE RESPONSE CHARACTERISTICS OF FEEDING SCHEDULES IN RATS. G.J. Coleman and R. Francis. (Spon: G.A. Bell) Department of Psychology, La Trobe University, Bundoora, Vic., 3083., Australia.

There is much evidence for a circadian oscillator in the rat which is distinct from the SCN (supra-chiasmatic nuclei) based, light-dark (LD) entrained oscillator (Boulos, Z., Rosenwasser, A. & Terman, M., Behavioural Brain Research, 1:36-65, 1980).

Recently, we have found that the Australian marsupial Sminthopsis macroura froggatti exhibits pronounced phase-shifts in free-running activity rhythms following 3 days of food deprivation (Coleman, G., O'Reilly, H. & Armstrong, S. (Neuroscience Abstracts, 15:816, 1985). It is not known what the mechanism for this phase-shift is nor is it known whether the phase shift is SCN mediated. A close inspection of previously published data from our laboratories (Clarke, J. & Coleman, G., Physiology & Behavior, 36:105-113, 1986) indicates that laboratory rats may also show such phase-shifts in activity rhythms following 3 days of food deprivation. In an unpublished study, we have found that a prior meal-feeding schedule is not necessary for deprivation-induced phase shifts to occur in Sminthopsis macroura froggatti. It remains to be determined whether the time of day at which food is presented followng deprivation has an effect on the induced phase-shift.

Our published data suggest that the phase shift in activity following deprivation occurs on the day at which food is reinstated. Thus the availability of food may serve as the stimulus for the phase shift. This is supported by the data from meal-feeding experiments where a daily presentation of a 2-hour meal could produce a daily phase shift in the meal-associated oscillator thereby producing entrainment as reflected in anticipatory activity.

Two experiments were conducted. In the first, Long Evans rats aged 29 days were maintained on 12:12 cycle LD for 15 days, then placed in constant dark (DD) for 36 days. During DD, rats were deprived of food for 3 days on two occasions. Following reinstatement of food at various times of day, no phase shifts were observed. Rats were then placed in constant light (LL) of 30-40 lux. When a 3-day deprivation was followed by food reinstatement, phase shifts were observed.

In a second experiment, Long Evans rats aged 95 days were placed in a 12:12 LD cycle for 15 days, then LL of 10 lux was introduced. After 15 days LL, all rats were deprived of food for 3 days and food was reinstated at different times of the day, 4 hours apart. Phase shifts in activity were observed, and a phase response curve (PRC) was constructed. This curve was characterized by considerable between-rat variability, and a much longer phase delay component than phase advance.

It was concluded that deprivation induced phase-shifts in Long Evans rats can only occur in LL and that the PRC of food reinstatement is less stable than that for light.

REGENERATION II

291.1 WHITE MATTER ASTROCYTES. DO THEY PRODUCE A NON-PERMISSIVE SUBSTRATE FOR AXONAL GROWTH. A. Bignami, D. Dahl, V. Gilad and G. Gilad. Veterans Administration Medical Center and Harvard Medical School, Boston, MA.

Most observations concerning failure of regeneration in the CNS could be readily explained if myelinated white matter rather than the glial scar constituted an impenetrable barrier for axonal growth: (i) Abortive regeneration in spinal cord tracts following transection. (ii) Extensive axonal sprouting and synaptic reorganization in adult spinal cord and cerebral gray matter following partial deafferentation. (iii) Growth of CNS axons into peripheral nerve graft through glial scars at the brain graft interface. (iv) Inability of regenerating posterior spinal roots to penetrate into spinal cord white matter. (v) Extensive regeneration in two non-myelinated CNS tracts (olfactory nerve and hypothalamohypophysial tract).

If myelinated white matter is in fact an impenetrable barrier for axonal growth, the following characteristics should be expected for the hypothetical repellent: (i) White matter specificity. (ii) Extracellular or cell surface localization. (iii) Late appearance in ontogeny as axons continue to grow until relatively late in development (e.g. cortico-spinal axons only reach the lumbar spinal cord on post-natal day 9 in the rat).

Brain specific hyaluronectin (BHN), the main component of hyaluronectin, a glycoprotein fraction isolated by Delpech and Havalent from human brain by affinity chromatography on hyaluronate-Sepharose (J. Neurochem. 36:855, 1981), appears to fulfill these criteria: (i) White matter specificity; within spinal cord white matter, immunocytochemically localized BHN forms a fine mesh surrounding individual myelinated axons paralleling the distribution of astrocytes as revealed by GFAP localization (Bignami and Dahl, PNAS 83:3518, 1986; J. Neurocytol. 15:671, 1986). (ii) Extracellular or cell surface localization; all hyaluronate-binding proteins so far reported in the literature are either extracellular or cell surface proteins. (iii) Late appearance in ontogeny; BHN first appears in white matter after the onset of myelination.

Experiments conducted in tissue culture are compatible with this hypothesis. Cells dissociated from fetal rat spinal cord or postnatal rat cerebellum were seeded on coverslips coated with polylysine on one half and with a hyaluronectin preparation on the other. Poor cell attachment was observed and neurite outgrowth was blocked on hyaluronectin. Neurites, which grew abundantly on the polylysine substrate, did not extend to the hyaluronectin coated surface. Thus, if BHN fills the space between myelinated fibers, it would effectively prevent axonal growth. Supported by N.I.H. grant NS 13034 and by the Veterans Administration.

291.2 PROTEINS OF RAT CNS WHITE MATTER AND OF MYELIN FORMING OLIGODENDROCYTES ARE POTENT INHIBITORS OF NEURITE OUTGROWTH AND OF CELL LOCOMOTION. P. Caroni* and M. E. Schwab. Brain Research Institute, University of Zürich, CH-8029 Zürich, Switzerland.

Extensive neurite outgrowth is not found in differentiated CNS tissue of higher vertebrates. Lacking favorable substrates, trophic factors or presence of inhibitors are possible underlying mechanisms.

Primary cultures of dissociated rat optic nerve contain cells which are strictly avoided by growing neurites as well as by migrating fibroblasts. Such cells are identified as myelin forming oligodendrocytes, as they are $GalC^+$, mAb $A_2B_5^-$, mAB O_4^+, and myelin basic protein positive. Myelin fractions from rat spinal cord adsorbed to PLYS-coated culture dishes represent a highly non-permissive substrate for neurite outgrowth for sensory and sympathetic neurons in presence of NGF, and for neuroblastoma cells stimulated with dibutyryl cAMP. Likewise, spreading of a number of cells, including 3T3-fibroblasts was inhibited. Non-permissivity is not found in PNS myelin fractions nor in cell membrane fractions from other tissues and is completely abolished by mild proteolysis. Non-permissive substrate components can be extracted with detergent and reconstituted in highly active form in artificial lipid vesicles. Active vesicles are formed from SDS-PAGE separated proteins of about 250kD and 30kD. Spreading-inhibiting proteins are effective in amounts lower than 10 ng/cm^2 of culture dish. High yields of active proteins are recovered from inhibitory oligodendrocyte-containing cultures, but not from Schwann cell cultures nor from a number of tissues including liver, kidney, and skeletal muscle. Addition of 250kD proteins to permissive liver or PNS myelin protein in ratios of up to 1/100 and subsequent reconstitution results in inhibitory mixed liposomes. Monoclonal antibody against 250kD inhibitor protein weakly but specifically stains the surface of myelin-forming oligodendrocytes in culture. Antibody adsorbtion partially neutralizes inhibition of cultured oligodendrocytes and of CNS myelin. Inhibitor protein is insoluble in the absence of detergent and is not a highly negatively charged proteoglycan. We hypothesize that such inhibitors might effectively prevent neurites from growing along "mature" CNS fiber tracts.

291.3 TISSUE CULTURE STUDIES INDICATE THAT MYELINATION BY OLIGODENDROCYTES OBTAINED FROM ADULT TISSUE IS SUPPRESSED BY ASTROCYTES. C.L. Rosen, P.M. Wood* and R.P. Bunge, Depts. Biol. and Anatomy/Neurobiol., Washington University, St. Louis, MO 63110.

Whereas remyelination is known to occur in the central nervous system (CNS), the degree of remyelination varies after different demyelinating injuries. Because the tissue response to demyelinating injury in the CNS often involves astrocytes (AS) as well as oligodendrocytes (OL), the role of the AS in fostering or inhibiting remyelination has been debated. The influence of AS on OL function was assessed by comparing OL proliferation and myelination in cultures of neurons and added purified adult OL (N+OL) with and without added purified type I AS (N+OL+AS). Pure neuronal cultures were prepared from embryonic rat dorsal root ganglia (Wood and Williams, Dev. Brain Res. 12:225, 1984). OL (immunostained for galactocerebroside) were purified by fluorodeoxyuridine treatment of crude glial cultures prepared from adult rat spinal cord and contained fewer than 2% AS (immunostained for glial fibrillary acidic protein). AS were purified from postnatal rat cerebral cortex (Noble et al., J. Neurosci. 4:1892, 1983). After preparation of the neuronal cultures, they were seeded with 10^5 purified OL; 1-2 weeks later AS were added to some of those N+OL cultures. After an additional 4-5 wks the cultures were stained with Sudan black and the number of OL and the amount of myelination determined by light microscopy.

Extensive OL proliferation and myelination was observed in control N + OL cultures which contained at most a few 100 AS. Very few OL and essentially no myelination was seen in N+OL+AS cultures which contained a monolayer of confluent AS. To determine if this inhibition of ODC function was mediated by cell contact or by soluble factors, OL proliferation and myelination were studied in N+OL cultures fed with type I AS conditioned medium treated to restore original nutrient levels (ACM). In addition, OL function in dialyzed ACM and boiled ACM was assessed. The results showed that factors in ACM effectively blocked OL proliferation and myelination. In dialyzed ACM the number of OL was nearly the same as in controls but myelination was only slightly improved. In boiled ACM the number of OL remained low and only a little myelin was seen. These results suggest that type I AS release at least 2 factors which can affect OL functions: one with a MW of $< 10{,}000$ which blocks OL proliferation, and another with a MW of $> 10{,}000$, which is heat stable, and blocks myelination. In this culture system type I AS suppress OL function; because this suppression appears to be mediated by a soluble agent it may be possible to isolate and identify the responsible factor(s). (Supported by RG1118 from the National Multiple Sclerosis Society.)

291.4 PREFERENTIAL REINNERVATION OF MOTOR NERVES BY REGENERATING MOTOR AXONS. T.M.Brushart.Dept. of Orthopaedics, Johns Hopkins Hospital, Baltimore, Maryland 21205.

Regeneration of axons into inappropriate Schwann cell tubes may adversely affect the outcome of peripheral nerve suture. However, the degree to which motor axons reinnervate sensory Schwann cell tubes, and vice versa, has not been determined. These experiments quantify the sensory and motor neurons regenerating across a proximal nerve suture into terminal sensory and motor branches. The rat femoral nerve was cut and sutured proximally where sensory and motor axons intermingle. Horseradish peroxidase (HRP) was then applied distally to the sensory or motor branch to identify reinnervating neurons.

Four experimental groups each contained 20 female Sprague-Dawley rats: adult (12-14 wks, 250 gms), juvenile (3 wks, 50 gm), adult rotation, and juvenile rotation. In adult and juvenile groups both proximal femoral nerves were severed, precisely realigned, and sutured, while in the rotation groups the distal stumps were rotated 90° before suturing. Eight weeks were allowed for regeneration. HRP was then applied to one sensory branch and the opposite motor branch. Animals were perfused with fixative 48° later and the lumbar spinal cords and dorsal root ganglia were sectioned at 80u and processed with tetramethyl benzidine to demonstrate HRP within neurons. Labeled cells were counted by an observer unaware of which branch had received HRP, and the counts were subjected to paired T test analysis. Control experiments determined the number of sensory and motoneurons innervating the femoral nerve in normal animals and the number and total axoplasmic area of myelinated axons in the sensory and motor branches.

More motoneurons were labeled from the motor branch than from the sensory branch in all groups; the difference was significant for juvenile ($p = .0002$) and juvenile rotation ($p = .029$) animals. Sensory neuron labeling was significantly greater from the sensory branch in all groups ($p = .016$). The motor findings suggest a specific interaction between regenerating motor axons and Schwann cell tubes leading to the motor branch. The sensory findings could be a random response to the greater number of myelinated axons in the sensory branch, and are thus more difficult to interpret.

Preferential motor reinnervation was significant in juvenile animals but not in adults. The superior results of nerve suture in children, previously attributed to age-related central reorganization, may therefore also reflect peripheral factors. Definition of the precise mechanism of preferential motor reinnervation and its augumentation, especially in adults, could improve the prognosis of injury to nerves containing both sensory and motor axons.

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291.5 TEMPORAL CHARACTERIZATION OF FUNCTIONAL RECOVERY IN MICE AFTER SCIATIC NERVE REPAIR WITH BIORESORBABLE GUIDES. M. Tang*, Psychology Dept., Rutgers Univ., New Brunswick, NJ 08903, R. Madison & R. Sidman, Neurosci. Dept., Harvard Med. School, Boston, MA 02215, F. Mares* & R. Tang*. Polymer Lab., Corp. Tech., Allied-Signal Inc., Morristown, NJ 07960.

The objective of repairing damaged nerves is to restore function to the affected parts. Except for EMG data, there is sparse information on recovery of function following reconnection of peripheral axons. We now report an attempt to characterize temporally the recovery of sensory motor functions following sciatic nerve repair with bioresorbable polymer nerve guides (Guides 0A1 and 0B1, Allied-Signal Inc.).

24 adult, male C57BL/6J mice were trained to walk on a revolving plane (motor function). Latency to withdraw from a 104^{o}F stimulus was also determined for both hind limbs (sensory function). After training, the sciatic nerve was exposed and transected at mid-thigh. The two stumps were sutured into 0A1 or 0B1 bioresorbable nerve guides (N=8 ea.) to yield a final gap distance of 4mm. Solid guides were used for the remaining 8 mice to prevent regeneration (negative control).

Motor functions were severely affected (40-60%) in all animals at 3 weeks post-surgery. Performance of the 0A1-guide mice recovered fully to pre-surgical levels by the 12th week. Animals with the 0B1 guide recovered to only 80%, even by week 24. Slight improvement was noted in the negative controls, probably due to practice effects. Correlation (r) between motor function and the # of myelinated axons in the regenerated nerve cable revealed S-shaped functions for both repaired groups. Sensory functions recovered steadily throughout the 24-week period to a mean of 85 and 94%, but negative controls, although showing insignificant sensory improvements initially, achieved similar levels of recovery by week 24. For all groups, zero-order r's were obtained between the degree of sensory recovery and the number of myelinated axons.

These data suggest that 1) sensory recovery is independent of regeneration and probably reflects collateral innervation, 2) regenerated nerves support motor function, the degree of recovery dependent on the type of guide and 3) a critical number of regenerated axons is needed before motor function recovers.

291.6 IDENTIFICATION AND CHARACTERIZATION OF cDNA CLONES TO GENES ASSOCIATED WITH PERIPHERAL NERVOUS SYSTEM REGENERATION. H.D. Shine, A.W. Sandrock, W.D. Matthew and L. Villa-Komaroff. Depts. of Neuropath. and Neurobiol., Harvard Med. School and Dept. of Neurosci., Children's Hospital, Boston, MA 02115.

Axons of the peripheral nervous system (PNS) readily regenerate after injury in response to molecules in the PNS environment. While well characterized molecules such as laminin may play important roles, evidence suggests that the regenerative capacity of the PNS is mediated by molecules not yet defined. We have used a combination of hybridoma and molecular biological technologies in an effort to identify and characterize novel regeneration-associated molecules.

To identify molecules resident in the regenerating PNS that may support axonal regeneration a panel of monoclonal antibodies was raised to molecules differentially expressed during regeneration. Mice were tolerized by immunizations with a crude preparation of normal rat peripheral nerve concurrent with injections of the anti-mitotic agent, cyclophosphamide. The mice were then re-immunized with a crude membrane preparation of peripheral nerve that had been cut 4 to 7 days before removal and their spleen cells fused with myeloma cells to produce hybridomas. One antibody, designated RN3B3, localized to Schwann cell basal lamina and blocked axonal growth in a bioassay (Matthew and Sandrock, Neurosci. Abstr. 11:1254, 1985).

To characterize the RN3B3 antigen we used the antibody to identify cDNA clones in an expression library that should contain its message sequence. The cDNA library was created in the bacteriophage lambda gtll to mRNA isolated from developing (1 day-old) rabbit peripheral nerve. The use of the lambda gtll system permits identification of clones of interest by immunochemical means in addition to standard DNA-DNA hybridization methods. Rabbit neo-natal nerve was used to insure that enough tissue was available for efficient mRNA isolation. We reasoned that genes expressed during nerve regeneration would be expressed during development. Immunocytochemical analysis demonstrated that RN3B3 stained neo-natal sciatic nerve. Twenty-two clones were identified via immunochemical screening of approximately 500,000 recombinant bacteriophage. Single-strand probes generated from the sequences recognize message in peripheral nerve and in other tissues shown to immuno-stain with RN3B3. Their sequences are not homologous to sequences presently in the GenBank data base suggesting that they represent novel proteins.

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291.7 A TUBULAR PROSTHESIS FOR NERVE REPAIR: EFFECTS OF BASEMENT MEMBRANE MATERIALS, LAMININ, AND POROSITY. R. Madison. Departments of Neuropathology and Neuroscience, Harvard Medical School, Childrens Hospital, Boston, Ma. 02115.

Since Bunger's initial report in 1891, entubulation repair has been used to repair severed peripheral nerves. Although many different materials have been used to fabricate such prostheses, none have yielded completely satisfactory results. The current study used nerve conduits fabricated from collagen materials and assessed the effects of porosity, and laminin alone or in conjuction with other basement membrane components. Purified laminin was obtained from human placenta. Laminin in conjunction with other basement membrane components (Matrigel, Collaborative Research) was a generous gift of Drs. Kleinman and Martin, NIH.

Thirty-two adult C57BL/6J male mice, four in each of eight groups, received sciatic nerve transections and entubulation repair as detailed previously (Madison et. al., Exp. Neurol., 95:378-390, 1987). Collagen based nerve conduits were fabricated by American Biomaterials Corporation, Plainsboro, N.J. (see also abstract by Shu-Tung Li). Four weeks following entubulation repair animals were perfused with fixatives, the nerve conduit dissected out, and processed for plastic embedding. Semi-thin sections were cut at mid-tube level, stained with toluidine blue, and the number of myelinated axons was determined with a computer assisted microscope. The number of myelinated axons for each group (mean ± std. dev.) is given in brackets following the description of the particular nerve conduit.

All nerve conduits consisted of Type I collagen and/or the following: 1)collagen alone with small pores, (see abstract by Shu-Tung Li) [861±462]; 2)#1 plus purified laminin protein 1:1 (W/W) [1358±454]; 3)#1 plus purified laminin protein 7:1 [1319±96]; 4)#1 plus Matrigel 2:1 (W/W) [1022±506]; 5)#1 plus Matrigel 1:1 [1511±172]; 6)#1 plus Matrigel 1:5 [1505±146]; 7)collagen alone with large pores [3963±1990]; 8)control unoperated mice [3851±196].

The results suggest that increasing concentrations of Matrigel added to Type I collagen nerve conduits or increasing their porosity increases the number of myelinated axons regenerating across the transection site. Analysis of variance and Student-Neuman Keuls comparisons shows only the large porous conduit group to be significantly different from the others. Retrtograde labeling studies are in progress to determine if this increase in the number of myelinated axons represents a greater number of primary motor and sensory neurons that are able to send an axon through the nerve conduit. Supported by NIH grant NS22404.

291.8 Porous Collagen Nerve Conduits for Nerve Regeneration: In Vitro Characterization Studies. Shu-Tung Li (SPON: M.E. Frank) American Biomaterials Corporation, Plainsboro, NJ. 08536

It is well recognized that the functional recovery of an injured nerve depends on the cellular environment. In order to facilitate nerve regeneration, collagen-based tubular guiding conduits were developed. This study was specifically designed to test the effect of the membrane permeability of the guiding conduit on nerve regeneration. I report here the results of in vitro characterization studies of the conduits. The results of in vivo performance of these conduits will be presented in the following paper by Dr. Roger Madison.

Tubular nerve conduits with different pore sizes were fabricated from purified bovine tendon collagen. In order to eliminate material related variations, collagen prepared from the same batch was used to fabricate both types of conduits. Surface and cross-sectional morphology were examined by scanning electron microscopy (SEM). The permeability of the membrane to glucose (MW=180) and to bovine serum albumin (BSA) (MW=68,000) was measured as follows: The collagen conduits of various diameters were filled with a .25%(w/v) glucose or a 1% BSA solution in 0.9%NaCl. The ends of the conduits were ligated and the conduits were then incubated in 15-20 ml of the same solution in the absence of the molecules of interest. The diffusion of glucose and BSA across the membrane was measured spectrophotometrically. Anthrone was used as a reagent for glucose and Coomassie Brilliant Blue G-250 was used as a dye reagent for BSA.

SEM of the small pore conduit showed a uniform structure throughout the cross-section of the conduit membrane. SEM of the large pore conduit showed a two-layered structure, a thin inner layer with tightly packed collagen fibers and an outer layer of open structure. The lumen surface of the small pore conduits had a typical film-like morphology. The lumen surface of the large pore conduits consisted of bundles of collagen fibers intermingled in a multi-layered mesh structure. The results of permeability studies showed that both types of conduits were permeable to glucose. The equilibrium concentration was reached in one hour and six hours for the large pore and small pore conduit, respectively. However, only the large pore conduit was permeable to BSA. Approximately 50% of the BSA diffused across the large pore conduit in 24 hours, whereas none of the BSA diffused across the small pore conduit.

The selective permeability of the conduit membrane to molecules of a defined size provides an experimental tool to investigate the effect of the local cellular environment on nerve regeneration in a systematic, quantitative and mechanistic manner.

291.9 D.C. ELECTRICAL FIELDS DO NOT INFLUENCE MOTOR RECOVERY FOLLOWING RAT TIBIAL NERVE TRANSECTION. J.M. Kerns and J.A Gramm . Department of Anatomy, Rush Medical College, Chicago, IL 60612

Our previous studies have shown that D.C. electrical fields promote the early stages of nerve regeneration following sciatic neurectomy. In the present study, we sectioned only the tibial nerve branch on both sides in eight adult rats to prevent toe-chewing. The nerves were anastomosed with 10-0 sutures. The treatment side received a battery implant delivering 10 uA to the lesion site with the cathode distal, while the contralateral control side had a neutral implant. The twitch tension of the digital flexors was monitored at weekly intervals and compared to preoperative values. At 77 dpo the battery was removed and both distal tibial branches were dipped in HRP for 1 hr, with a 72 hr period prior to perfusion fixation. The lumbosacral spinal cord was removed and sectioned (50 um) for TMB incubation and motoneuron cell counts. At no time during the recovery period was there a side difference in the twitch tension amplitude. The relative index of recovery at 77 dpo was 0.66 (0.05) and 0.61 (0.03) for the treated and untreated sides respectively. This corresponds to a mean ratio of 1.10 (t=0.96). At 80 dpo the mean number of labeled motoneurons was 1372 (68) on the treatment side and 1306 (33) on the no-treatment side. Again, the ratio between sides of 1.06 was not significantly different (t=0.9). The results from these two analytical methods had a correlation coefficient of 0.60. We conclude that the motor recovery in a single nerve fascicle which is transected and conventionally repaired, is not enhanced by the application of a continuous D.C. electrical field at the delivered dose. These and other experimental models and methods are being investigated further. Supported by NIH grant NS 19769 and BRSG S07 RR05477.

291.10 NERVE REGENERATION CHANGES WITH FILTERS OF DIFFERENT PORE SIZE. C.B. Jenq, L.L. Jenq* and R.E. Coggeshall Marine Biomed. Inst. and Depts. Anat. & Neurosci. and Physiol. & Biophys., Univ. Tex. Med. Br., Galveston, TX 77550.

An experimental reason for placing stumps of a transected nerve in an impermeable tube is that substances derived from the nerve stumps are pooled and separated from cells and soluble substances in the body in general. Our previous work showed that regeneration was improved when the impermeable tube was made permeable by cutting macroscopic holes in its side. To begin exploring the reasons for the improvements, we covered the holes in the perforated tubes with filters of 2 different pore sizes, 5.0 µm and 1.2 µm, and quantitated the regenerated axons in the present study. The results shown in the following table are myelinated (MY) and unmyelinated (UN) axon counts from rat nerves at 8 weeks after the sciatic nerves were transected and the stumps separated with an 8mm gap.

REGEN. NERVE	GAP		DISTAL STUMP		SURAL		NMG	
	MY	UN	MY	UN	MY	UN	MY	UN
5.0µm pore	7183 ±1194	17230 ±2607	11523 ± 935	15636 ±3758	850 ±347	2320 ±712	281 ± 93	515 ±241
1.2µm pore	6317 ±1281	13306 ±5108	9206 ±2218	9459 ±2493	448 ±222	1248 ±270	193 ± 10	461 ± 50
Perforated tube	6528 ±1406	21924 ±4727	8468 ±1461	16463 ±5736	814 ±156	2049 ±367	256 ± 73	494 ± 35
Impermeable tube	4642 ±1266	10446 ±1853	7046 ±2262	7638 ±3277	491 ±268	1136 ±420	236 ±154	463 ±266

NORMAL NERVE	SCIATIC		SURAL		NMG	
	MY	UN	MY	UN	MY	UN
N = 8	7905 ± 395	15647 ±1510	973 ±141	3456 ±338	317 ± 53	403 ±106

(SCIATIC: sciatic nerve. SURAL: sural nerve. NMG: medial gastrocnemius nerve)

In the paradigm with the 5.0 µm pore size, cells from the connective tissue space invaded through the millipore filter and mingled with the regenerated nerve in the tube, whereas with the 1.2 µm pore size, no cells were found in the filter. These findings suggest that cells from the general connective tissue should be considered when designing experimental procedures to maximize the regeneration potential of regenerating axons. (Supported by Muscular Dystrophy Association, NIH grants NS17039, NS11255, and the Florence and Marie Hall Endowment).

291.11 PREPARATION OF ACELLULAR NERVE FOR GRAFTING BY COCULTURE WITH MACROPHAGES. A.K. Gulati*, A. M. Behzadian* and T.R. Swift* (SPON: M.J. Mulroy). Departments of Anatomy and Neurology, Medical College of Georgia, Augusta, GA 30912.

It has been proposed that a suitable graft material for peripheral nerve gap repair be composed of basal lamina tubes lying parallel to one another. Earlier methods used in preparing such grafts have included various chemical and mechanical treatments. The present study describes an in vitro method to prepare acellular basal lamina nerves by coculturing them with macrophages. Inbred Fischer rats were used and under chloral hydrate anesthesia the sciatic nerve was cut to allow in situ degeneration. Six to eight weeks later the degenerated tibial and peroneal nerves were removed and cut into 1 cm pieces. Some of these nerves were repeatedly frozen in liquid nitrogen and thawed and then placed individually in culture dishes with RPMI medium supplemented with 20% fetal bovine serum. Five million peritoneal macrophages obtained from another Fischer rat also were included in each dish. The remaining nerves were not frozen and thawed but placed in culture as above for comparison. Nerves for each group were removed after 1,3,5,7 and 12 days in culture for light and electron microscopic analysis. Schwann and perineurial cells in forzen and thawed nerves disintegrated and their cellular debris absorbed by the cocultured macrophages. By 7 days in culture these nerves were completely devoid of cellular elements. However, basal lamina of Schwann cell and perineurium persisted as hollow tubes as determined by laminin antibody staining and electron microscopy. Presence of well organized collagen fibrils was also observed in the endoneurium and perineurium. Analysis of cultured nerves which were not frozen and thawed revealed presence of many Schwann cells, arranged in columns, as well as perineurial cells. Presence of basal lamina and collagen fibers was also observed in the endoneurium and perineurium of unfrozen nerves. These results show that nerves devoid of any cellular structures but composed of basal lamina and connective tissue can be prepared after in vitro coculture with macrophages. Nerves prepared by this in vitro method may serve as suitable graft material for peripheral nerve gap repair.

291.12 CONDUCTION PROPERTIES OF NERVE FIBERS REGENERATED ACROSS GAP BRIDGED BY BIODEGRADABLE POLYMER MATRIX I.V. Yannas,* C. Krarup,* A. Chang,* T.V. Norregaard,* N.T. Zervas, and R. Sethi.* Fibers and Polymers Lab., Mass. Inst. Technology, Cambridge MA 02139, Division of Neurology, Brigham and Women's Hospital, Boston MA 02115 and Division of Neurosurgery, Mass. General Hospital, Boston MA 02114.

An electrophysiological procedure has been used to monitor the extent of physiological recovery of rat sciatic nerve which was cut, generating a 10-mm gap. The gap was grafted with a silicone tube filled with a highly porous, biodegradable collagen-glycosaminoglycan (CG) polymer. A previous study showed that regeneration of a morphologically adequate nerve occurred across a 15-mm gap in silicone tubes which contained CG polymer but not in empty tubes (Yannas, I.V., Orgill, D.P., Silver, J., Norregaard, T.V., Zervas, N.T. and Schoene, W.C., Trans. Soc. Biomaterials 8:146, 1985). Maximal electrical stimulus pulses, 0.1 ms in duration were delivered to the regenerating nerve at the sciatic notch near the hip and at the tibial branch near the ankle. The recording electrode (concentric needle electrode, TECA CF-25) was placed in the plantar muscles. Action potentials were photographed from an oscilloscope. We followed the restoration of conduction in longitudinal studies in a group of 7 rats which were grafted with a silicone tube filled with CG polymer. These rats were grafted contralaterally with empty silicone tubes (controls). No response was registered in the first measurement, performed 3 wks after surgery. The first response was recorded with filled tubes at about 11 wks. Only one of the empty tubes showed evidence of regeneration, about 14 wks after surgery. When a response was first recorded the distal motor latency was 2-3 times longer than normal, the conduction velocity across the gap was less than 40% normal and the amplitude of these early responses averaged less than 5% normal. In subsequent measurements the distal motor latency gradually recovered to near normal, the conduction velocity to near 60% normal and the amplitude recovered to about 20% normal. Even after 25 weeks of follow up only one of the 7 empty tubes showed evidence of regeneration. We conclude that the procedure described adequately monitors the restoration of conduction in the sciatic nerve. These results extend earlier morphological findings on the ability of certain CG polymers to support regeneration of sciatic nerve across a long gap.

VISUAL CORTEX V

292.1 **FLATTENING VISUAL CORTEX AT IMAGE RESOLUTION: QUANTITATIVE COMPUTER RECONSTRUCTION OF THE MACAQUE OCULAR DOMINANCE COLUMN PATTERN** *E.L. Schwartz, A. Shaw* and D. Weinshall** Computational Neuro. Lab. NYU Med. Ctr. 550 1st Ave NY NY 10016 and Courant Inst. of Math. Sciences.

We have previously described an algorithm designed to flatten cortex with minimal distortion or error [1]. This algorithm operates on a three dimensional polyhedral model of the surface of cortex, composed of perhaps several thousand small triangles. This level of detail is sufficient to provide a good approximation to the surface geometry of a cortical structure. However, the level of detail of a polyhedral model (e.g. roughly .5 mm^2) is not sufficient to represent details such as the columnar pattern of V1.

In the present work, we describe an algorithm for constructing an image resolution flattening of Macaque visual cortex. We illustrate this algorithm with a specimen of (Macaque) striate cortex, stained for cytochrome oxidase following enucleation of one eye. The cytochrome oxidase data from coronal sections cut at 40 μ clearly indicated the ocular dominance column pattern. The design goal was to reconstruct this pattern, at the full 40 μ resolution of the digitized sections, in three dimensions. A polyhedral model of striate cortex was then constructed from these data and it was numerically flattened. This yielded a planar model of the cortex, for which the median distance error was 5%. Next, a three dimensional model of layer IV of the stained cortex was constructed. This procedure required the construction of the full three dimensional cortical model at image resolution, and then the use of a "brain peeler" or digital tangential microtome, to "peel" lamina of the model cortex until layer IV was isolated as a thin (40μ) three dimensional section. A computer graphic of a "peel" representing much of the occipital pole will be demonstrated. Finally, the 3D "brain peel", which is a digital image of stained layer IV of cortex, was image mapped into the flattened two dimensional model of the cortex. The result of these steps was a two dimensional image of the ocular dominance column pattern of striate cortex, at 40μ resolution, with 5% metric error. The cortical representation of the optic disk, as well as full details of the columnar pattern, are clearly produced.

This work represents the completion of a system of computer aided neuro-anatomy, which is adequate to manipulate cortical specimens on the scale of an entire occipital pole, at a level of detail sufficient to resolve functional architecture, in both three dimensions and two dimensions. This work allows questions related to the metric structure of visual cortical areas (e.g. topography, columnar geometry, etc.) to be studied with full quantitative rigor.

1. E. Schwartz and B. Merker. IEEE Comp. Graph. and App. 6:36-44 (1986)
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292.2 **A NEW METHOD FOR MEASURING THE VISUOTOPIC MAP FUNCTION OF STRIATE CORTEX:VALIDATION WITH MACAQUE DATA AND POSSIBLE EXTENSION TO MEASUREMENT OF THE HUMAN MAP** *D. Weinshall * and E. L. Schwartz(SPON:E. Flamm)* Computational Neuro. Lab. NYU Med. Ctr. 550 1st Ave NY NY 10016 and Courant Inst. of Math. Sciences.

The observation and measurement of the visuotopic map of primate visual cortex is a classical experimental activity. A number of psychophysical (e.g. vernier acuity, visual acuity, Panum's area, stereo acuity, motion thresholds) and anatomical (e.g. retinal cell densities) measurements bear at least a qualitative relationship to the presumed curve of cortical magnification. However, there is no accurate and direct method for estimating human magnification factor; and even for the case of monkeys, where microelectrode and 2DG experiments have been performed, there is still uncertainty in this area. What is the correct functional form for the primate map? What is the variance of this estimate across a population?

In order to address these issues, we have constructed [1] a computer generated planar approximation to the surface of Macaque striate cortex (median flattening error = 5%). This eliminates errors associated with cortical surface curvature. We then implemented a numerical algorithm which generates the unique isotropic map from the retinal hemisphere to the surface of the cortex, constrained by a single interior point (e.g. the representation of the optic disk) and a direction at this point. We used this algorithm to analyze three different hemispheres (one from our lab, computer flattened; and two from previously published data from other labs). The same analysis was used in each case, which was based on the assumption that the striate map is isotropic (i.e. conformal: this means that magnification factor does not depend *locally* on direction). Although the assumption that Macaque V1 is locally isotropic is the basis of this analysis, this is an assumption which is supported, at least approximately, by several recent experimental studies [2]. We further support this conclusion by showing that the variance and range of map parameters generated by our numerical/anatomical method is comparable to those between existing microelectrode studies. In other words, given as data a flattened V1 and a single topographic location (and direction) within it, we are able to numerically estimate cortical magnification about as well as other existing methods.

Since our numerical-anatomical method is similar in its results to current micro-electrode measurements of V1 topography, it should be possible to apply this method to human visual cortex, and to obtain an estimate of the human map function with a precision which is comparable to current measurements of magnification factor in monkeys.

[1]E. Schwartz and B. Merker. IEEE Comp. Graph. and App. 6:36-44 (1986)
[2]A particular conformal map, based on the complex logarithm, has been stated to be a good approximation to the V1 map in a number of recent experiments: Dow et al, J. Neurosci. 5:890-902; Van Essen et al, Vis. Res. 24:429-448; Tootel et al, Science 227:1066.
[3]Supported by AFOSR #85-0235 and System Development Foundation

292.3 EMERGENCE OF CYTOARCHITECTONIC DIFFERENCES BETWEEN AREAS 17 AND 18 IN THE DEVELOPING RHESUS MONKEY.

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There are striking differences in laminar patterns of neurons in areas 17 and 18 of primate neocortex. In addition to qualitative differences between these visual areas, it has been reported that the number of neurons in 30-μm-wide columns of cortex is about 2.5 times greater in area 17 than in area 18 of adult monkeys (Rockel et al., Brain **103**:221, 1980). We have used computer-aided methods to study the emergence of cytoarchitectonic differentiation of these two areas in developing rhesus monkeys, and have tested the possible contributions of neuron death and differential expansion of cells and neuropil in the development of this marked cortical heterogeneity.

Comparisons of neuron number and density were made in areas 17 and 18 of twelve monkeys ranging in age from embryonic day E80 to birth (E165), in 4 neonates, and in 10 adults. Particular care was made to quantify sites on single sections in which curvature of occipital cortex was minimal. Sites were generally 1 to 2 mm on either side of the 17-18 border. Video-enhanced differential interference contrast microscopy, in combination with a video-overlay system, was used to get accurate counts of Nissl-stained neurons. Series of adjacent fields, 30-μm wide, extending through the full thickness of cortex were counted and summed.

We found that at maturity the number of neurons per radial probe is on average 1.5 times greater (range from 1.3 to 2.2) in area 17 than in area 18 of ten adult monkeys. Variation in the ratio of neurons per radial unit is substantial. No relation was evident between the ratio and the retinotopic locale that we examined. The higher values in area 17 are mainly due to the greater density of neurons in the supragranular layers II and III, and to a lesser degree to a greater density of neurons in sublaminae of layers IV and VI. As early as E85 the boundary separating 17 and 18 can be recognized in Nissl-stained tissue, nearly two weeks before the last neurons destined for layers II and III have been generated (Rakic, Science **183**:425, 1974). At this age there is a clear distinction between layers V and VI in areas 17 and 18. Nonetheless, counts between E85 and E99 revealed no consistent difference in the number of cells per radial unit in 17 and 18 (n=4). Ratios ranged from 0.9 to 1.1. In contrast, in prenatal animals between E118 and birth (n=5), the 17-18 ratio was only slightly less than the adult average (1.4 vs 1.5). The ratios appear to change most rapidly over a period of less than 1 month—from E100 to E120. This change is associated with the breaking up of the undifferentiated cortical plate into layer IV and its sublaminae, with the emergence of the supragranular layers, and with expansion of the deep cortical layers. The quantitative changes between areas 17 and 18 are not due to the selective elimination of neurons in area 18 since cell death is insignificant prior to E118 in both areas. Furthermore, the incidence of cell death is somewhat greater in area 17 than in area 18 in cases examined at E118 and E128, and is largely limited to supragranular layers.

The emergence of cytoarchitectonic differences between areas 17 and 18 correlates well with the ingrowth of thalamic fibers into the cortical plate (Rakic, Nature **261**:467, 1976) and with the emergence of the mature pattern of cellular lamination. The results indicate that the quantitative difference between area 17 and area 18 is due principally to a differential increase in neuropil that causes greater horizontal (areal) expansion of area 18 than of area 17 between E100 and E120.

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292.4 DISTRIBUTION, DENSITY AND ULTRASTRUCTURE OF SYNAPSES IN THE VISUAL CORTEX IN MONKEYS DEVOID OF RETINAL INPUT FROM EARLY EMBRYONIC STAGES.

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Early bilateral enucleation in rhesus monkey embryos markedly reduces the number of geniculocortical axons and surface area of the striate cortex, but has little effect on its thickness, cytoarchitectonic layering pattern (Rakic and Williams, Abstr. Soc. Neurosci., '86), or on the laminar distribution of several neurotransmitter receptors (Rakic et al., Abstr. Soc. Neurosci., '87). Here, we examine the effect of this procedure on synaptoarchitecture of the striate cortex.

Binocular enucleation was performed at embyonic (E) days E67 and E59, prior to the growth of geniculocortical fibers into the developing cortical plate (Rakic, '76, Nature, 261:467). Fetuses were returned to the uterus, delivered at term (E165) and sacrificed at 3 months and 3 years, respectively. In experimental animals and in age-matched controls, 3 to 6 vertical probes were prepared. Each probe consisted of about 100 overlapping electron micrographs spanning the thickness of the cortex in the posterior bank of the calcarine fissure. All synapses were counted and classified according to our standard protocol (Bourgeois and Rakic, Abstr. Soc. Neurosci., '83). In agreement with the previous study, the striate cortex had normal thickness, and a normal complement of layers and sublayers. The mean density of synaptic contacts per unit of neuropil across all cortical layers in the striate cortex of the two operated animals was similar to that in their corresponding age-matched controls (ca. 30 contacts per 100um^2 of neuropil). The proportions of synaptic contacts on dendritic spines (ca. 75%) and shafts (ca. 25%) were unchanged in all cortical layers of enucleated animals, except in layer IVC of the 3 year-old operated animal, where these proportions were reversed. The proportion of symmetric versus asymmetric synapses and their mean length was within the normal range of variability. The normal range of density, size and proportion of synapses in area 17 of early enucleates came as a surprise given that the number of geniculate neurons which project to the striate cortex was drastically reduced and that they never received any information from the retina. However, the results are in harmony with findings of similar synaptic densities recorded in functionally and anatomically distinct cytoarchitectonic areas that receive different types and amounts of thalamic input (Rakic et al., Science, '86, 232:232). Our observations indicate that the density of synaptic contacts per unit of neuropil may develop to a certain optimal level in the cortex, regardless of the number or type of thalamocortical connections and their functional properties. Supported by EY02593 and Program Project NS22807.

292.5 INNERVATION OF MONKEY STRIATE CORTEX BY PHYSIOLOGICALLY IDENTIFIED AND HRP-FILLED THALAMOCORTICAL AFFERENTS

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Single thalamocortical axons were recorded in the white matter or layer VI of the monkey striate cortex, and visualized by intraaxonal injection of HRP. Two axons were characterized - on the basis of their response to visual stimuli - as originating from magnocellular (MA) and two from parvocellular (PA) LGN neurons. Camera lucida or computer-assisted three dimensional reconstruction of the axons revealed that the MA axons had extensive arbors in layer IVCα, forming 2-3 distinct clumps at 200-400μm intervals, each being 400-800μm in diameter, elongated in the anteroposterior direction. The PA afferents terminated in layer IVCβ. One of them had two 150-200μm diameter clumps with approximately 200μm interval, the other had a single arbor of 200μm diameter. Collaterals to layer VI from both types of afferents were very sparse. The MA axons had at least twice as many boutons as the PA axons. Electron microscopy of 272 (115 MA, 157 PA) boutons of these axons revealed that all gave at least one synapse in case of both axons. The maximum number of synaptic contacts made by a single bouton was 5. The average number of synapses per bouton was found to be higher for the MA afferent (2.03) than for the PA axon (1.79). Most postsynaptic elements were tested for GABA in immunoreactivity by a postembedding immunogold procedure.

MA afferents				PA afferents			
Total	Somata	D.shafts	Spines	Total	Somata	D.shafts	Spines
229	3(1.3)	108(47.2)	118(51.5)	224	7(3.1)	74(33.0)	143(68.9)
				GABA(+)			
8.1%	100%	14.4%	0%	8.5%	100%	16.4%	0%

Numbers in table refer to number of synapses, those in brackets are percentages of total. Numbers in lower line indicate percentages of targets immunoreactive for GABA.

Both types of axon contacted a much higher percentage of dendritic shafts than X and Y axons in the cat (Freund et al., J.comp.Neurol.242:263-274, 1985). A large number of the target dendrites contained lamellar bodies (an organelle similar to the spine apparatus in structure) near the site of the thalamic synapse. These showed the ultrastructural characteristics of spiny dendrites, and were invariably negative for GABA. The higher number of boutons of the MA axons together with a greater proportion of synapses/bouton means that the MA input to the striate cortex is magnified relative to the PA input.

292.6 LOCAL CIRCUIT NEURONS IN LAYERS 5B AND 6 OF MONKEY STRIATE VISUAL CORTEX.

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Using Golgi Rapid impregnations of infant monkey striate cortex, a study has been made of the organization of local circuit (LC) neurons in layers 5B and 6. These neurons have smooth or sparsely spined dendrites in the adult and the great majority contain the inhibitory neurotransmitter GABA; they comprise 15-25% of the neuron population in the cerebral cortex. The LC neurons of layers 5B and 6 differ from each other in their axon projections to the more superficial layers; LC neurons with dendrites restricted to layer 5B make axon projections to layers 2 and 3A, while LC neurons lying in layer 6 project to 5A, 4C, 4A and 3B. These LC axon projections parallel the different recurrent axon projections of pyramidal neurons in layers 5B and 6. Some varieties of LC neuron have axons and dendrites that pass between layers 5B and 6 suggesting inhibitory interaction occurs between the layers. Several varieties of the layer 5B LC neurons send axon trunks into the white matter, raising the possibility that inhibitory neurons may make efferent projections from this layer.

The diverse axon characteristics of LC neurons in layers 5B and 6 suggest the presence of a wide variety of these neurons in the deep layers. In lamina 5B 'chandelier' neurons are found with dendrites and characteristic axon arbors (targeting pyramidal neuron initial axon segments) restricted to layer 5B and the 5B-6 border. The junctional region of layers 5B and 6 seems to be a specialized region with 'basket' LC neurons lying within it with horizontally oriented dendrites and large diameter stout axon trunks running long distances within this border region emitting vertical terminal collaterals at intervals. These 'basket' neurons resemble similar neurons found in upper lamina 4Cα whose axons spread laterally in layers 4B and 4A; these neurons may form part of the substrate for direction selective responses which characterize layer 6 and the upper 4Cα-4B-4A region. Also in upper layer 6 and the 5B-6 border zone lie the cell bodies of the largest pyramidal neurons of striate cortex, whose axons innervate the motion sensitive cortical area MT and project to the superior colliculus. The somata of these giant pyramidal neurons are encased in true 'basket' arrays of terminal boutons arising from the axon trunks of a distinct population of 'basket neurons' lying in layer 6 with dendritic characteristics in common with 'chandelier' neurons.

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292.7 THE TWO TYPES OF IPSPs IN NEOCORTEX HAVE VERY DIFFERENT EFFECTS ON REPETITIVE FIRING OF PYRAMIDAL CELLS. L.R. Silva and B.W. Connors. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Two types of IPSP may be evoked onto pyramidal cells of the neocortex: 1) $IPSP_1$ is fast, Cl^--dependent, high conductance, and $GABA_A$-mediated; 2) $IPSP_2$ is prolonged, K^+-dependent, low conductance and possibly $GABA_B$-mediated. We have examined how each of these IPSPs modifies the input/output properties of cortical neurons. Coronal slices of rat somatosensory cortex and cat primary visual cortex were prepared and maintained in vitro. Intracellular recordings were made from pyramidal neurons in layers II-III. Intracellular current pulses (> 200 msec) of varying amplitudes were applied to generate repetitive action potentials.

Firing frequency of pyramidal neurons shows strong accomodation. The frequency vs. current (f/I) function for the first interspike interval was initially very steep (mean of 492 Hz/nA), but fell abruptly to a secondary slope of 87 nA a few tenths of a nA above action potential threshold. The f/I function for steady-state firing yielded a single, nearly linear slope across the entire current range, with a mean of 31 Hz/nA. GABA, focally applied to the recorded cell, strongly activates $GABA_A$ receptors. Small GABA applications caused a profound increase in threshold (the current intensity needed to evoke a single spike) and the moderate applications completely eliminated single or repetitive spikes at currents up to 3 nA. Similarly, during $IPSP_1$ the threshold for one spike was greatly increased, and repetitive spikes were abolished. $IPSP_1$ completely suppressed steady state firing at all currents tested.

Baclofen specifically activated $GABA_B$ receptors and mimicked $IPSP_2$. In baclofen, thresholds were somewhat increased, and the primary slope of the initial f/I was shifted to the right by a mean of 0.26 nA. In addition, the steepness of this slope was increased to a mean of 792 Hz/nA. At high current intensities, the firing rate for the initial spikes in a train was actually slightly higher in the presence of baclofen than in its absence. In contrast, steady-state firing was slightly depressed at all currents. $IPSP_2$ effects were similar to those of baclofen.

We conclude that the two types of IPSP have very different functions in the neocortex: $IPSP_1$ profoundly depresses firing during both phasic and tonic stimuli. In contrast, the effects of $IPSP_2$ are more subtle; thresholds for phasic and tonic firing are increased, but the sensitivity of initial firing rates is increased within a low and narrow current range. In addition, $IPSP_1$ provides precise temporal control of firing, whereas $IPSP_2$ exerts a more protracted modulation. Supported by the NSF, NIH and the Klingenstein Fund.

292.8 SEROTONERGIC SYNAPSES IN MONKEY PRIMARY VISUAL CORTEX. A.D. de Lima*, F.E. Bloom and J.H. Morrison (Spon: W. Young) Division of Preclinical Neurosciences and Endocrinology. Research Institute of Scripps Clinic, La Jolla, CA 92037.

Efferent fibers from the serotonergic raphe nuclei are known to reach the entire neocortex where they presumably release serotonin. The macaque neocortex receives a very dense serotonergic innervation, which reaches its highest density in layer IV of primary sensory regions such as primary visual cortex. We studied the morphology and distribution of serotonergic fibers and synapses in the primary visual cortex of adult cynomolgus monkeys using an antibody against serotonin and the avidin-biotin method. In addition we quantified the laminar distribution of labeled varicosities by light microscopy using computer assisted counting programs (EMMA; W.G. Young et al., Neurosci. Abstr., 1985). In accordance with previous studies in this species, we observed that although serotonin immunoreactive fibers were labeled in all cortical layers, at least three bands with higher density of innervation could be readily recognized at low magnifications. The most dorsal band, also the widest (approx. 0.6 mm), was coincident with layers IIIB to IVC-alpha. A second band, fairly thin (approx. 0.1 mm), was coincident with sublamina VA. Ventrally, layer VIB formed a third dense band of innervation (approx. 0.3 mm thick). Among these regions, fibers and varicosities were most numerous in sublayers IVB and IVC-alpha ($3870/mm^2$). The lowest number of varicosities was in layer I ($685/mm^2$). At the electron microscopic level, synaptic contacts were observed through the entire thickness of area 17, with higher frequency in layer IV. The labeled synaptic varicosites (mean diameter = 545 nm) were packed with synaptic vesicles and formed type I synaptic complexes (mean length = 242 nm) presenting conspicuous post-synaptic densities. Dendritic shafts were the most common post-synaptic target of the labeled synapses. Among these post-synaptic dendrites, which were characteristically of small diameter (mean = 355 nm), profiles with ultrastructural features of both spiny and smooth dendrites were observed. The small diameter of most of the post-synaptic dendrites and the absence of axosomatic synapses indicates that distal dendrites are preferentially contacted by serotonergic varicosities. In conclusion, while direct identification of the postsynaptic neurons will be required for complete characterization of this circuitry, these data suggest that serotonergic interactions in visual cortex are directed more towards the distal dendrites of granular and subgranular neurons than to targets in the supragranular layers.

292.9 THE IN VITRO VISUAL CORTICAL SLICE IN RODENT AS A MODEL SYSTEM FOR STUDYING CORTICAL MICROCIRCUITRY, REVEALED BY CURRENT SOURCE DENSITY ANALYSIS (CSD). G. Vaknin, T.J. Teyler. Dept. of Neurobiolgy, N.E. Ohio Univ. Col. of Med., Rootstown, OH 44272.

Some of the advantages of the in vitro cortical slice in studying cortical microcircuitry are stability and reproducibility of the evoked response, the precision in which one can place the recording and stimulating electrodes and the ability to change the media surrounding the tissue. One of the limitations of the visual cortical slice is the discreteness in stimulating the white matter. The following study is an attempt to overcome this limitation. We have used Current Source Density (CSD) analysis to study neocortical microcircuitry in 400um thick coronal and saggital slice of rat and mouse visual cortex. A 60um diam. microbipolar stimulating electrode was positioned in the white matter (dorsally in coronal slice, ventrally in saggital slice) or in the gray matter (every 200um from surface to white matter). A laminar field potential profile was recorded in area OCl perpendicular to white matter (spacing of 100um in rat and 50um in mouse) , from which 2nd nearest neighbor one dimensional CSDs were calculated. In coronal slices recordings were made on-line (perpendicular to stimulation site) and off-line (200, 400um lateral; 200, 400, 600um medial). In saggital slices the same procedure was used in the rostral-caudal axis. In the experiment in which gray matter was stimulated the recordings were made 300-400um off-line (lateral) in both coronal and saggital slices.

The CSD analysis revealed the following pattern of activity: A) coronal slice white matter stimulation, recording off-line 200 to 400um.,(medially),: the earliest sinks in layer IV are seen in some cases. The sinks in layer III with the corresponding sources above are prominent and seen in every profile. Sinks in layer I and II are not seen in most cases. The infragranular layers are characterized by massive sinks in layer VI drawing its current from above. Recording laterally reveals only the infragranular activity. B) saggital slice, white matter stimulation, recording off-line 250 to 350um., (caudally),: the supragranular layers are characterized by massive sinks in layers I and II with corresponding sources in layers III and IV. The infragranular layers are characterized by a large sink in layer V which draws its current from layer VI. Recording more caudally or rostrally results in diminished infragranular activity, the supragranular activity is still prominent but smaller. C) gray matter stimulation and recording: in both coronal and saggital slices, stimulation in layers I/II reveals sinks in layers I/II. As the stimulating electrode is lowered to layer IV/V additional sinks appear at corresponding depths. Below layer IV/V, additional sinks are seen, but supragranular sinks decrease.

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292.10 THE DISTRIBUTION AND CELLULAR LOCALISATION OF MUSCARINIC ACETYLCHOLINE RECEPTOR SUBTYPES IN THE DEVELOPING CAT STRIATE CORTEX. Glen T. Prusky, Christopher Shaw*, and Max S. Cynader. Departments of Psychology and Physiology and Biophysics, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

The selective affinity of the muscarinic acetylcholine receptor (mAChR) antagonist pirenzepine (PZ) for the M_1-mAChR, and the mAChR agonist oxotremorine-M (OXO-M) for the M_2-mAChR have been demonstrated in membrane receptor binding experiments. In this study we investigate the binding site characteristics, laminar distribution and cellular localisation of M_1 and M_2-mAChR during development of the cat striate cortex using $[^3H]$PZ and $[^3H]$OXO-M as selective ligands with in vitro receptor autoradiography.

$[^3H]$PZ (M_1) binding sites are primarily localised in the superficial and deep cortical layers of adult cat striate cortex (layers 1-3 and 5-6) with the most dense labelling in layer I and a distinct band in layer V. $[^3H]$OXO-M (M_2) binding sites also avoid the middle cortical layers, but labelling with this ligand is most prominent in layers V and VI with less pronounced binding in layers I and II.

Surgical isolation of a portion of striate cortex in adult animals does not reduce $[^3H]$PZ or $[^3H]$OXO-M binding in the deafferented cortex, but neuron-specific excitotoxic lesions of striate cortex with quinolinic acid abolish both populations of mAChR. This shows that neither M_1 nor M_2-mAChR are located on neuronal afferents terminating within striate cortex, but rather that they are associated with intrinsic cortical neurons.

Immediately following birth, M_1-mAChR are specifically localised in cortical layer IV. The number of M_1-mAChR increases in this and other layers, achieving a homogeneous distribution across all cortical layers by postnatal day 30. Between days 30 and 50 this distribution alters and the adult pattern of binding develops with dense labelling in the superficial and deep cortical layers, while layer IV contains relatively few M_1-mAChR. M_2-mAChR are also localised in layer IV at birth, but layer VI is also densely labelled at this time. The M_2 receptors continue to increase in number in layers IV and VI and are still concentrated in these layers until day 60. Thereafter, the receptors redistribute and achieve their adult pattern by day 90. Thus, although both M_1 and M_2-mAChR populations reverse their specific laminar distribution during the critical period for visual development, the time-course of this redistribution is several weeks later for M_2-mAChR than for M_1-mAChR.

The differential time-course of redistribution of these two classes of mAChR may be relevant to the mechanisms of critical period plasticity in kitten visual cortex.

292.11 CELLULAR LOCALIZATION OF RECEPTOR POPULATIONS IN CAT VISUAL CORTEX USING QUINOLINIC ACID LESIONS C. Shaw*, G. Prusky, F. Van Huizen* and M. Cynader (SPON: R. Brown) Department of Psychology, Dalhousie University, Halifax, N.S., Canada, B3H 4J1

We have previously described the laminar and areal distributions of a number of neurotransmitter receptors in cat visual cortex (Shaw et al., Brain Res. Bull., 16:661, 1986). We now extend our localization of these receptors to the cellular level by using quinolinic acid (QA), an excitotoxin which selectively destroys neurons and their processes, while sparing glia as well as axons and axon terminals originating outside the lesion zone (Schwarcz et al., Science, 219:316, 1983).

A series of 1 μl QA injections (300 nmol/μl saline, pH. 7.4) were made in the visual cortex of adult cats using a Hamilton microsyringe. Injections were made so as to distribute the 1 μl QA to all cortical laminae. Animals were sacrificed 4 - 14 days after QA injections and the visual cortex processed for electron microscopy (EM) or in vitro receptor autoradiography. QA damage was found in all cortical laminae although the areal extent of damage appeared greatest in layer VI. EM at 4 and 14 days revealed a general and severe loss of neurons and the presence of cellular 'debris' in the affected zone. Axon terminals (which made no synaptic contacts), fibers of passage and glia were noted in approximately normal proportions as compared to unlesioned zones. After 14 days survival some proliferation of astrocytes was noted.

In vitro autoradiography in the lesion zone revealed a drastic decrease in the following receptors: the various putative subtypes of muscarinic acetylcholine (ACh) (labelled with ^{3}H-QNB, ^{3}H-NMS, ^{3}H-pirenzepine, ^{3}H-oxotremorine-M), A_1-adenosine (^{3}H-CHA), cholecystokinin (^{3}H-CCK-5), NMDA/quisqualate/kainate (^{3}H-glutamate, ^{3}H-AMPA, ^{3}H-kainate) $GABA_B$ (^{3}H-baclofen) and in the voltage-sensitive calcium channel binding site (^{3}H-PN-200). In contrast, nicotinic ACh (^{3}H-nicotine), $GABA_A$ (^{3}H-muscimol) and benzodiazepine (^{3}H-FNZ) receptors were either unaffected or increased in number 4 days after QA injections. At 14 days however, $GABA_A$ and benzodiazepine receptor densities were reduced.

The receptors which disappear 4 days after QA injections presumably represent those located on neurons or neuronal processes. Those receptors which survive must reside on axon terminals, fibers of passage, cellular debris and/or glia. The loss of certain receptors at 14 days ($GABA_A$ and benzodiazepine) may suggest a secondary and more selective loss after QA of the cellular elements with which these receptors are associated.

The present results extend our description of receptor loci to the cellular level and provide a more comprehensive view of cortical 'chemical circuitry'.

292.12 DIFFERENCES IN THE RICHNESS OF THE ENVIRONMENT INDUCE CHANGES IN THE RECEPTIVE FIELD PROPERTIES OF CAT VISUAL CORTEX NEURONS. C. Beaulieu and M. Cynader. Dept. of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada. B3H 4J1.

In a recent study on the cat visual cortex, it was demonstrated that the size and the number of synaptic contacts associated with flat vesicles (FS synapses) are affected by the richness of the animal's environment during development (Beaulieu and Colonnier Soc. Neurosci. Abs. 11: 226, 1985). Since the vast majority of FS synapses are GABAergic (Somogyi and Hodgson, J. Histochem. Cytochem. 33: 249, 1985) and many of the properties of visual cortex neurons are GABA dependent, it has been suggested that these morphological synaptic changes induced by the richness of the environment correlate with differences in cortical receptive field properties. In the present study, this has been verified by recording the action potentials of area 17 cells in two groups of cats raised either in isolation (impoverished condition, IC) or in a colony (enriched condition, EC).

To date, 152 cells have been recorded in EC and IC cats. Quantitative estimations have been made of spontaneous activity and of orientation and direction selectivities. Responses were quantified using a PDP/11 computer.

Spontaneous activity is not significantly affected by the richness of the environment. However, orientation tuning is not as sharp in IC animals (mean half width at half height is equal to 51^o) as in EC cats (32^o). Moreover, the decrease in the cellular responsivity at 90^o from the preferred orientation is not as pronounced in IC cats. At the orthogonal orientation, only 70% of the impoverished cells show activity decreases of at least half of their maximal response while this proportion reaches 99% in enriched cats. In addition, modest diminutions in the proportion of direction-selective cortical cells are found in IC animals. It appears however, that this selectivity is less affected by the richness of the environment than the width of the orientation tuning.

These physiological differences support the hypothesis of a structure-function relationship between FS synapses and the receptive field properties of the visual neurons. (Supported by MRC and NSERC Canadian grants to M.C. and by FRSQ postdoctoral fellowship to C.B.)

292.13 RECOVERY OF CYTOCHROME OXIDASE ACTIVITY IN THE ADULT MACAQUE VISUAL SYSTEM AFTER TERMINATION OF IMPULSE BLOCKAGE DUE TO TETRODOTOXIN: (SPON: L.K. Vaughn). E.W. Carroll* and M. Wong-Riley. Depts. Basic Sci., Marquette U. Sch. of Dent., and Anat. & Cell. Biol., Med. Coll. of Wis., Milwaukee, WI 53233.

Previously, we have shown that the blockage of impulse transmission with intravitreal injections of tetrodotoxin (TTX) led to significant yet reversible changes in cytochrome oxidase (C.O.) activity in the adult cat visual system (Wong-Riley & Riley, '83), and the effects of TTX in the monkey visual system (Wong-Riley & Carroll; '83,'84). The present investigation sought to examine the morphological basis of recovery in C.O. activity in the macaque striate cortex by following the time course and quantifying the areal recovery in the metabolically active laminae II-III "puffs". Nine adult monkeys (M. mulatta) were monocularly injected with 19ug TTX in 10 ul D.W. every 3-4 days for 1wk, 2wks and 4wks. Recovery times were: 6wks for 1wk TTX; 4wks, 5wks and 9wks after 2wks of TTX; and 4wks for 4wks TTX. Animals were perfused and tissues reacted for cytochrome oxidase histochemistry as in our previous studies. Serial tracings were made of the puffs, and areal measurements (N=100/animal) made using a digitizing pad. Results were contrasted with normal and saline injected controls and TTX treated animals. Retinae and dorsal lateral geniculates (dLGN) of recovery (R) animals were compared with corresponding TTX_2 treated animals. In controls, average puff area was 50,176 um^2 (saline controls=49,277 um^2). Retinae of all recovery animals were similiar to controls; however, with only 4wks recovery, slightly diminished C.O. activity was still evident in the inner plexiform layer of the retina, dLGN and lamina IVC. With additional recovery time, little if any differences were detected in the retina, dLGN and cortex. After 1wk of TTX_2 the average size of "affected" puffs had decreased to 36,965 um^2 (26% decrease). 1wkTTX/6wksR: average area of "recovered" puffs had increased to 47,496 um^2 (95% of control). With 2wks TTX, affected puffs averaged 33,337 um^2 (34% decrease). 2wksTTX/4wksR: average area of TTX recovered puffs was 45,528 um^2 (92% control). 2wksTTX/ 5wks and 9wksR: as ocular dominance columns were not visible, areas represent an average of recovered and unaffected puffs. After 5wks and 9wksR, puffs averaged 50,914 um^2 and 53,054 um^2 respectively (comparable to controls). 4wksTTX/4wksR: average area of recovered puffs was 45,528 um^2 (92% control) while unaffected puffs averaged 51,615 um^2. These results indicate that, given sufficient time for recovery from intravitreal TTX injections, and based on morphological criteria, there is complete recovery of the C.O. activity in the puffs after retinal impulse blockage. (Supported by NIH EY05439; MWR).

292.14 EFFECT OF MONOCULAR LID-SUTURE, ENUCLEATION AND RETINAL IMPULSE BLOCKAGE ON THE VOLUME OF CYTOCHROME OXIDASE-RICH PUFFS IN THE ADULT MACAQUE STRIATE CORTEX. T.C. Trusk and M.T.T. Wong-Riley, Dept. of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, WI, 53226.

Monocular enucleation and retinal impulse blockage with tetrodotoxin (TTX) have been shown to selectively decrease the cross-sectional area of cytochrome oxidase (C.O.)-rich puffs within laminae II and III of macaque striate cortex (Horton, 1984; Wong-Riley & Carroll, 1984). The affected puffs lie in exact register with lightly-stained bands in layer IVC, where afferent input from the deprived eye predominates. The effect of prolonged lid-suture on C.O. staining patterns in the adult is less clear. In this study, we compared the volumes of supragranular C.O.-rich puffs from the primary visual cortex of adult *Macaca fascicularis* that had been monocularly deprived by either 2 weeks of enucleation (ME), 2 weeks of retinal impulse blockage (intravitreal injections of 19 ug TTX in 10 uL water every 3-4 days), or lid-suture for periods ranging from 1 to 2.5 years. The volume of C.O.-reactive tissue within puffs was obtained from three-dimensional reconstructions of striate cortex sectioned tangentially and processed for C.O. histochemistry. Consistent with previous findings, puffs in all visually deprived monkeys were arranged in alternating rows of large, dark- and small, lightly-stained puffs. The center-to-center spacing of puffs both within and between rows was consistent indicating little evidence of tissue shrinkage. The volume of C.O.-reactive tissue within control puffs from large, darkly-reactive rows was similar in all visually deprived monkeys (0.0112, 0.0122, and 0.0124 mm^3 in ME, TTX and lid-sutured macaques, respectively). The volume of treatment puffs from small,lightly reactive rows decreased by nearly 50% in ME- (0.0068 mm^3) and TTX-treated animals (0.0063 mm^3), and was reduced by 25% in lid-sutured monkeys (0.0088 mm^3). Preliminary results indicate that these reductions in volume result from less reaction product in peripheral regions of the treatment puffs. In lamina IVC, light- and darkly-reactive bands in register with treatment and control puff rows were evident in ME- and TTX-treated monkeys. In contrast, C.O. staining in layer IVC of lid-sutured monkeys was heavy and homogeneous throughout. It is worth noting that this result is not consistent with the observation of lamina IVC banding in younger macaques lid-sutured for shorter periods (Hendry and Jones, 1986; Horton, 1984). These results suggest that the mature macaque visual cortex responds differentially to these forms of visual deprivation. The effects of long term lid-suture in adult macaques are more prominent in supragranular striate cortex, reflecting a special type of functional deprivation in the fully-developed visual system. [Supported by NIH EY07016 (TCT) and EY05439 (MWR).]

292.15 THE EFFECT OF 2-AMINO-4-PHOSPHONOBUTYRATE (APB) ON INDUCED CONTRAST PERCEPTION IN MONKEYS. R.P. Dolan and P.H. Schiller. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA. 02139.

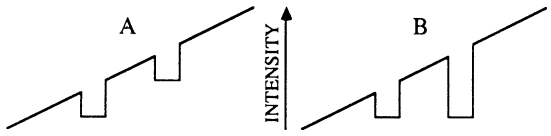
The phenomenon of induced contrast perception, whereby the perceived brightness of a stimulus is dependent on the surrounding intensity, is well documented in humans. The present studies were designed to determine the role of background in the perception of induced contrast in the monkey. The contribution of the ON channel in this phenomenon was assessed by selective blockade with APB.

An adult rhesus monkey was trained to discriminate the lighter or darker of two stimuli. Trials were initiated when the monkey fixated a small centrally placed black square, after which two stimuli would appear to the left and right of fixation. The background was either homogeneous or graded in intensity from left to right. One of the two stimuli was varied in intensity and the two stimuli were considered to be equal in perceived brightness when the choice between them became random. After characterization of the animal's performance, an intravitreal injection of APB was made under halothane anaesthesia and the monkey was tested with the other eye patched.

The results show that the apparent brightness of the stimuli prior to application of APB depended primarily on the difference in intensity between each stimulus and the immediate background rather than on the absolute intensity of the stimuli. Thus in diagram A below the two stimuli would be perceived as equally dark whereas in B the stimulus on the right would be perceived as much darker. Following APB administration performance on the light stimuli decreased markedly. Performance on the dark stimuli showed only a minor deficit, and as before the injection, the animal's perception depended on the difference between the stimuli and the background and not on absolute intensity.

These findings show that for dark stimuli induced contrast perception does not depend on the ON channel, suggesting that the center-surround mechanisms assumed to be involved are created independently within each of the two channels.

Supported by NIH EY00676 and NSF BNS8310399.



292.16 COLUMNAR ORGANIZATION OF VISUAL CORTEX IN DOLPHIN BRAIN. P. J. Morgane, M. S. Jacobs and I. I. Glezer. Worcester Foundation for Expt. Biology, Shrewsbury, Mass. 01545, Dept. Pathology, NYU Dental Center, New York, N.Y. 10010, Dept. Anatomy, City University of New York Medical School, New York, N.Y. 10031.

We have used computer assisted image analysis techniques to study the visual cortical areas in the dolphin brain which have been mapped physiologically by the Russian workers (Supin et al., 1978). By use of cytoarchitectural analyses we identified two types of cortex in the visual areas of the lateral gyrus: (1) heterolaminar cortex corresponding to areas in and bordering the entolateral sulcus from which primary type visual evoked responses were obtained and (2) homolaminar cortex corresponding to areas lateral and medial to the entolateral sulcus from which secondary type visual evoked responses were obtained. Using computer assisted morphometric methods we demonstrated for the first time in the dolphin cortex a columnar type of organization with both major and minor columns being found in both types of cortex. Our determinations of the columnar organization of dolphin visual cortex relates to the cytoarchitectonic structure of columns and these should not at this stage be considered equivalent to physiological columnar organization as quantified by immunofluorescence and other techniques. In our studies comparative analyses were done between dolphin, human and bat visual cortices. In dolphins the major columns averaged 168 μ in diameter while in human striate cortex these cytoarchitectonic columns measured 105 μ in diameter indicating that we are measuring only a component of the larger physiological column which average 600-1000 μ in the human. The small architectonic columns average 20 μ diameter in the dolphin versus 15 μ in human striate cortex. The frequency of small columns per mm of cortex in dolphin is approximately one-half the value for the human whereas the frequency of the major columns is about two-thirds the human value. The diameter of the minor columns in bat brain is larger than these columns in human area 17 but smaller than in both types of dolphin visual cortex. The major columns in visual cortex of the bat are intermediate in size between human and dolphin. These studies indicate, even in models of the so-called "initial" brain organization represented by the bat and dolphin that the same fundamental vertical organization of cortical cellular elements prevails. The radial cytoarchitectonic columns revealed in the dolphin appear to be components of larger columns such as the dominance columns in human visual cortex which reach approximately 1 mm in diameter. Further studies are in progress to more precisely establish the boundaries of the major and minor columns in both types of visual cortex we have identified in the dolphin. Additionally, it is important to examine the organization of columns in a brain such as in dolphins where the primary afferent input is to layer I rather than layer IV. (Supported by NSF Grant 85-45732 and NIH Grant HD 06364).

292.17 SPECIFIC FEATURES OF BLOOD-BRAIN BARRIER ULTRASTRUCTURE IN DIFFERENT REGIONS OF THE DOLPHIN BRAIN(Stenella coeruleoalba). Glezer I.I., Morgane P.J. and M.S. Jacobs. CUNY Med. School, New York, NY 10031, Osborn Laboratories of Marine Sciences of New York Aquarium. New York Zoological Society, Brooklyn, NY 11224, Worcester Foundation for Exp. Biology, Shrewsbury, MA 01545.

The ultrastructural characteristics of endothelial and perivascular glial cells were investigated in the visual cortex of the lateral gyrus, head of the caudate nucleus of the striatum and in the cerebellar cortex of the anterior cerebellar lobe(vermis) of the dolphin brain(Stenella coeruleoalba). It was found thatall three areas sampled for transmission electron microscopy have some specific features of the ultrastructure of the blood-brain interface. Thus, only in the cortex did we find both tight and adhesive junctions present in the endothelium and in the pericapillary glial belt. Also, the endothelial junctions are longest in the neocortical capillaries and artereioles as compared to these in striatum and cerebellum. In the caudate nucleus the endothelial junctions are much shorter and represented by both tight and adhesive types, whereas pericapillary glio-glial junctions are represented only by adhesive types(zonulae and maculae adherentes). In the cerebellar cortex endothelial junctions have the same characteristics as in the striatum. However, pericapillary glio-glial junctions there differe significantly from these in cortex and striatum and show an elaborate system of gap junctions. The tight and adhesive types of juctions between pericapillary glial cells are not found in the cerebellar cortex of the dolphin. Thus, in bothtelencephalic regions(cerebral neocortex and striatum) of the dolphin brain the blood-brain interface is characterized by the presence of double barrier(endothelial and glial), whereas in the metencephalon(cerebellum) there is only endothelial barrier. Our data might be tentatively interpreted as an evidence of higher specialization of the blood-brain barrier in the telencephalic structures of the dolphin brain as compared withstructures of the brain stem. This specialization might be related to the total aquatic adaptation of the dolphins evolved in the ecological niche which demands deep diving and fast surfacing for biological success of these aquatic mammals.

Supported by NSF grants BNS 84-14523, BNS 85-45732 and NIH grant HD 06364.

292.18 COLUMNAR MULTIPLICATION/MODIFICATION HYPOTHESIS: RELATION TO NEOCORTICAL EVOLUTION IN CETACEA. Jacobs M.S., Glezer I.I. and P.J. Morgane. CUNY Med. School, New York, NY 10031, Osborn Laboratories of Marine Sciences of New York Aquarium. New York Zoological Society, Brooklyn, NY 11224, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Columnar organization of the neocortex has been found in all investigated terrestrial mammals(Mountcastle, 1957, 1979). Comparing columnar organization of the dolphin neocortex with that of terrestrial mammals, we can tentatively speculate that in neocortical phylogenesis two main processes have taken place: 1) Multiplication of the columns, that eventually may have resulted in an expansion of the neocortex, and 2) structural modification of the columns that may be associated to differentiation of the neocortex into areas and subareas. The different emphasis on either multiplication or modification of the columns may have resulted in the presence of four modes of neocortical evolution and subsequently in the presence of four types of neocortical organization in extant mammals.

Thus, in the mode which we define as conservative and present in basal Insectivora and Chiroptera both multiplication and modification of the archetypal columns have been minimal and neocortex retained main features of the so-called "initial" brain. In the mode that is defined as progressive-conservative and that may be found in the lower species of most mammalian orders, modification of the columns has taken place mainly, while multiplication has been moderate. These have resulted in the appearance of small and medium-sized neocortices with well-differentiated areal and subareal divisions. In the mode that is defined by us as progressive and present in higher species of most mammalian orders, both modification and multiplication of the columns appear to have been most intensive and resulted in the development of the large neocortices with extremely differentiated areas and subareas. In the fourth mode which we define as conservative-progressive, found only in extant Cetacea, modification of the archetypal columns appear to be minimal; whereas, multiplication of these columns has been extreme resulting in the development of a highly expanded neocortex characterized by many conservative features. A comparison of whales with the most progressive types of terrestrial mammals shows that from an "initial" brain line, there have evolved two quantitatively large brain lines with absolutely different qualitative characteristics of neocortex.

Supported by NSF grants BNS 84-14523 and BNS 85-45732 and NIH grant HD 06364.

292.19 ORGANIZATION OF THE GENICULOCORTICAL PROJECTION IN TURTLES. K.A. Mulligan and P.S. Ulinski. Dept. Anatomy, University of Chicago, Chicago, IL 60637.

Visual cortex in turtles is located in a cortical area known as D2 (Heller and Ulinski, '87) and contains cells responsive to small, moving stimuli anywhere in visual space (Mazurskaya, '73). These cells differ from those in the primary visual cortex of mammals which respond to stimuli in restricted areas of visual space. Their wide-field receptive fields make it likely they are involved in analyzing stimuli moving throughout visual space. Thus, this and the following abstract deal with the mechanisms through which turtle cortical neurons receive information from all points in visual space.

Cells in the dorsal lateral geniculate complex have restricted visual receptive fields (Boiko, '80) and project to visual cortex through the lateral forebrain bundle. Geniculocortical axons were labeled in an *in vitro* wholebrain preparation by iontophoretic or pressure injections of horseradish peroxidase into the lateral forebrain bundle as it passes beneath the floor of the lateral ventricle. Cortical wholemounts were maintained in oxygenated turtle Ringer's solution for up to twelve hours and then flattened, fixed and reacted using diaminobenzidine as the chromagen. Labeled axons could be traced in relatively straight or gently curved trajectories from all along the cortex's ventrolateral edge for up to 2 mm through both the lateral (pallial thickening) and medial parts of visual cortex. Individual axons are fine, unbranched and bear fusiform varicosities along their entire lengths. Detailed analysis of twenty-four well-labeled axons shows varicosities are small (mean $\pm$ S.D. of long diameter = 1.4 $\pm$ 0.9 um) and spaced irregularly at intervals ranging from less than 10 um to greater than 70 um (mean $\pm$ S.D. = 16 $\pm$ 17 um). Single axons display small variations in the number of varicosities per 100 um segment, but there is no consistent variation as a function of distance along the axon.

The results show that the geniculocortical projection in turtles is organized in a point-to-line fashion. Each geniculate cell projects via an unbranched, varicose axon to a line of cortical cells throughout the medio-lateral extent of the visual cortex. All of the cortical cells along the line should, thus, share the restricted receptive field of that geniculate neuron.

The next abstract continues the analysis by considering the relationship between neurons at a given cortical locus and neurons in the dorsal lateral geniculate complex.

292.20 REPRESENTATION OF VISUAL SPACE IN THE VISUAL CORTEX OF TURTLES. P.S. Ulinski and K.A. Mulligan. Dept. of Anatomy, University of Chicago, Chicago IL 60637

The study reported in the preceding abstract demonstrates that neurons in the dorsal lateral geniculate complex of turtles project to the visual cortex via a point-to-line projection. Since the geniculate complex contains a point-to-point representation of the retinal surface (with the nasotemporal axis of the retina represented along the rostrocaudal axis of the geniculate), the point-to-line nature of the geniculocortical map raises the issue of how visual space is represented in turtle visual cortex.

This question was addressed using the retrograde transport of horseradish peroxidase *in vivo* to determine the spatial relation between neurons in the geniculate complex and the visual cortex. Turtles received iontophoretic injections of horseradish peroxidase in restricted loci within the visual cortex. After survival times of seven days, visual cortex was reconstructed from transverse serial sections to accurately plot the center of each injection site, and the distribution of retrogradely labeled neurons was plotted in the ipsilateral geniculate complex. Injections near the rostral pole of visual cortex label neurons near the caudal pole of the geniculate complex. Injections at progressively more caudal loci label neurons at progressively more rostral loci in the geniculate complex. Injections near both the medial and lateral edges of the visual cortex label neurons along a dorsoventral band in the geniculate complex.

Thus, there is a systematic representation of the horizontal meridian of visual space along the rostrocaudal axis of the visual cortex. However, the point-to-line character of the geniculocortical projection effects a collapse of each vertical meridian such that cortical cells receiving information about stimluli at any given horizontal eccentricity will receive convergent information from all points along that vertical meridian. Since physiological studies show that each cortical neuron is also responsive to stimuli at all points along the horizontal meridian, there must be intracortical projections that effect a subsequent collapse of the horizontal meridian. We suspect that this two-step construction of wide-field receptive fields allows turtle cortical neurons to respond preferentially to visual stimuli that appear successively at two disjunct horizontal eccentricities within visual space.

292.21 THE DISTRIBUTIONS OF SUBSTANCE P-LIKE AND CHOLECYSTOKININ-LIKE IMMUNOREACTIVITY WITHIN THE VISUAL WULST IN PIGEONS. T. Shimizu, H.J. Karten and K.T. Keyser*. Dept. of Neurosciences, School of Medicine, M-008, Univ. of California, San Diego, La Jolla, CA 92093.

The visual wulst of birds is a laminated dorsomedial telencephalic elevation with many similarities to mammalian visual cortex. The major laminar components of the wulst, from dorsal surface inward, include the hyperstriatum accessorium (HA), the nucleus intercalatus hyperstriatum accessorium (IHA), the hyperstriatum intercalatus superior (HIS), the lamina frontalis suprema (LFM), the hyperstriatum dorsale (HD) and the lamina frontalis superior (LFS). Thalamic efferents terminate in HD and IHA. Efferents from the wulst to the tectum arise from HA.

Immunohistochemical techniques were used to examine the distributions of substance P-like immunoreactivity (SP-LI) and cholecystokinin octapeptide-like immunoreactivity (CCK8-LI) within the visual wulst in pigeons (*Columba livia*).

Neurons containing SP-LI were found in HA, the most superficial layer of the wulst. SP-LI neurons were also observed at the dorsolateral edge along the wulst, and the medial portion of the hyperstriatum ventrale dorsoventrale (HVdv). Fibers and terminals containing SP-LI were also found within the above areas. A large number of intensely immunoreactive terminals and fibers were observed in the areas located mediodorsal and lateral to HD, the deepest layer within the wulst. The ventral portion ("core") of HD was only lightly stained and contained fine SP-positive fibers. In addition to the visual wulst, the area parahippocampalis (APH) and hippocampus (HP), medial to HA, contain numerous neurons and fibers that show intense SP-LI.

Using *in situ* hybridization, we localized mRNA for preprotachykinins, precursors for substance P and substance K, in APH and the dorsolateral region of HA. The distributions of probe-labelled cells closely matched the results of the immunohistochemical study.

Intense CCK8-LI was found within neurons and fibers in HIS and HD. The hyperstriatum ventrale (HV), which is ventral to HD, also contains large numbers of diffusely distributed CCK8-LI neurons and fibers. A high density of the staining was observed in HD, LFS and along the anterior margin of HVdv. In addition, CCK8-LI was found in APH, HP and the medial portion of HV.

The diversity of transmitters/peptides within the visual wulst indicates that the constituent layers are biochemically, as well as cytoarchitectonically distinct.

292.22 PATTERN ONSET EVOKED POTENTIALS FROM STRIATE AND EXTRASTRIATE CORTEX. BW van Dijk and H Spekreijse. dept. of Visual Systems Analysis, the Netherlands Ophthalmic Research Institute, POBox 12141, 1100AC, Amsterdam, the Netherlands.

We have introduced a technique to find physiologically meaningful components in visually evoked potentials recorded with large electrode arrays attached to the occipital scalp (Maier et al., Vision Res., 27:165, 1987). This technique is employed on the pattern onset visually evoked potential to study the properties of the components attributed to different cortical areas. The stimuli used were checkerboard patterns that appeared and disappeared abruptly in a 300/500 ms duty cycle. The parameters of interest were element size, contrast and ocular input. Spatially filtered checkerboard patterns were also employed. Results indicate the presence of an acuity mechanism underlying the activity from the striate cortex. This mechanism responds to very small pattern elements, is monocular and the responses from this mechanism hardly depend on contrast. The activity from extra-striate cortex is from a contrast mechanism; it shows spatial frequency tuning, its responses saturate at relatively high contrast values and it receives binocular input.

293.1 PROXIMAL RETINAL CONTRIBUTIONS TO THE DARK-ADAPTED ERG IN THE CAT: A K^+-MÜLLER CELL MODEL. L. J. Frishman* and R. H. Steinberg (SPON: J. A. Fox) Depts. of Ophthalmology and Physiology, UCSF, San Francisco, CA 94143

At intensities above 5 log q deg^{-2} s^{-1}, the electroretinogram (ERG) comes from distal retina and consists of an initial negative-going a-wave followed by two positive components: PII (b-wave and dc component) and the c-wave. However, at near threshold intensities (3.0-3.5 log q deg^{-2} s^{-1}) the dark-adapted ERG obtained with 4 s flashes shows three clear negative components: a fast negative response at stimulus onset, a slow negative response that increases in amplitude for about 2 seconds, and a negative-going off response that come from proximal retina.

The fast negative response was previously shown to derive from the scotopic threshold response (STR), a negative-going proximal retinal response that inverts distally. It saturates about 2.5 log units below rod saturation and is distinct from the higher threshold PII in distal retina (Sieving, Frishman, and Steinberg, 1986).

The slow negative response, like the STR, also depends on threshold activity of proximal retina. Negative-going responses at 2-4 s after stimulus onset were maximal in proximal retina. Responses inverted in mid-retina, and were positive distally. The distal positive potential was not slow PIII since subretinal $[K^+]_o$, measured with K^+-selective microelectrodes, did not decrease at these low intensities. Nor was it a photoreceptor potential since intravitreal injections of APB (0.5-1mM vitreal conc.) blocked the entire negative dark-adapted ERG, including the off response. The later response resulted from a merging of the slow negative response and the rapid offset of PII.

The data support a K^+-Müller cell model for the negative responses. Light-evoked increases in K^+ were largest and fastest in proximal retina, and they followed the dynamic range of the negative responses. Intravitreal injections of Ba^{++} (≈3 mM), a K^+ conductance blocker, blocked the negative responses in the ERG and proximal retina. PII enlarged intraretinally, and dominated the ERG.

Inversion of the STR and slow negative response in distal retina suggests that current flow in the cat Müller cell is dominated at low intensities by a distally oriented current loop rather than by a proximally oriented loop toward the highly conductive vitreal endfoot, as described for frog (e.g. Newman and Odette, 1984). Domination by the distal loop appeared to increase over the time-course of the slow negative response, perhaps because sustained release of K^+ in proximal retina decreased the proximal loop by reducing the K^+ concentration gradient across the Müller cell's vitreal endfoot.

293.2 **EFFECTS OF COBALT AND LOW CALCIUM ON LIGHT-EVOKED $[K^+]_o$ CHANGES IN THE SUBRETINAL SPACE AND ON THE CHICK DC ELECTRORETINOGRAM.** R.P. Gallemore* and R.H. Steinberg. Depts. of Physiology and Ophthalmology, University of California, San Francisco, 94143.

While using Co^{2+} and low Ca^{2+} to suppress Ca^{2+}-dependent synaptic transmission in a preparation of chick retina-pigment epithelium-choroid, we observed significant effects on the potentials of the DC electroretinogram (DC ERG). Both Co^{2+} (2-3 mM) and low Ca^{2+} (0.18 mM, ten fold decrease) increased the amplitude of the two responses of the DC ERG that result from the light-evoked $[K^+]_o$ decrease in the subretinal space: the c-wave and the fast-oscillation trough (FOT). Monitoring of subretinal $[K^+]_o$ with K^+-selective microelectrodes showed that Co^{2+} and low Ca^{2+} increased the amplitude of the light-evoked $[K^+]_o$ decrease, and this can account for the observed increase in the c-wave and FOT. With maintained illumination there was also an increase in the rate of $[K^+]_o$ reaccumulation, which follows the $[K^+]_o$ decrease, and at light-offset the overshoot of $[K^+]_o$ was also increased. These results are consistent with Co^{2+} and low Ca^{2+} increasing the photoreceptors' dark current and, thereby, increasing both the photoreceptors' Na^+/K^+ pump rate and the photoresponse.

Strikingly, both Co^{2+} and low Ca^{2+} also increased the amplitude of the DC ERG light peak significantly, and shortened its time to peak. The light peak is a slow positive potential that originates from the retinal pigment epithelium as a basal membrane depolarization in response to a substance believed to be produced by the photoreceptors. The light peak is not thought to be caused by $[K+]_o$ changes in the subretinal space. Since the light-peak amplitude increases with a purported increase in the photoreceptors' dark current, these results provide support for a photoreceptoral origin for the light-peak substance. The increase in the light-peak amplitude suggests that the amount of light-peak substance produced by the photoreceptors could be a function of the photoresponse and/or the rate of the Na^+/K^+ pump.

293.3 **MÜLLER CELL INVOLVEMENT IN ELECTRORETINOGRAM GENERATION AND pH REGULATION IN THE VERTEBRATE RETINA.** R. Wen* and B. Oakley II, NBB Program and Depts. of ECE and Biophysics, Univ. of Illinois at Urbana-Champaign, Urbana, IL 61801.

We recorded light-evoked changes in Müller (glial) cell membrane voltage (V_m) and in $[K^+]_o$, as well as the electroretinogram (ERG), in the isolated retina of the toad, Bufo marinus. Our measurements of the V_m and ERG waveforms (latency, amplitude, and time course) provide strong support of the hypotheses that the ERG b-wave and slow PIII components are produced by a rapid depolarization and a slow hyperpolarization, respectively, of the Müller cell membrane. The Müller cell hyperpolarization seems to be produced by a light-evoked decrease in $[K^+]_o$, which, in turn, is produced by the rod photoreceptors.

Switching pH buffer systems between phosphate and bicarbonate produced dramatic changes in both the ERG and the light-evoked responses of Müller cells. In phosphate-buffered solutions (nominally HCO_3^--free), both the b-wave and the Müller cell depolarization were severely attenuated, and the response waveforms were dominated by the slow PIII component and the membrane hyperpolarization. Conversely, in bicarbonate-buffered solutions, both slow PIII and the Müller cell hyperpolarization were attenuated, and the response waveforms were dominated by the b-wave and the membrane depolarization. These observed effects appear to be due to changing intracellular HCO_3^- and/or pH in Müller cells. Acetazolamide inhibits carbonic anhydrase, which is located in Müller cells, and produces effects similar to increasing HCO_3^-. SITS produces effects similar to reducing HCO_3^-, most likely by blocking a Cl^-/HCO_3^- exchanger in the Müller cell membrane. The effects of increasing HCO_3^- may be due to blocking a specific K^+-conductance in Müller cells, since similar effects also were produced by superfusion with Cs^+, and Müller cell input resistance increased upon switching from phosphate-buffered solution to bicarbonate-buffered solution.

Aerobic glycolysis in rod photoreceptors produces lactic acid, and we suggest that the Müller cells may provide a mechanism to remove the resulting H^+, and thereby prevent changes in extracellular pH. Based on our results, it seems that protons enter the Müller cells down their electrochemical gradient, and that HCO_3^- enters via a Cl^-/HCO_3^- exchanger. Within the Müller cells, these ions form carbonic acid, which then is dehydrated by carbonic anhydrase to form CO_2 and H_2O. Therefore, we suggest that the Müller cells provide a mechanism to buffer changes in retinal pH. Supported by NIH grant EY04364.

293.4 THE EFFECT OF ACETAZOLAMIDE ON THE ERG b-WAVE OF THE SKATE. S.H. Hensley*, P.J. Linser* and J.L. Cohen. (SPON: R. Grubbs). Wright State University Biomedical Sciences Ph.D. Program, Dayton, Ohio 45435, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine 04672 and C.V. Whitney Marine Laboratory, St. Augustine, Florida 32086.

Work in our laboratory using monoclonal and polyclonal antibodies has demonstrated the presence of the enzyme carbonic anhydrase II in Muller cells in the retina of the skate, Raja oscellata. Carbonic anhydrase catalyzes the hydration of carbon dioxide, a reaction which is important in the regulation of intracellular pH. Since the Muller cells are thought to be the site of origin of the b-wave of the ERG, we examined the effect of acetazolamide, a potent inhibitor of carbonic anhydrase activity, on the b-wave of the electroretinogram of the skate.

Pieces of eyecup were incubated consecutively in each of the following solutions; (1) normal elasmobranch saline, (2) elasmobranch saline containing 500 uM acetazolamide, (3) normal elasmobranch saline. The ERG was recorded after each step. Acetazolamide decreased the b-wave amplitude by approximately 50% compared to control responses over a five log unit intensity range. The effect of acetazolmide was reversible, as the b-wave amplitude increased following washout in normal elasmobranch saline. To test if acetazolamide was acting on the photoreceptors, pieces of eyecup were incubated in aspartate to isolate the receptor potential (RP). Acetazolamide had no effect on the RP compared to the control response.

The results of this study indicate that inhibition of carbonic anhydrase activity affects the normal function of the Muller cells, resulting in a decrease in the amplitude of the b-wave. The data support the functional importance of the Muller cell in the generation of the b-wave. (Supported by a fellowship from the Lucille P. Markey Trust to J.L.C.)

293.5 **DIFFERENTIAL EFFECTS OF NEUROTENSIN ON RABBIT ERG OSCILLATORY POTENTIALS.** P. Olivier and F.B. Jolicoeur
Departments of Ophthalmology and Psychiatry , University of Sherbrooke, Québec, Canada, J1H 5N4.

The presence of neurotensin (NT) in the retina of a variety of animal species has been reported(Brecha et al.Neurosci. 6:1329, 1981). Although the physiological significance of retinal NT remains to be ascertained, we have shown recently that NT markedly decreased both A and B wave amplitudes without affecting implicit response times. (F.B.Jolicoeur and P.Olivier: Neurosci. Abstr.12: 640,1986). In order to further characterize the actions of NT on retinal responses to photic stimulation, the effects of various doses of NT on oscillatory potentials (OP) were examined.

Experiments were performed using adult pigmented rabbits, curarized, maintained under pentobarbital anesthesia and ventilated through a tracheostomy. Pupils were maximally dilated with cyclopentolate and neosynephrine drops. Animals were dark adapted for 60 min and baseline ERG recordings of four distinct OPs (1 to 4) were first obtained. Different groups of animals (n= 6) were then injected intravitreally in one eye with either 0.9 % NaCl, 0.1, 1.0 or 10.0 µg NT. Another group of animals received 10 µg of the generally inactive analogue [Ala ^{11}]- NT . For each animal, the fellow eye received a 0.9 percent NaCl solution. Volume of injections was 0.1 ml. ERG recordings were then performed at 15, 30, 60, 90, 120 and 150 min following injections.

Results revealed significant dose related reductions in OP 1 amplitudes which first appeared at 90 min and were still present at 150 min following NT administration. None of the NT doses significantly altered OP 2 amplitudes. Interestingly, OP 3 amplitudes were significantly enhanced with all doses of the peptide. These increases were first detected at 15 min and progressed throughout the experiment. On the other hand, OP 4 amplitudes were significantly decreased at 60 min following administration of the largest dose of NT. The implicit response times of each oscillatory potential were not affected by NT. Finally, the administration of 10 µg of the analog [Ala ^{11}]-NT did not affect the amplitudes nor the implicit response times of oscillatory potentials, indicating that the observed effects with NT can not be attributed to non-specific effects of intravitreal administration of a tridecapeptide.

Together, the present results demonstrate that NT has highly selective modulatory effects on retinal responses to photic stimulation. Furthermore, the differential effects of NT point to different mechanisms generating individual oscillatory potentials.

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293.6 **THE EXCITOTOXINS KAINIC ACID AND IBOTENIC ACID HAVE DIFFERENT EFFECTS ON GANGLION CELL ACTIVITY AND ELECTRORETINOGRAM (ERG) IN THE CAT RETINA.** A. W. Przybyszewski*, N. Sucher*, M. Hagner*, and O.-J. Grüsser. (SPON: J. J. Kulikowski). Dept. of Physiology, Freie Universität, Arnimallee 22, 1 Berlin 33, Germany-West.

In anaesthetized cats (35 mg/kg pentobarbital initial dosis, 3-6 mg/h pentobarbital infusion) corneal ERG and single unit activity from optic tract fibres were recorded before and up to six hours after intravitreous injections of kainic acid (KA) (1 mg/eye) or ibotenic acid (IBO) (0.5-1 mg/eye). The effects of these excitotoxins were compared with those of D- and L-2-amino-4-phosphonobutyrate (APB) and the quinolinic acid analog cis-2,3-piperidine dicarboxylic acid (PDA) *(Soc. Neurosci. Abstr., 12: 959, 1986).*

1) KA led to a change in the ERG commencing about 5 minutes after injection: b-wave amplitude decreased and its latency increased (also relative to the latency of the a-wave) with time. After 6 hours only a very small b-wave was evoked with high stimulus luminance. Concomitantly the slope of the b-wave intensity function decreased, while the a-wave became more prominent and the slope of its intensity function increased. Off-center ganglion cell activity (postinhibitory activation) showed a considerable decrease in the response to 10 ms flashes, while the flash-induced initial inhibition was not impaired and increased relative to the flash-induced excitation during the first hour after the KA injection.

2) In contrast to KA, ibotenic acid did not affect the ERG significantly, but did reduce the flash-induced postinhibitory activation and the spontaneous activity of off-center ganglion cells. The flash-induced activation in on- and off-center ganglion cells disappeared about 60-90 minutes after injection of IBO. This excitotoxin is believed to be metabolized into muscimol, a hypothetic GABA-agonist. In comparable doses, however, muscimol changed neither the ERG nor single unit activity.

3) The flash responses were transformed into power spectra (PS) and the frequency range between 6 and 200 Hz was analysed in detail. The excitotoxins changed the shape and maximum frequency of the PS and reduced the stability of PS sampled for successive flash responses.

4) In conclusion it seems that KA has direct and/or indirect effects on the neural activity in both the distal and proximal retina. Off-excitation was more sensitive than on-excitation and on-inhibition. IBO seems to interact primarily with receptors in the proximal retina and to have no effect on on-bipolar cells. The influence of these excitotoxins on the retinal network activity differs therefore from that of L-APB, which blocks the signal transmission through on-bipolar cells and D-APB, which seems to have a gliotoxic action on Müller-cells. PDA-effects on ganglion cell activity were reversible within about one hour and the ERG b-wave was only slightly diminished.

The work was supported in part by a grant of the Deutsche Forschungsgemeinschaft (Gr 161).

293.7 **Morphine attenuates rod and cone components of the human electroretinogram.** M.J. Jaffe , R. Rittmaster , R.J. Wyatt. Neuropsychiatry Branch, St. Elizabeth's Hospital (NIMH) and Developmental Endocrinology Branch (NIH).

Retinal opioid binding-sites have been found in the guinea pig, cow, monkey and human. Cells with enkephalin-like immuno-reactivity have been found in: i) the inner margin of the inner nuclear layer and, ii) several laminae of the inner plexiform layer. Despite the growing knowledge of opioid neuro-anatomy, however, their function within the retina is largely unknown. A probe that can be used to assess the functional effects of different neuroactive drugs on the retina of humans is the electroretinogram (ERG), a flash-evoked, mass electrical response.

In our study, six normal males with a mean age of 22.8 years (±s.d. 2.3) gave informed consent to have their ERG measured before and after the i.m. administration of 10 mg morphine. All subjects in all groups agreed to abstain from caffeine and tobacco. For all groups, eligibility criteria included for both eyes a best corrected visual acuity of 20/20 or better, normal confrontation visual fields, intraocular pressure, slit lamp and fundus examination. The ERG recordings were obtained using a ganzfeld stimul-ation system and bipolar Burian-Allen contact lens electrode. Isolation of the rod and cone components of the ERG and the oscillatory potentials have been previously described. The effects of morphine on the ERG were evaluated with an analysis of variance.

Morphine has a general attenuating effect upon the ERG signals mediated by both rod and cone processes of the human retina. Across the flash intensities tested, morphine resulted in a mean attenuation in rod b-wave amplitude of 11% ($p<0.01$) and a mean delay of 4% in its latency ($p<0.0001$).

Responsivity of cone mechanisms was also attenuated by morphine. Under conditions of dark-adaptation, the latency of the cone a-wave was delayed by 3% ($p<0.01$) across the intensities tested. An attenuating effect of morphine on photoreceptors of humans is corroborated by a similar delay of 5% ($p<0.001$) on the light-adapted cone a-wave. Morphines's attenuating effect on cone processing is further supported by the 8% delay ($p<0.01$) of the 'blue cone' b-wave across the intensities tested. The oscillatory potentials (OPs), believed to represent feedback circuits within the retina, are delayed by morphine. The latency of each of the first 3 oscillatory potentials is increased by 2% ($p<0.01$ for OP1 and $p<0.0001$ for OP2 and OP3) across the intensities tested.

Our ERG results suggest that systemically administered morphine does cross the blood-retina barrier and reduces the gain of the retina's responsivity to light flashes. Possible interactions with the dopamine D-2 autoreceptor will be discussed.

293.8 **EFFECTS OF SYSTEMIC AND INTRAOCULAR ADMINISTRATION OF MESCALINE ON FLASH EVOKED POTENTIALS IN THE INTACT, AWAKE RAT.** J.T. Eells and D.M. Wilkison. Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI 53226

Mescaline is a phenethylamine hallucinogen capable of eliciting profound perceptual distortion including visual hallucinations. We have previously reported a reduction in the primary component of the flash evoked potential (FEP) by mescaline. This report details further investigations of the mechanism and possible sites of action of mescaline in the visual system.

Hooded Long Evans rats were anesthetized with pentobarbital and indwelling epidural screw electrodes were stereotaxically implanted over the right and left visual cortices at points 7 mm posterior to bregma and 4 mm lateral to the midline. Ground and reference electrodes were placed 2 mm anterior and 2 mm lateral to bregma. Following a one-week recovery period recording sessions were initiated. FEPS were recorded in response to 10 µsec flashes at a rate of 0.4 Hz. Mescaline was administered intraperitoneally (20 mg/kg) or intraocularly (1 µl of a 400 mg/ml solution under anesthesia). Input/output curves at 3 luminescence intensities were established prior to drug administration and at defined intervals thereafter. The averaged FEP consisted of a primary (P20-N30) component and a secondary (P50-N70-P100) component.

Mescaline significantly attenuated the amplitude of the primary (P20-N30) component of the FEP at all three stimulus intensities. This effect was maximal 60 min after drug administration. Intraocular injection of mescaline produced a similar degree of inhibition with a maximal effect observed 20 min after drug administration. To determine if the mydriatic actions of mescaline contributed to the observed reduction in FEP; the effects of atropine and phenylephrine, alone and in combination with mescaline, on the FEP were examined. Atropine (1%ophthalmic solution), atropine, methyl nitrate (0.1 mg/kg,ip) and phenylephrine (2.5%ophthalmic solution) had no effect alone on the FEP. However, both atropine and atropine methyl nitrate antagonized the effect of mescaline on the FEP. Phenylephrine did not.

These data suggest that mescaline increases the threshold and slope of the visual response to flash in the rat. The magnitude and the specificity of the effects of mescaline on the P20-N30 primary response suggest that the drug produces deficits in conduction through the retino-geniculato-cortical system. Furthermore, these effects may be mediated by an action of mescaline at the level of the retina which may be modulated by cholinergic systems. Supported by NIDA, R01 DA 03785.

293.9 INFLUENCE OF GABACULINE TREATMENT ON GABA IMMUNOREACTIVITY IN RAT RETINA. M.H. Makman*, J.F. Cubells and S.U. Walkley (SPON: N.S. Sharpless). Depts. of Neuroscience, Biochemistry and Molecular Pharmacology, Albert Einstein Col. of Med., Bronx, NY 10461

In the mammalian retina γ-aminobutyric acid (GABA) appears to be synthesized exclusively in a subset of amacrine cells. However, many other retinal neurons and also Muller cells can accumulate GABA and degrade GABA by the action of GABA-transaminase (GABA-T). Gabaculine (gbc) is an enzyme-activated irreversible inhibitor of GABA-T. We recently demonstrated that subcutaneous (s.c.) injection into rats of gbc produces a progressive and long-lasting inhibition of GABA-T as well as a progressive and sustained increase in the level of GABA in the retina (Cubells et al., J. Pharm. Exp. Therap., 238:508 (1986); Brain Res., in press). We report here the influence of gbc treatment on the amount and localization of GABA in rat retina as visualized by GABA immunocytochemistry.

Long-Evans rats were treated with 10 mg/kg gbc (s.c.) for 2 or 8 hours. Posterior orbits were sectioned for peroxidase immunocytochemistry with a highly specific GABA antibody. Immunocytochemical controls were performed on sections incubated in normal serum. Sections were examined under the light microscope for peroxidase reaction product.

In the control retinas GABA immunoreactivity (GABA-IR) was detected most prominantly in cell bodies fairly regularly spaced in the inner aspect of the inner nuclear layer (INL). Also there was a prominant network of GABA-IR in the inner plexform layer (IPL). Some GABA-IR was evident in the ganglion cell layer (GCL). However GABA-IR was almost totally absent from the outer nuclear layer (ONL). After treatment with gbc for 2 hrs GABA-IR in the retina was markedly increased overall, with an additional increase from 2 to 8 hrs. Distribution of GABA-IR in retinas of the gbc-treated animals was strikingly different from the controls. Following gbc treatment the number of cell bodies exhibiting intense GABA-IR was markedly increased. Numerous processes containing GABA-IR were now evident coursing through the ONL. In addition considerable GABA-IR now appeared to be in processes coursing vertically across layers. GABA-IR was also increased at the inner aspect of the GCL.

Thus, gbc-treatment causes a major change both in the distribution and amount of endogenous GABA within the retina. The qualitative changes are highly suggestive of a major accumulation of GABA in Muller cells, in turn permitting exposure to GABA of certain neurons that normally have little or no access to GABA. Hence, gbc treatment would qualitatively as well as quantitatively alter GABAergic function in retina. These considerations may also apply to GABAergic function in brain following GABA-T inhibition, e.g., when GABA-T inhibitors are used in man for treatment of epilepsy.

293.10 THE EFFECTS OF APB ON RETINAL SYNTHESIS AND RELEASE OF GABA: EVIDENCE THAT ON-CHANNEL BIPOLAR CELL ACTIVITY INFLUENCES RETINAL GABAERGIC TRANSMISSION. J.F. Cubells, C. Ndubuka* and M.H.Makman*. Depts. of Biochemistry, Neuroscience and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461

Subcutaneous (s.c.) administration of gabaculine (gbc), an irreversible inhibitor of GABA-transaminase, produces a linear increase in retinal GABA for up to 4 hours post-injection (Cubells et al., Brain Res., in press). We report here the effect of light on the post-gbc synthesis of retinal GABA in vivo, as well as the effects of 2-amino-4-phosphonobutyrate (APB) on retinal GABA synthesis in vivo and on release of newly synthesized 3H-GABA from retinal synaptosomes in vitro. APB is a glutamate receptor agonist previously found to selectively block the light response of on-channel bipolar cells.

Rats were given 10 mg/kg gbc s.c.; 15 min afterwards they were placed in a completely darkened room, held under ambient laboratory lighting, or were ether-anaesthetized and given 3 nmoles of APB intraocularly (i.o.) in one eye and 3 ul of 0.9% NaCl in the other. Rats given i.o. injections were then placed in darkness or held under ambient light. Rats were killed 1.25 hrs after gbc injection and retinal GABA measured. For in vitro experiments, rat retinal synaptosomes were preincubated with 3H-glutamate, aliquots of resuspended washed synaptosomes incubated in the presence of APB or other agents and the 3H-GABA released into the medium then determined.

Retinal GABA levels were higher in rats held in the light than in those placed in darkness after s.c. gbc (62.9 ± 1.7 vs 54.4 ± 1.9 nmol/retina). Initial (pre-gbc) GABA levels were the same in light and dark (approximately 23 nmol/retina). In gbc-treated rats held in the light, the GABA level in retinae from APB-injected eyes was diminished compared to that from saline-injected eyes (49.6 ± 1.8 nmol/retina vs 57.8 ± 1.1 nmol/retina). In contrast, when gbc-treated rats were placed into darkness, GABA levels were not different in retinae from APB vs saline-injected eyes (47.4 ± 1.0 vs 47.6 ± 1.8 nmol/retina). In vitro, APB exerted a small stimulatory effect on synaptosomal GABA release: Release of newly synthesized 3H-GABA was 3.4% in the presence of 1.0 mM APB, compared to 2.9% for the control, 7.6% with 0.01 mM kainate and 7.7% with 1.0 mM glutamate. The in vitro results further suggest that APB inhibition of GABA synthesis in vivo is not due to a direct action of APB on GABA-synthesizing amacrine cells.

It is concluded that retinal GABA synthesis is stimulated by light. Furthermore, this stimulation may be indirect and specifically caused by activity of on-channel bipolar cells. Hence APB is inhibitory in the light but not in the dark when on-bipolars are already hyperpolarized by endogenous transmitter. (T32 GM7288)

293.11 QUANTITATIVE ANALYSIS OF THE DISTRIBUTION OF GAD- AND GABA-LIKE IMMUNOREACTIVITY WITHIN THE TURTLE RETINA: A DOUBLE-LABEL STUDY. W.D. Eldred and L. Hurd, Dept of Biology, Boston University, Boston, MA, 02215

This double-label study details the coexistence of GABA and GAD in subpopulations of retinal cells in the turtle, Pseudemys scripta elegans. Sections were treated with antisera directed against GAD and GABA. The fluorescent markers FITC and TRITC, conjugated to appropriate secondary antibodies, were used to distinguish the two different primary antibodies.

GAD immunoreactivity was seen in cell bodies in the inner and outer regions of the inner nuclear layer (INL), and in processes within the inner plexiform layer (IPL) and outer plexiform layer (OPL). GABA immunoreactivity was seen in cell bodies in the inner and outer regions of the INL and within the ganglion cell layer (GCL), and in processes in the OPL and IPL. The intracellular labeling of these antibodies apparently differed in that the GABA antibody labeled the entire somata of the cells while the GAD antibody was concentrated only in the cytoplasm and not the nucleus.

Both GABA and GAD positive neurons gave rise to processes which arborized in the IPL, although they were observed to have different stratification patterns. We quantified the labeling of the cells near the inner border of the INL, and used a scanning densitometer (SD) to quantify the stratification patterns of the labeling within the IPL. The SD peaks have been described on the basis of their relative position within a normalized IPL sample, where L0 would represent the INL/IPL border and L100 the IPL/GCL border. Analysis of GAD labeling yielded seven distinct bands, with peaks centered at positions L8, L18, L28, L42, L57, L74, and L88. GABA yielded five bands, centered at positions L14, L26, L63, L79, and L90. Four of the five GABA bands were statistically identical to corresponding GAD bands, with the fifth GABA band, at L14, being the exception. GABA antibody failed to label bands at positions corresponding to the GAD bands at L8 and L42.

Cell counts of labeled cell bodies at the IPL/INL border have shown a 67% correspondence between the labeling seen with GABA and GAD antibodies, with more GABA than GAD positive cells. At least two anatomically distinct cell types have been observed which ramify in characteristically different lamina: one in the proximal bands and one in the distal bands. Our research indicated, that in the turtle retina the GABA and GAD antisera we used produced labeling which was significantly different in the INL and IPL. This research supported by EY04785 to WDE.

293.12 DOPAMINERGIC AND INDOLEAMINE ACCUMULATING AMACRINE CELLS BOTH EXPRESS GABA-LIKE IMMUNOREACTIVITY IN CAT RETINA. H. Wässle and M.H. Chun*, Max-Planck-Institut für Hirnforschung, Deutschordenstr. 46, D-6000 Frankfurt 71, W. Germany

In the cat retina immunoreactivity for TH (tyrosine hydroxylase) revealed a population of amacrine cells with large cell bodies and a preferred stratification close to the inner nuclear layer (Oyster et al., 1985, PNAS 82, 6335-6339). They occur at a very low density (0.1% of all amacrine cells) and seem to be a cell type common to all mammalian retinae.

GABA-like immunoreactivity was found in approximately 30% of all amacrine cells. Consecutive 1 µm thick semithin sections were incubated in antisera to either TH or GABA. In 3 out of 4 TH-like immunoreactive amacrine cells there was also GABA-immunoreactivity present. Hence it is quite likely that the majority of dopaminergic amacrine cells of the cat retina express GABA-like immunoreactivity.

Uptake of 5-HT (5-hydroxytryptamine) following injection of 20 µg into the vitreous, labelled approximately 10% of all amacrine cells and at least two morphologically different types could be discerned (Wässle et al., 1987, J. Neurosci.). Following 5-HT uptake, consecutive 1 µm thick semithin sections were incubated in antisera to either 5-HT or GABA. 91% of all 5-HT accumulating amacrine cells expressed GABA-like immunoreactivity. Since in the cat retina endogeneous 5-HT could not be found by immunocytochemistry, one has to consider the possibility that some GABAergic amacrine cells take up indoleamines.

In rabbit retina uptake of 5-HT following injection into the vitreous labelled approximately 6% of all amacrine cells. When consecutive semithin sections were incubated with antisera to either 5-HT or GABA, 75-80% of all 5-HT accumulating amacrine cells expressed GABA-like immunoreactivity. This confirms a similar study by Osborne and Beaton (1986, Brain Res. 382, 158-162) and makes it likely that both serotonin-accumulating amacrine types of rabbit retina (Vaney, 1986, Science 233, 444-446; Sandell & Masland, 1986, J. Neurosci. 6, 3331-3347) express GABA-like immunoreactivity.

293.13 CHOLINERGIC AMACRINE CELLS OF THE RABBIT RETINA EXPRESS GABA- AND GAD-LIKE IMMUNOREACTIVITY. N. Brecha, D. Johnson, L. Peichl*, H. Wässle. Departments of Anatomy and Medicine, UCLA School of Medicine, Los Angeles, CA 90024, Max Planck Institute für Hirnforschung, Frankfurt, West Germany.

In the rabbit retina, several histochemically defined cell populations have been identified, including those containing peptides, dopamine, acetylcholine and GABA. Cholinergic amacrine and displaced amacrine cells have been described and these cells selectively accumulate the fluorescent dye 4,6 diamidino-2-phenylindole (DAPI). GABAergic amacrine and displaced amacrine cells have also been identified. The present studies have examined the distribution and co-existence of the cholinergic and GABAergic cell populations.

Normal, optic nerve sectioned and intraocularly DAPI-treated retinas, some pretreated with colchicine, were fixed in a paraformaldehyde and glutaraldehyde solution and subsequently processed by immunohistochemical methods using antibodies to GABA, glutamic acid decarboxylase (GAD) and choline acetyltransferase (CAT). Double label studies demonstrated that all DAPI accumulating cells in the ganglion cell layer (GCL) and prominently labeled DAPI accumulating cells in the inner nuclear layer (INL) contained either GABA or GAD immunoreactivity. In the GCL, GABA or GAD immunoreactive cells which did not accumulate DAPI were also identified and in mid-peripheral retina, these cells make up 10-20% of the total GABA immunoreactive cell population. These cells were characterized by medium to large size somata. In optic nerve sectioned retinas, there was no apparent loss in the number of DAPI accumulating or small size GABA immunoreactive cells. Quantitative studies of the GCL from whole mounted retinas demonstrated that displaced amacrine, GABA and CAT cell populations have a parallel distribution across the retina. GABA and CAT immunoreactive cell density is highest in central retina over the visual streak (GABA, 1150 cells/sq mm; CAT, 850 cells/sq mm) and lower in mid-peripheral (GABA, 400 cells/sq mm; CAT, 350 cells/sq mm) and peripheral retina (GABA, 270 cells/sq mm; CAT, 250 cells/sq mm). CAT immunoreactive and the majority of GABA immunoreactive cells have an identical appearance characterized by a round, small cell body and both of these immunoreactive cell populations have similar spacing in all retinal regions. In summary, these studies demonstrate that cholinergic amacrine cells form a subpopulation of the GABAergic amacrine cell population in the rabbit retina.

We wish to thank Drs. Streit, Oertel and Epstein for antibodies to GABA, GAD and CAT, respectively. Supported by EY 04067 and a Sloan Fellowship to NB and the Max Planck Institute.

293.14 SYNAPTIC ORGANIZATION OF CHOLINERGIC AMACRINE CELLS IN THE RABBIT RETINA. T. Millar* and I.G. Morgan. Centre for Visual Sciences and Research School of Biological Sciences, Australian National University, GPO Box 475, Canberra City, ACT 2601, Australia.

Cholinergic amacrine cells were detected by immunohistochemistry in the rabbit retina using an antiserum prepared against affinity-purified chicken choline acetyltransferase (Johnson, C.D. and Epstein, M.L. J. Neurochem. 46: 968, 1986). At the light microscope level, immunoreactive cell bodies were detected in the inner nuclear layer and the ganglion cell layer, and there were two immunoreactive synaptic layers in the inner plexiform layer. These corresponded well to the putative cholinergic amacrine cells detected in the rabbit retina by localizing sites of acetylcholine synthesis (Masland, R.H. and Mills, J.W. J. Cell Biol. 83: 159, 1979), as starburst cells in Golgi studies (Famiglietti, E.V. Brain Research 261: 138, 1983), by selective neurofibrillar staining (Vaney, D.I., Peichl, L. and Boycott, B.B. J. comp. Neurol 199: 373, 1981) and by DAPI-labelling (Masland, R.H., Mills, J.W. and Hayden, S.A. Proc. R. Soc. 223: 79, 1984. At the electron microscope level, immunoreactivity was associated with processes in the inner plexiform layer which formed clusters within the dense synaptic bands observed at the light microscope level. The processes were derived from amacrine cells since they formed conventional synapses onto processes of ganglion cells and non-immunoreactive amacrine cells. Inputs to the cholinergic processes were observed from bipolar cells, but no input was observed from non-immunoreactive amacrine cells. A feature of the synaptic organization of the cholinergic cells was the occurrence of synapses between immunoreactive profiles within the clusters - a feature also observed in the chicken and goldfish retinas. These features need to be taken into account in models of how the cholinergic amacrine cells are involved in generating directional selectivity at the ganglion cell level.

293.15 INTRAOCULAR ADMINISTRATION OF ETHYLCHOLINE MUSTARD AZIRIDINIUM ION (AF64A): EFFECTS ON CHOLINERGIC AMACRINE CELLS OF THE RABBIT RETINA. A.W. Spira[1], T. Razniewska[1], R. Goldade[1], and I.G. Morgan[2]. [1]Dept. of Anatomy and Lions' Sight Centre, The University of Calgary, Calgary, Alberta, T2N 4N1; [2]Dept. of Behavioural Biology, Australian National U., Canberra, ACT 2601, Australia.

The introduction of the choline analog ethylcholine mustard aziridinium ion, AF64A, (ECMA) into the central nervous system has a profound effect on several important properties of cholinergic neurones (Rylett, B.J. and Calhoun, E.H., J. Neurochem., 34:713, 1980; Fisher, A. et al., J. Pharmacol. Exp. Ther. 222:140, 1982). The retina, with its readily accessible and richly endowed set of well-characterized cholinergic neurones, constitutes a favourable model for investigation of the structural, functional and developmental effects of this neurotoxin. Recent studies of the effect of ECMA on cholinergic neurones in the chick retina (Millar, T.J., et al., J. Neurosci. 7:343, 1987) have demonstrated a loss of acetylcholinesterase and of choline acetyltransferase-like immunoreactivity (ChAT-IR) from the mirror-symmetrical amacrine cells of the inner nuclear (INL) and ganglion cell layers (GCL), and from their processes in the inner plexiform layer (IPL). We have now investigated the effect of intraocular injections of ECMA in anesthetized albino New Zealand rabbits to determine whether the effects on mammalian cholinergic retinal neurones are comparable to those of the chick. Retinas were examined one or four weeks after administration of ECMA on two successive days at a dosage of 30nmoles/10ul injected volume of saline or 10% dimethylsulfoxide (DMSO). Acetylcholine (ACh) levels were assayed by hplc with electrochemical detection (Potter, P.E. et al., J. Neurochem. 41:188, 1983). Tissues were processed for histology and for localization of ChAT-IR in sections and whole-mounts. The primary anti-ChAT serum used was that developed by Johnson and Epstein (J. Neurochem. 46:968, 1986). ECMA (30nmoles) produced a retinal lesion which caused minimal, histological changes, while effecting a sharp reduction in both assayable acetylcholine and ChAT-immunoreactivities. Reduction of ACh by ECMA/saline, as compared to opposite vehicle injected eyes, was 40% at one week post-injection and was sustained at that level at four weeks. ECMA/DMSO resulted in a sustained 25% loss. Histologic alterations were limited to transient changes in photoreceptor outer segments. ChAT-IR was considerably reduced in the half retina adjacent to the injection site. Viewed in both sectioned and intact whole-mounts, it was deficient in the cholinergic INL cells and in the corresponding sublamina 'a' of the IPL. Amacrine cells within the GCL, in contrast, were much less affected at this dosage, retaining ChAT-IR in perikarya and their processes in sublamina 'b' of the IPL. The lesion induced by ECMA appears to be long lasting; it differs from that produced in the chick in primarily involving one of the two symmetrical classes of amacrine cells. It may therefore prove useful in studies of the functional characteristics of rabbit cholinergic cells.

293.16 THE MECHANISM OF DIRECTION SELECTIVITY IN RABBIT RETINAL GANGLION CELLS

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The computational mechanism of On-Off direction selective (DS) rabbit retinal ganglion cells was investigated by comparing their responses to low contrast drifting gratings with the predictions of three basic types of theoretical models: (1) correlation-type second order models such as those proposed for direction selectivity in the fly (Poggio and Reichardt, 1973), which have recently been applied to human psychophysical results (van Santen and Sperling, 1984), (2) threshold models that discriminate the different maximum amplitudes of intracellularly summed responses to preferred vs. null direction stimuli (Grzywacz and Koch, 1987), and (3) a rectified correlation model proposed to take into account segregated On and Off pathways in the retina (Grzywacz and Koch, 1987). Biophysically plausible implementations of these theoretical models that predict different DS cell responses to low contrast drifting gratings may be made in some detail for On-Off DS ganglion cells in rabbit retina because key electrotonic and morphological parameters have been previously defined by intracellular recording and staining (Amthor et al., 1984).

Our experimental results show that DS cell responses to drifting sinusoidal gratings cannot be accounted for by a pure second order (correlation-type) mechanism; Fourier analysis of PST histogram responses to single sine wave gratings reveals significant power at higher than second order harmonics at contrasts as low as 1%, where direction selectivity is still significant. Extant biophysical and morphological data suggest that this may be due to rectifications associated with the segregation of On and Off pathways.

Thresholds are unlikely to be the nonlinearity underlying the DS mechanism because responses to drifting phase locked multiple sinusoidal gratings are not better than the sum of the responses to the isolated components, despite the fact that the summated waveform of two gratings should at some points be more superthreshold than the individual components. The threshold model is also inadequate to explain our finding that in cells which have sufficient maintained firing, statistically significant direction selectivity can exist at very low contrasts that is invariant to the phase angle between pairs of gratings, although phase angle strongly effects the maximum total amplitude of the summated gratings.

The model in which rectified inputs interact by a multiplicative correlation mechanism seems to be most consistent with our results.

Supported by EY05070 and the Sloan Foundation.

293.17 **SYNAPTIC DRUGS INJECTED INTO THE VITREOUS AFFECT THE RETINAL CONTROL OF TURTLE EYE MOVEMENTS.** M. Ariel. Departments of Behavioral Neuroscience and Psychiatry, and Center for Neuroscience, University of Pittsburgh. Pittsburgh, PA 15260.

The analysis of receptive field properties of Directionally Sensitive (DS) retinal ganglion cells during synaptic drug application has shown that similar drugs have similar effects across species. For example, retinal application of the GABA antagonist picrotoxin enables DS cells to respond equally to stimulus movement in all directions, for both rabbit and turtle. GABA antagonism thus blocks DS processing in the vertebrate retina. This conclusion was useful in the interpretation of the effects of intravitreal picrotoxin on eye movement control in the cat, reported last year at this meeting. Similar experiments have now been repeated for turtle and extended to other drugs which are being studied in this laboratory during in vitro recording DS retinal ganglion cells.

Following subcutaneous injections of lidocaine at cranial pressure points, a turtle, Pseudemys Scripta, was placed in a plexiglass box with its head fixed at the center of sets of horizontal and vertical Helmholtz coils. A contact lens search coil was placed on the anesthetized cornea of either eye to measure eye position. Following control records of spontaneous and optokinetic eye movements under various stimulus conditions, intravitreal injections of 20 uL were made through a 30 gauge needle with a Hamilton syringe. Within 30 minutes, the contact lens was replaced on the eye and its movements were recorded while the stimulus conditions were repeated.

As with the cat and rabbit, intravitreal injection of 3.3 mM picrotoxin resulted in a spontaneous nystagmus by that eye with a temporal-to-nasal slow phase. Another selective GABA antagonist, bicuculline, has a very similar effect, and has been found to be effective at concentration in the syringe as low as 34 uM. Assuming a substantial dilution of the injection within the vitreous and retina, these drug induced eye movements are a sensitive indicator of the functioning of DS cells.

For both GABA antagonists, recovery is observed by the day after injection. Similar to rabbit and cats (without visual cortex), the effect of these GABA antagonists was potent enough to block light evoke movements in response to nasal-to-temporal monocular stimulation. However, unlike those mammals, each eye of the turtle may move independently. Thus, during monocular drug application, only the injected eye is affected, whereas the other (unyoked) occluded eye serves as a simultaneous control.

Neostigmine, an acetylcholinesterase inhibitor, increased eye movements at low doses and yet blocked them at high doses. Amino-Phosphono-Butyrate (5.4 mM), which block ON cells in other vertebrate retinas, also blocks visually evoked nystagmus in turtle. These results support the view that drugs which effect DS retinal ganglion cells also modulate pathways involved in subcortical reflexive eye movements. **(Supported by NIH grant EY0578).**

293.18 THE ON CHANNEL IS NECESSARY FOR OPTOKINETIC NYSTAGMUS AT HIGH SPATIO-TEMPORAL FREQUENCIES IN GOLDFISH. P.J. DeMarco*, J.D. Nussdorf*, D.A. Brockman* and M.K. Powers. Dept. of Psychology, Vanderbilt University, Nashville, TN, 37240.

The vertebrate visual system can be subdivided into ON and OFF channels, but differences in function between these channels remain obscure. We assessed the behavioral consequences of blocking ON channel activity with 2-amino-4-phosphonobutyric acid (APB) by measuring the optokinetic nystagmus reflex (OKN) of the goldfish (Carassius auratus). APB was injected bilaterally into the eyes of fish to yield vitreal concentrations of 10, 100, 500, or 1000 uM; controls received saline carrier solution. Two square wave gratings (0.039 & 0.19 cycles/degree visual angle) and a homogeneous field served as stimuli. OKN response was assessed by counting the number of saccadic eye movements over 3-5, 30 sec trials for each stimulus condition. OKN was measured for approximately two weeks following APB treatment. A dose response function revealed that deficits in OKN occurred for animals that received high doses of APB and were exposed to the high spatial frequency stimulus. Fish receiving 1000 uM APB showed a significant decrease in OKN response to the high spatial frequency on post-injection days 0 and 1, and were fully recovered by post-injection day 3. In this experiment, stimuli were rotated about the animals at a constant speed of 72 deg/sec. This translates to temporal frequencies of 2.8 cycles/sec for the low spatial frequency grating and 14 cycles/sec for the high spatial frequency grating. When stimulus gratings were counterbalanced for temporal frequency, disruption in OKN was only apparent for the high spatial frequency rotating at high temporal frequency. These results imply that when high spatial frequencies are presented at high temporal frequencies, blocking the ON channel significantly decreases OKN response. We conclude that: 1) high concentrations of APB disrupt the processing of high spatiotemporal information by the visuomotor system and, 2) the disruption is transient, lasting no longer than 4 days. For the goldfish, processing by the ON channel is necessary to maintain the OKN reflex at high spatiotemporal frequencies. Supported by NIH grant EY03352, BRSG SO7-RR07201 and the Department of Psychology Honors Fund.

293.19 SPATIOTEMPORAL CHARACTERISTICS OF THE RETINAL GANGLION CELLS OF THE FROG. M.L.Mallon* and E.Micheli-Tzanakou. Department of Biomedical Engineering, Rutgers University College of Engineering, Piscataway, New Jersey 08855

In this study the latencies of the spatial components of the ganglion cell Receptive Fields (RFs) are examined for information pertaining to neural pathways in the retina for each of those spatial components.

Multiunit extracellular recordings were performed on ganglion cell fibers terminating in the superficial layers of the optic tecta of medium size grass frogs (Rana Pipiens). The units were separated using an amplitude discrimination method and the RFs were mapped using semi-automated scanning techniques. The response matrix produced is smoothed and linearly transformed into our intensity matrix which is further analyzed by a clustering method[1] to separate the RF into its spatial components. These spatial components which approximate the center, surround and outer surround are then used as stimuli and the latencies in addition to responses are collected for each cluster.

Data were collected for 33 recording sites for a total of 86 neurons. The data show a strong anticorrelation between the magnitudes of responses and the latencies. A weighted increase in latency from center to surround to outer surround of the RF was observed. A simple neural network is proposed for each of the spatial components that fits the experimental data.

References:
1. Micheli-Tzanakou, E.: "Visual Receptive Fields and Clustering." Behavior Research Methods and Instrumentation. 15 (6) 553-560 (1983).

293.20 MODELS OF MOVEMENT SENSITIVITY IN TYPE-2 GANGLION CELLS OF FROG RETINA. J. L. Teeters* (SPON: M.A. Arbib). Dept. of Computer Science, University of Southern Calif., Los Angeles, CA 90089-0782

Most ganglion cells in frog retina are movement sensitive. Their response (firing rate) is porpotional to the velocity of the stimulus raised to a constant exponent, $R = k * V^c$. Simulation done by others indicate that models consisting of a differentiation of bipolar cell signals, followed by rectification and spatial summation onto the ganglion cell can explain this velocity dependency. (See O.J. Grusser, U. Grusser-Cornehis, In Frog Neurobiology, R. Llinas & W. Precht. eds, Springer-Verlag 1976, Page 396 for review.)

We use computer simulation to examine the functional validity of these models from two standpoint: The stability of the models in response to parameter changes, and their ability to account for response properties other then velocity dependence. Our current emphasis is the type 2 ganglion cell. The velocity response of the model seems to be very stable to parameter changes. Halving or doubling most time constants does not significantly degrade the behavior. However other response properties (such as response to moving stimuli under stroboscopic illunimation, the response to stationary stimuli being enhanced by previous movement, local adaptation, and erasability) are not accounted for. Extensions to the basic model to encompass these other phenomena are being made. The goal is the development of single model which simultaneously accounts for many response properties. Research supported in part by the NIH under grant 7 R01 NS24926-01 from National Institute of Neurological and Communicative Disorders and Stroke and by the San Diego Supercomputer Center.

294.1 **PURIFICATION OF RAT RETINAL GANGLION CELLS BY PANNING**
B.E.Silverstein* and L.L.Y.Chun* (SPON: V.Tran). Department of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Studies of neurons and their interactions with other cell types often require pure neuronal cell populations. Previous methods of enrichment for ganglion cells from whole-retina dissociates include density gradient centrifugation (Beale *et al.*, 1983; Sarthy *et al.*, 1983) and use of a fluorescence activated cell sorter (Armson and Bennett, 1983). These procedures are time consuming and yield at best a cell population containing 75% retinal ganglion cells (RGCs).

Isolation of a highly pure (>98%) population of RGCs from a whole-retina cell suspension was effected by the technique of antibody-mediated plate adhesion ("panning") developed by Wysocki and Sato (1978). Plastic petri dishes are prepared by sequential incubation with affinity purified anti-mouse immunoglobulin and then with mouse monoclonal supernatant containing antibody directed against the Thy1.1 antigen. Retinas are dissected *in situ* from P8 rats, dissociated using the proteolytic enzyme papain (Lam, 1972; Bader *et al.*, 1978; Huettner and Baughman, 1986), and triturated. The resulting suspension of single cells is incubated on the panning plates. After removal of the non-adherent cells, the plates are gently washed. The adherent cells are then dislodged by pipetting medium against the plate, yielding a suspension of purified Thy1.1-positive cells.

Samples from the suspensions of whole-retina cells, non-adherent cells, and putative purified RGCs were labeled with anti-Thy1.1 antibody and analyzed with flow cytometry. The whole-retina cell suspension contained 0.35% Thy1.1-positive cells, corresponding to 135,000 Thy1.1-positive cells/retina. The non-adherent and RGC suspensions had 0.14% and 98% Thy1.1-positive cells, respectively. Thus, there was a 280 fold enrichment of Thy1.1-positive cells. The final yield of RGCs was 80,000 cells/retina or 60% of all Thy 1.1-positive cells in the whole-retina dissociate.

Because the retina contains macrophages, which might adhere to the panning plate nonspecifically, as well as other Thy1.1-positive cells, the purity of the RGC population is being studied immunohistochemically. Antibodies directed against GFAP, vimentin, lymphocytes, and macrophages are being used to probe the RGC population for contaminating cell types. However, virtually all cells label with antisera directed against neuron specific enolase and neurofilaments.

This simple, inexpensive, panning procedure rapidly yields a large number of highly pure RGCs. Retinal dissociates from six rats may be prepared and panned in under five hours, yielding 10^6 RGCs.

294.2 **MAb 2-54: AN ANTIBODY THAT BINDS TO A 38 KDa SYNAPTIC VESICLE PROTEIN IN THE RETINA.**
V. Gaur*, D. Possin*, W. Eldred and P.V. Sarthy (SPON: K. Chan). Depts. Ophthalmology and Physiology & Biophysics, University of Washington, Seattle, WA 98195, and Dept. of Biology, Boston University, Boston, MA 02215.

During a search for monoclonal antibodies that bind to specific cell types in the rat retina, we have obtained an antibody, 2-54, that binds only to the two synaptic layers in the retina. MAb 2-54 showed broad species cross-reactivity and labeled retinas from human, monkey, cow, rat, mouse, Xenopus, turtle, goldfish, and Anolis. In certain retinas, a difference in the staining of the two plexiform layers was noted. For example, in the mouse retina, the IPL was strongly stained while the OPL was barely stained, whereas with Anolis the reverse was true. A closer examination of the OPL staining in the monkey retina suggested labeling of both rod and cone terminals.

Ultrastructural localization was carried out using an avidin/biotin-peroxidase pre-embedding technique. The antibody was found to label presynaptic terminals in the plexiform layer and the reaction product was associated with synaptic vesicles. In the turtle retina the amacrine terminals were particularly strongly labeled.

The distribution of the antigen was also investigated in other areas of the nervous system as well as in other organs. Cryostat sections of rat spinal cord, cerebellum, cerebrum, hippocampus, adrenal gland, kidney, and diaphragm were screened. In most brain areas, 2-54 gave a punctate staining in regions containing nerve terminals while the white matter was negative. In the cerebellum, immunoreactivity was seen as small dots in the molecular layer and as large, irregular patches in the granule cell layer. In the spinal cord, staining was once again restricted to nerve terminals. There was no staining of the adrenal gland, kidney, and diaphragm.

Double-diffusion assays showed that MAb 2-54 was an IgG_3. Western blotting of rat retinal homogenates suggested that the antibody binds to a 38 KDa protein. Its ultrastructural localization to vesicles, broad cross-reactivity, and M_r of the antigen indicated that the antibody may be directed against a previously characterized synaptic vesicle protein, Synaptophysin. Comparative Western blotting studies using a commercially available (Boeringer Mannheim) MAb to bovine Synaptophysin showed that the antigens recognized by the two antibodies co-migrated. Further biochemical studies are underway to determine whether 2-54 is directed against Synaptophysin. Nevertheless, the tissue distribution of 2-54 staining is different from that reported for Synaptophysin MAbs, and therefore suggests binding to a new epitope on the Synaptophysin molecule. Developmental studies show that the pattern of binding of 2-54 and that of another synaptic vesicle antibody, SV48, are different in the rat retina. (Supported by EY03664 and EY01730)

294.3 **LOCALIZATION OF GABA RECEPTORS ON PHOTORECEPTOR SYNAPTIC TERMINALS IN GOLDFISH AND CHICKEN RETINAS BY IMMUNOCYTOCHEMISTRY.**
S. Yazulla, K.M. Studholme*, J. Vitorica* and A.L. DeBlas. Dept. Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

A large body of physiological and pharmacological evidence indicates that GABA is a feedback transmitter from a class of horizontal cells to cone photoreceptors in the retinas of nonmammals. However, GABA receptors have not yet been demonstrated on photoreceptors by morphological techniques. We have used a mouse monoclonal antibody (mAb)62-3G1 to GABA receptors in the retinas of chicken and goldfish at the light microscopical level. The mAb 62-3G1 was produced by immunizing BALB/c mice with the $GABA_A$/benzodiazepine receptor complex that had been purified by affinity chromatography on immobilized Ro7-1986/1. The mAb 62-3G1 immunoprecipitated the receptor complex and reacted in immunoblots with the 57,000 Mr peptide which is the [^{3}H]muscimol binding subunit of the receptor complex (Vitorica, Park and DeBlas, Eur. J. Pharmacol., in press). Cryostrat sections were incubated in mAb and processed by a standard indirect PAP method. In both species, GABA receptor immunoreactivity (GABAr-IR) was distributed in a laminar pattern throughout the inner plexiform layer (IPL), similar to that previously shown for GABA-IR and GAD-IR. However, in the outer plexiform layer (OPL) GABAr-IR was restricted to the synaptic terminals of photoreceptors, unlike GABA-IR and GAD-IR which are localized to horizontal cells. Photoreceptor inner segments, nuclei and outer segments were not labeled by GABAr-IR. In chicken, there were two layers of labeled synaptic terminals in the OPL. We could not determine if only cone terminals or if both rod and cone terminals were labeled by GABAr-IR. In goldfish retina, cone synaptic terminals were intensely labeled by GABAr-IR. Rod terminals appeared to be unlabeled or much reduced in label. Cones, unlike rods, are thought to receive GABAergic inhibition from horizontal cells in nonmammalian retinas. The absence of GABAr-IR labeling of horizontal cells suggests that this mAb labels GABA synaptic receptors rather than high affinity GABA transporters in the outer retina of chicken and goldfish. This is the first morphological evidence for synaptic GABA receptors located on cone photoreceptor synaptic terminals.

294.4 **MONOCLONAL ANTIBODIES TO RABBIT RETINAL PHOTORECEPTORS OR MÜLLER CELLS.**
W. H. Tsai[1], J. N. Hokoc*[2], A. P. Mariani*[2] and M. Nirenberg[1]. [1]Laboratory of Biochemical Genetics, NHLBI and [2]Laboratory of Neurophysiology, NINCDS. National Institutes of Health, Bethesda, MD 20892.

Retinal tissue from adult New Zealand white rabbits was dispersed by gentle homogenization and crude particulate fractions were injected into the spleens of Balb/c mice. Three days later the spleen cells were harvested and fused with P3X63Ag8.653 mouse myeloma cells. The antibodies synthesized by the 435 hybridoma cell lines produced were screened by immunohistochemistry using radial sections of fixed adult rabbit retina and peroxidase-antiperoxidase and indirect immunofluorescent methods.

The initial screening of antibody specificity revealed 8 monoclonal antibodies that are specific for a single cell type or a layer of the retina; i.e., 3 antibodies are specific for photoreceptor outer segments, 1 for photoreceptor cell soma, 1 for the outer synaptic layer, 2 for Müller cells, and 1 for cell soma in the ganglion cell layer. In addition, 1 antibody is specific for photoreceptor outer segments and photoreceptor cell soma, 1 antibody recognizes photoreceptor outer segments and Müller cells, and 2 antibodies bind to the outer limiting membrane of retina and to cell soma in the ganglion cell layer. Many antibodies also were found that recognize antigens that are distributed in other, more complicated patterns in retina.

294.5 **MONOCLONAL ANTIBODIES THAT RECOGNIZE SUBPOPULATIONS OF CELLS IN THE GANGLION CELL LAYER OF THE CAT'S RETINA.** N. Tumosa, P.D. Spear, L. Kahan*, Dept. of Psychology, Neurosciences Training Program, Dept. of Physiological Chemistry, and Hybridoma Facility, University of Wisconsin, Madison, WI 53706

Physiological studies have shown that there are three relatively distinct functional classes of ganglion cells in the cat's retina. These classes, termed X, Y, and W cells, form the basis of parallel and separate functional outputs from the retina to visual areas of the brain. Morphological studies of the cat's retina also have identified separate classes of ganglion cells based on soma size and dendritic branching patterns. There is good evidence that the largest of these morphological cell types, the alpha cell, corresponds to the physiological Y-cell. The development of a specific marker for alpha/Y cells would be of great assistance in determining their role in vision. To that end, we have attempted to produce a monoclonal antibody specific for alpha/Y cells in the cat's retina.

We first separated cat retinal cells on the basis of soma size. We then injected 2.3 x 10^5 of the largest cells (25-40 um diameter) intrasplenically into a mouse. Several days later the spleen cells were fused with NS-1 mouse myeloma cells. Antibody producing hybridomas were cloned and IgM antibodies were tested for their reaction with the original immunogen of large retinal cells. Antibodies that showed specificity were placed overnight on 25 um cryostat-cut transverse sections of cat's retina. Rinsed tissue was then incubated with peroxidase-labeled goat anti-mouse IgM for 1 hour. Hydrogen peroxide and diaminobenzidine were used to visualize the monoclonal antibody labeled cells.

Many of the monoclonal antibodies reacted with retinal cells; nine were chosen for further analysis. All nine reacted with cell somas in the ganglion cell layer (GCL) and most also reacted with processes that appear to be dendrites. Seven of the antibodies reacted only with cells in the GCL. One of these reacted with a large heterogeneous population of cells; the other six reacted with more homogeneous subpopulations of GCL cells. For instance, one antibody reacted only with very large cells in the GCL whereas others reacted only with smaller cells. Two antibodies reacted with cells in the inner nuclear layer (INL) as well as a subpopulation of cells in the GCL. One of these antibodies reacted with many INL cell somas but labeled no processes. The other reacted with only a few INL cell somas and with processes extending into the inner plexiform layer from both INL and GCL cells. None of the antibodies reacted with elements in any other layer of the retina. Further studies are under way to identify the retinal cell types recognized by each antibody.

294.6 SEROTONIN ANTAGONISTS REDUCE THE EFFICACY OF HORIZONTAL TO GANGLION CELL TRANSMISSION IN THE INNER RETINA OF THE RABBIT. S.C. Mangel, W.J. Brunken and R.F. Miller. Department of Ophthalmology, Washington University School of Medicine, St. Louis, Missouri 63110.

In the rabbit retina, serotonin ($5\text{-}HT_2$) antagonists reduce the spontaneous activity and the on-components of the light-evoked responses of all classes of brisk ganglion cells (Brunken and Daw, 1986). Because the on-surround of off-center cells is affected by these antagonists and because rabbit horizontal cells contribute to the surround of ganglion cells (Mangel and Miller, 1987), we studied whether horizontal cells or the horizontal cell input to ganglion cells is also affected by serotonin antagonists.

In our experiments, LY53857, a $5\text{-}HT_2$ antagonist, was added to the superfused rabbit eyecup, while horizontal and ganglion cells were simultaneously monitored with intracellular and extracellular electrodes, respectively. The light-evoked responses of horizontal-ganglion cell pairs were tested with flashing spots, annuli and slits of light. In some drug applications, sinusoidally-modulated current was injected into a horizontal cell while the extracellular spike activity of a nearby ganglion cell was monitored.

As reported previously, LY53857 (10-80 uM), reduced the spontaneous activity and the on-components of the light responses of ganglion cells. During these effects, the membrane potential and light-evoked responses of horizontal cells remained unchanged, as did the b-wave of the ERG. Later occurring effects on horizontal cells, such as increases in light response amplitude, were also observed. These findings suggest that during the effects of LY53857 on ganglion cells,the serotonin antagonist acts predominantly in the inner retina. Furthermore, a dramatic reduction in the modulation of ganglion cell discharge induced by horizontal cell polarizations during application of the $5\text{-}HT_2$ antagonist was observed. These findings thus suggest that serotonin antagonists can reduce the efficacy of horizontal cell to ganglion cell transmission at an inner retinal site.

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294.7 THE EFFECTS OF GABA AND SEROTONIN ON THE RESPONSE PROPERTIES OF RETINAL HORIZONTAL CELLS OF THE SKATE. J.L. Cohen Dept. of Anatomy, Wright State University School of Medicine and School of Science and Engineering, Dayton, Ohio 45435 and the Mount Desert Island Biological Laboratory, Salsbury Cove, Maine.

The retina of the skate has been shown to possess neurons that contain the neurotransmitters GABA and serotonin (Brunken et al., J. Comp. Neur. 243:1-12, 1986). GABA was found in an interplexiform type cell, while serotonin was located within amacrine cells. We therefore wanted to determine the effect of GABA on the membrane potential, and light-evoked activity of the horizontal cell. We also wanted to determine if serotonin played a role in the activation of the GABAergic interplexiform cells, since it has been found in amacrine cells which may be presynaptic to the GABA-ergic interplexiform cell (Brunken et al., 1986).

Intracellular recordings were made from the superfused eyecup preparation of the skate, Raja oscellata. Horizontal cells were identified by their characteristic response to specific types of light stimuli. They were encountered between 75 and 120 microns below the retinal surface and had resting potentials in the dark between -20mv and -40mv. These cells responded to light with graded hyperpolarizations that increased in amplitude with increasing stimulus diameter. In order to keep the retina in the dark adapted state, stimulus intensities used were approximately one log unit above threshold.

When a 3 minute pulse of 500 uM GABA flowed over the retina, the membrane potential of the cell depolarized approximately 12 mv. As the membrane potential depolarized, the response amplitude of the cell increased. Changes in the waveform of the response was also seen. The response became faster, a transient began to appear in the initial part of the hyperpolarization and the waveform became more complex. As the GABA was washed out with control Ringer's, the membrane potential started to hyperpolarize until it returned to the control resting potential. During this recovery period, the amplitude of the light evoked responses also decreased until it returned to control levels.

Application of 500 uM serotonin to the retina caused a depolarization of the membrane potential approximately 13mv. At the same time there was an increase in the amplitude of the light evoked responses.

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294.8 N-ACETYLASPARTYLGLUTAMATE LOCALIZED BY IMMUNO-ELECTRON MICROSCOPY IN PUTATIVE SYNAPTIC VESICLES IN THE INNER PLEXIFORM LAYER OF THE AMPHIBIAN RETINA. L. C. Williamson* and J. H. Neale (SPON: D. Eagles). Dept. of Biology, Georgetown University, Washington, D.C. 20057.

N-Acetylaspartylglutamate (NAAG) is found specifically and in relatively high concentration in the nervous system. We have reported the use of antisera and affinity purified antibody prepared against NAAG to localize this molecule within specific subpopulations of peripheral and central neurons. In the amphibian retina, NAAG-IR is concentrated in some, but not all, amacrine and bipolar neurons as well as their neurites in the inner plexiform, outer plexiform and inner segmental synaptic layers (Br. Res. 406:397, 1987). In contrast to data we have obtained in the mammalian retina, there is little evidence for NAAG in frog retinal ganglion cells.

In the present study, we have examined the fine structural localization of NAAG-IR within the amphibian inner plexiform layer, a synaptic neuropil containing ganglion cell, amacrine and bipolar neurites. Frogs, Rana pipiens, were fixed by perfusion with carbodiimide and paraformaldehyde. Retinas were removed and postfixed prior to embedding in LR White resin. Ultrathin sections were stained with primary antibody against NAAG and IR was visualized by immunogold labeling. NAAG-IR was observed within some but not all vesicles associated with apparent presynaptic endings in the inner plexiform layer. Only a portion of the vesicles within individual synaptic endings were labeled suggesting the possibility of its co-release with other molecules. Some synapses within the neuropil exhibited no labeled vesicles. At the light microscopic level, NAAG-IR is distributed throughout the cell body and neurites of a subpopulation of amacrine and bipolar cells. Consistent with this result, NAAG-IR was found associated at the ultrastructural level with a number of non-synaptic structures in some inner nuclear layer neurons.

Data on the activation of NMDA receptors by NAAG and on the role of NMDA receptors in amphibian amacrine and bipolar neuron function, together with these ultrastructural results, provide support for the hypothesis that NAAG participates in retinal neurotransmission. Studies of NAAG biosynthesis and release from frog retinal cells in vitro have been initiated. (supported by NIDA grant DA 02297)

294.9 ENKEPHALINS AND DYNORPHINS IN MAMMALIAN RETINA. K.L. Jones* and D.W. Hoffman. Neurochemistry Lab., SIU Sch. of Med., Springfield, IL 62708.

Neuropeptides have been identified in amacrine cells in the retinas of several vertebrate species, where they may serve as neurotransmitters or neuromodulators mediating the action of amacrine cells on the receptive field organization of complex ganglion cells. Several proenkephalin A-derived neuropeptides (the enkephalins), have been identified in the retina of the guinea pig (cavia porcellus), but not in other mammalian retinas, such as the OM strain white rat, despite the fact that opiate receptors have been reported in the rat and other mammalian retinas.

The other neuropeptides which can be endogenous ligands for these opiate receptor binding sites are other enkephalins, or the proenkephalin B-derived peptides (the dynorphins/neoendorphins). Their presence may serve to resolve the discrepancies which currently exist between immunochemical studies of opioid peptides in mammalian retina, and the results of opiate receptor binding studies.

Retinas are removed from anesthetized animals and rapidly sonicated in HPLC buffer, which is composed of 7.5 mM trifluoroacetic acid in 25% acetonitrile. Samples are centrifuged and injected onto a reverse phase column, and eluted with the above HPLC buffer at 0.6 ml/min. Fractions are collected and assayed for peptides by RIA. Preliminary data indicates that although dynorphin B is present in some mammalian retinas, the levels are low and variable. Dynorphin A 1-17 appears to be present in much higher concentrations than dynorphin B. Using combined HPLC-RIA, we have consistently found significant levels of dynorphin A 1-17 in retinas of cat, rabbit, and monkey. This may be the predominant form of the prodynorphin-related peptides in mammalian retina.

Animal	N	Dynorphin A 1-17 (pmol/retina)
Monkey	2	1.48
Rabbit	12	0.81
Cat	6	0.48

Acknowledgements: This work was supported by EY06180 (DWH).

294.10 **LOCALIZATION OF MET-ENKEPHALIN-ARG-PHE IN THE VERTEBRATE RETINA.** Y.Y. Thomas Su. Center for Biotechnology, Baylor College of Medicine, 4000 Research Forest Drive, The Woodlands, Texas 77381

It is well established that the structure of proenkephalin A contains four copies of Met-enkephalin (ME) and one copy each of Leu-enkephalin (LE), the heptapeptide ME-Arg-Phe (ME-7) and the octapeptide ME-Arg-Gly-Leu (ME-8). Previous studies from this laboratory have demonstrated the presence, localization, biosynthesis and release of ME in the vertebrate retina. In this communication we report the localization of the heptapeptide ME-7 in the chicken retina.

Isolated eye cups were fixed in buffered (0.1 M phosphate buffer, pH 7.4) 4% paraformaldehyde overnight at 4°C. The retinas were then isolated from the posterior eye cups and cut into small rectangular-shaped pieces. Each piece was embeded in agarose and 75 μm thick transverse sections prepared with a vibratome. The sections were preincubated in 2% normal goat serum in PBS for 2 hours and then incubated in polyclonal anti-ME-7 (1:500 in PBS) overnight at 4°C. The sections were rinsed 3 times for 10 minutes each in PBS and then incubated in a biotinylated goat anti-rabbit IgG (1:200 in PBS) for 2 hours at room temperature. The sections were then rinsed 3 times for 10 minutes each before incubation in the avidin-biotin complex (ABC, 1:100 in PBS) for 2 hours at room temperature. After a 1 hour rinse, the sections were stained with a DAB solution (20 ml PBS, 60 μl H_2O_2, 10 mg diaminobenzidine tetrahydrochloride) for 10 to 20 minutes. The staining reaction was terminated by a 10 minute wash in PBS. The specificity of the immunostaining was determined by incubating retinal sections in antibody which had been preabsorbed with excess ME, LE, ME-Arg, ME-7, ME-8, or β-endorphin (100 μg/ml of antiserum).

An examination of retinal sections revealed that ME-7-like immunoreactivity was localized to a subpopulation amacrine cells in the inner nuclear layer. ME-7 stained pear-shaped cell bodies were situated in the second and third tiers of cells from the border of inner nuclear and inner plexiform layers. These cells sent their processes into sublaminae 1, 3 and 4 of inner plexiform layer. No ME-7 immunostaining was detectable when antibody was preabsorbed with excess ME-Arg or ME-7. However, excess ME, LE, ME-8 or β-endorphin exhibited no effect on ME-7 staining. These observations, therefore, suggested that the immunostaining observed in the tissue is specific for ME-7 and/or ME-Arg.

The distribution of ME-7-like immunoreactivity observed in the present study corresponds quite well with previous reports on the distribution of ME-like immunoreactivity in the avian retina. However, more studies are needed to elucidate their relationships.

Supported by NIH Grant EY03701 and Retina Research Foundation (Houston).

294.11 THE DEVELOPMENT OF CRF-CONTAINING NEURONS IN THE RAT RETINA. D. Zhang and H.H. Yeh, Dept. Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

We have been studying a system of corticotropin releasing factor (CRF)-containing neurons in the rat retina. In the rat retina, CRF has been localized to amacrine cells in the inner nuclear layer and ganglion cells in the ganglion cell layer (Skofitsch and Jabcobowitz, 1984). Recent anatomical evidence from our laboratory, however, suggests the possibility that the majority of CRF-containing cells in the ganglion cell layer may in fact be displaced amacrine cells. To help reveal the identity of CRF-containing neurons, we have examined their developmental profiles as part of our anatomical analysis of the CRF system in the rat retina.

We examined immunohistochemically the development of CRF-containing retinal neurons in Long Evans rats of different ages (embryonic day-20 to postnatal day-19). Beginning with the day of birth, littermates were used for each developmental series; one rat pup from each litter was sacrificed on a selected postnatal day by transcardiac perfusion with ice-cold phosphate buffer under chlorpent anesthesia and then enucleated. The retinas were quickly isolated, prepared as wholemounts, immersed in 4% paraformaldehyde for two hours and stored in cryoprotectant (Watson et al., 1986) so that tissue from a complete developmental series could be processed simultaneously for immunohistochemistry. One wholemount from each rat pup was subsequently embedded in epon to obtain transverse sections.

The first CRF-immunopositive cells were observed by postnatal day-4, when wholemount preparations revealed faintly staining perikarya limited largely to the central (posterior) region of the developing retina. By postnatal day-6, CRF immunoreactivity became more evenly distributed throughout the retina. Transverse sections indicated that, at the earliest detectable time, CRF-immunopositive cells were situated both in the ganglion cell layer and in the innermost aspect of the neuroblastic cell mass destined to become the inner nuclear layer. This is similar to the laminar distribution of CRF-containing cells in the adult rat retina. However, a striking feature in development appears to be shift in the pattern of distribution of these cells. Over the first two postnatal weeks, both the density of CRF-containing cells in the inner nuclear layer and the ratio of cells in the inner nuclear layer to those in the ganglion cell layer decrease. This is consistent with the notion of a perikaryal translocation during development and we postulate that a subpopulation of immature amacrine cells become displaced to the ganglion cell layer. By eye-opening (postnatal day-15), such dynamic developmental changes appear to stabilize and, by postnatal day-19, CRF-containing neurons become morphologically mature.

Supported by a grant from the Rochester Eye and Human Parts Bank and NIH grant NS 24830 to HHY and by a U. Rochester Program in Biology and Medicine Fellowship to DZ.

294.12 SYNAPSES FROM BIPOLAR CELLS ONTO TYROSINE HYDROXYLASE IMMUNOREACTIVE AMACRINE CELLS IN CAT AND RABBIT RETINAS J.N. Hokoc* and A.P. Mariani* (SPON: M.T. Caserta) Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892

Dopaminergic amacrine cells in the vertebrate retina had long been characterized as "interamacrine" as they were only found to be pre- and post-synaptic to other amacrine cells based on uptake studies. Immunohistochemistry with antibodies directed against tyrosine hydroxylase (TH), the rate limiting enzyme in the catecholamine synthetic pathway, revealed synapses from bipolar cell axon terminals to TH-containing neuronal processes at ribbon synapse in the rhesus monkey retina (Hokoc & Mariani, 1987). In fact, these ribbon synapses accounted for the majority of the synaptic input to this transmitter phenotype. This finding provided evidence for a direct, light-mediated pathway from the outer plexiform layer to dopaminergic amacrine cells in the inner plexiform layer and, thus challenged the notion of the dopaminergic amacrine cell type as "interamacrine". In order to determine if the finding of synapses from bipolar cells to dopaminergic amacrine cells could be generalized to other species, we therefore studied the synaptic organization of dopaminergic amacrine cells in the retinas of cat and rabbits with electron microscopy of TH immunoreactivity. In both species, the TH-containing processes possessed small clear vesicles and were presynaptic to nonimmunoreactive amacrine cells. Synapses onto TH-immunoreactive profiles were from bipolar axon terminals at ribbon synapses and from nonimmunoreactive amacrine cells also. These synapses were located in the outermost stratum of the inner plexiform layer. Ribbon synapses onto dopaminergic amacrine cells in retinas of cats and rabbits now demonstrate that the original finding in the primate can be generalized to at least two other species and therefore may be a significant feature in the retinas of many other vertebrates as well.

J.N. Hokoc is on leave from the Federal University of Rio de Janeiro.

294.13 TYROSINE HYDROXYLASE-LIKE IMMUNOREACTIVE AMACRINE CELLS IN THE LARVAL TIGER SALAMANDER RETINA. S.Z. Yang*, C.B. Watt, D.M.K. Lam, and S.M. Wu. Center for Biotechnology, Baylor College of Medicine, The Woodlands, Texas 77381 and Department of Ophthalmology, Baylor College of Medicine, Houston, Texas 77030.

Recently we have initiated studies that systematically examine the structural organization of classical transmitter-specific cell populations in the larval tiger salamander retina (Watt et al., 1987). In the present study, immunocytochemistry was used to examine the organization of tyrosine hydroxylase-like (TH) immunoreactive cells. The antibody to TH was obtained from Eugene Tech and studies were performed using the avidin-biotin method (Vector Laboratories).

Of 728 TH-immunostained cells observed in transverse cryosections obtained from various retinas, 90% were situated in the innermost cell row of the inner nuclear layer and were designated as amacrine cells. Their cell bodies were round- to pyramidal-shaped. The processes of these cells were found to distribute in sublayers 1, 3 and 5 of the inner plexiform layer. The remaining 10% of immunostained cells were found in the ganglion cell layer and were tentatively designated as displaced amacrine cells. Their cell bodies were round- to oval-shaped, and their processes were observed to ramify in sublayers 1, 3, and 5 of the inner plexiform layer.

An examination of whole-mount retinas revealed the size, distribution and density of TH-immunostained cells. TH-amacrine cells ranged from 17 to 19 microns in diameter with the vast majority measuring 18 microns. Displaced TH-amacrine cells were somewhat larger and ranged from 18 to 20 microns in diameter. Both TH-immunostained amacrine and displaced amacrine cells were distributed throughout central and peripheral regions of the retina. The density of TH-amacrine cells was 49±13 cells per mm^2. The density of displaced TH-amacrine cells was not determined in the whole-mount retina, although an examination of transverse sections revealed that they composed 10% of TH-cells. Studies are presently examining the morphology of dendritic fields of TH-immunoreactive amacrine and displaced amacrine cells.

This work is supported by NIH EY05622 (CBW), EY02423 (DMKL), EY04446 (SMW) and by the Retina Research Foundation (Houston).

294.14 **GABA-ERGIC GANGLION CELLS IN THE RABBIT RETINA.** **C.B. Watt, B.C. -Y. Yu, D.M.K. Lam, and K.R. Fry.** Center for Biotechnology, Baylor College of Medicine, The Woodlands, Texas 77381.

Previous studies using glutamic acid decarboxylase (GAD) immunocytochemistry and high affinity ^{3}H-GABA uptake autoradiography have suggested the presence of gamma-aminobutyric acid (GABA) in populations of ganglion cells in the rabbit retina. The recent development of an anti-GABA polyclonal antibody has provided an additional tool to investigate the localization of GABA in the central nervous system. We used the GABA antibody in combination with a ganglion cell-specific monoclonal antibody, AB5 (Fry, et al., Brain Research 338:360, 1985), to examine if GABA is present in ganglion cells of the rabbit retina (Yu, et al., Invest. Ophthalmol. Vis. Sci. (Suppl.) 28:349, 1987). These studies revealed that GABA was localized to large, medium and small ganglion cells.

As a continuation of these studies, we have utilized other markers of GABA-ergic activity to further investigate GABA-ergic ganglion cells in the rabbit retina. Both GAD-immunocytochemistry and high-affinity ^{3}H-GABA uptake autoradiography labelled large, medium and small cells which had the appearance of ganglion cells. These techniques also labelled fibers in the nerve fiber layer. Furthermore, ^{3}H-GABA-labelled processes were observed to enter and travel in the optic nerve itself. Subsequent double-label studies utilizing ^{3}H-GABA uptake in combination with the AB5 ganglion cell-specific antibody were performed to definitively identify those ganglion cells which exhibit high affinity uptake of ^{3}H-GABA. These studies revealed that large, medium and small ganglion cells specifically accumulate ^{3}H-GABA. Double-label studies utilizing GAD immunocytochemistry in combination with AB5 immunocytochemistry are presently underway to determine if GAD is present in ganglion cells of the rabbit retina. Future studies are directed towards determining the central projection sites of GABA-ergic ganglion cells.

This work is supported by NIH grants EY05622 (CBW), EY02608 and EY02423 (DMKL), EY06469 (KRF), and the Alberta Heritage Foundation (KRF).

294.15 RELEASE OF DOPAMINE AND GAMMA-AMINOBUTYRIC ACID FROM THE RETINA FOLLOWING DIISOPROPYLFLUOROPHOSPHATE. A.W. Kirby, A.T. Townsend*, R.G. Stafford*, C.D. Pope* and T.H. Harding*. U.S. Army Aeromedical Research Laboratory, Ft. Rucker, AL 36362.

We previously have demonstrated a preferential loss in the visual evoked response (VER) to low spatial frequency stimulation in cats and rabbits following administration of diisopropylfluorophosphate (DFP), an anticholinesterase agent. Although the most likely mechanism is the prolonged action of acetylcholine (ACh) at cholinergic synapses in the visual pathway, it has become apparent that acetylcholinesterase (AChE) inhibition does not always correlate well with observed changes. This is explained easily if putative neurotransmitters other than ACh are involved following exposure to DFP. Previous neurochemical work from our lab has demonstrated changes in dopamine (DA) and gamma-aminobutyric acid (GABA) both in retina and cortex following administration of DFP. ACh, as well as DA and GABA, has been localized mainly to amacrine cells in the retina. Based upon histochemical localization by others, cholinergic amacrine cells are not in position to alter DA or GABA levels through increased synaptic activity. Therefore, we undertook these experiments to directly investigate release of DA and GABA from the retina.

Seven adult cats and 5 adult rabbits were anesthetized and 50 µCi of ^{3}H-DA or ^{3}H-GABA injected into the vitreous. Following one hour of incubation, the eye was enucleated and the anterior segment and lens removed. The remaining eye cup was rinsed in 54 ml oxygenated Ames buffer for 30 minutes to remove excess label, trimmed to about a 15 mm square (including area centralis), and mounted on a holder. The holder then was transferred through a series of buffer-containing vials, spending 2 minutes in each. DFP was added to 4 vials on the plateau of the release curve. To determine the amount of labeled material released, one ml of buffer from each vial was mixed with 5 ml of scintillation fluid and radioactivity counted with a scintillation counter. Studies thus far have been done without any light modulation.

For the 4 vials containing DFP we observed a biphasic change in release of both DA (4 rabbit eyes; 6 cat eyes) and GABA (6 rabbit eyes; 3 cat eyes). Release was increased consistently in the first vial containing DFP and then decreased. Some experiments were done under conditions to block synaptic transmission during DFP exposure (high Mg^{2+} and low Ca^{2+}). The biphasic change persisted in those experiments (2 rabbit eyes-GABA; 1 cat eye-DA). For one cat eye, 2 mM ACh was substituted for DFP and there was no effect on DA release. Although preliminary, these experiments support the lack of synaptic contact between cholinergic and DA or GABA amacrine cells in cat and rabbit retina. Futhermore, they suggest a direct effect of DFP on neuronal membranes of DA and GABA neurons.

294.16 **IDENTIFICATION OF A DOPAMINE-ACCUMULATING GANGLION CELL TYPE IN THE CAT'S RETINA BY *IN VITRO* FLUORESCENCE.** D.M. Dacey. Dept. of Ophthalmology, Univ. of Washington, Seattle, WA 98195.

Anesthetized cats received intravitreal injections of dopamine and the indoleaminergic transmitter analogue 5,7 dihydroxytryptamine. The eyes were removed after 3-8 hrs and the retinas were dissected in oxygenated Ames medium and mounted flat in a superfusion chamber on the stage of a light microscope. When retinas treated in this way are observed under blue epifluorescence illumination, an intense yellow-green fluorescence is present in distinct subpopulations of neurons in the inner nuclear layer (INL) and ganglion cell layer (GCL), and in their dendrites in the inner plexiform layer (IPL). The complete dendritic morphology of these cells was demonstrated by intracellular injections *in vitro* of lucifer yellow and rhodamine conjugated horseradish peroxidase (HRP). One population of fluorescing neurons corresponds to the dopaminergic amacrine cells shown previously with immunohistochemical and histofluorescence techniques. These cells are located in both the INL and the GCL and give rise to sparsely branched dendrites that narrowly stratify at the outer border of the IPL. Another population of cells is restricted to the ganglion cell layer; they have larger cell bodies and a less intense fluorescence than the dopaminergic amacrines. Intracellular HRP injections into these cells show that they comprise a single morphological type of ganglion cell with the following properties. The spherical cell body ranges in size from about 15 µm near the central area to about 20 µm in the retinal periphery. Dendritic field size increases from 150-200 µm at 0.5 mm from the central area to 500-600 µm in the retinal periphery and is thus about twice the size of a beta cell at any given eccentricity. The axon is medium caliber, about 1 µm in diameter. The dendritic tree is narrowly stratified at the outer border of the IPL (25-30 µm from the cell body when measured *in vitro* after lucifer yellow fills) and shows a characteristic branching pattern marked by gently recurving and meandering dendrites that establish an elliptical field. The dendritic branches do not fill the field uniformly but show a relatively high density and regular spacing in the proximal half of the tree and more sparse, less regular spacing in the distal half. HRP fills of a cluster of adjacent cells show that the distal half of the dendritic field overlaps with its neighbors. In the region of overlap dendrites of neighboring cells interdigitate so that, despite the variation in density across a single field, a constant density and spacing across the plexus of dendrites for the cell population is achieved. As for alpha and beta cells, dendritic field size increases as cell density decreases so that the amount of dendritic overlap also remains constant from center to periphery. Preliminary estimates of cell density based on cell-to-cell spacing suggest that the dopamine-accumuating ganglion cells comprise about 10% of the total ganglion cell population in cats. (Supported by USPHS-NEI grant EY-06098.)

294.17 CATECHOLAMINERGIC GANGLION CELLS IN THE RETINA OF THE PIGEON. K.T. Keyser*, L.R.G. Britto and H.J. Karten. Dept. of Neurosciences, UCSD, La Jolla, CA 92093 and Dept. Physiol. and Biophys., Institute of Biomedical Studies, Sao Paulo State Univ., 05508 Sao Paulo, Brazil.

Tyrosine hydroxylase (TH) converts tyrosine to DOPA and is considered a reliable marker for catecholaminergic neurons. We used a rabbit antiserum directed against tyrosine hydroxylase to investigate the distribution of catecholamines in the retina of the pigeon Columba livia.

Two populations of neurons in the ganglion cell layer (GCL) exhibited TH-like immunoreactivity; a lightly stained type which was not included in this analysis and a more intensely stained type. The heavily stained somata were about 12 um in diameter and, in some sections, could be seen to extend processes into the middle and outer laminae of the inner plexiform layer (IPL). In addition, in many cases the cells gave rise to a single long process which could be followed in the optic fiber layer toward the optic nerve head.

The TH-positive cells in the GCL were present in low density throughout the retina. In the ventral periphery of the retina, there were about 10 TH-positive cells/mm^2. This figure increased to 20-25 cells/mm^2 in an oval central area that included the fovea. There was also a region of higher density (20-22 cells/mm^2) in the central red field. The density decreased in the remainder of the superior nasal and temporal areas to the level found in the ventral periphery.

Multiple injections of rhodamine labelled latex microspheres into the optic tectum resulted in retrograde labelling of a large number of ganglion cells throughout the retina. Many of the TH-positive cells in the GCL also exhibited rhodamine fluorescence. This indicates that these cells project centrally and therefore represent a population of retinal ganglion cells.

Tyrosine hydroxylase immunoreactivity is present in fibers in many areas of the optic tectum including the retinorecipient layers. Some of this may be attributable to central catecholaminergic nuclei. Unilateral enuculation results in little apparent change in the pattern of staining in these areas. This result may be due to both the low numbers of TH-positive ganglion cells and the extensive projections of central catecholaminergic cell groups to the optic tectum. Supported by EY06890 and DAMD 17-86-C-6093/Livingston.

294.18 CHOLINOCEPTIVE NEURONS IN THE RETINA OF THE CHICKEN: AN IMMUNOHISTOCHEMICAL STUDY OF THE NICOTINIC ACETYLCHOLINE RECEPTORS. T.E. Hughes, K.T. Keyser*, P.J. Whiting#*, J.M. Lindstrom#, and H.J. Karten. Dept. of Neurosciences, UCSD, La Jolla, CA 92093 and #Receptor Biology Lab, The Salk Institute for Biological Studies, La Jolla, CA 92138

Choline acetyltransferase (ChAT) antibodies have been used to identify and characterize the presumptive cholinergic neurons in the vertebrate retina. However, the postsynaptic cholinoceptive neurons have been more difficult to identify.

We used monoclonal antibodies directed against the nicotinic acetylcholine receptor (nAChr) of bovine muscle (mAb 210) or chick brain (mAb 270) to investigate the distribution of presumptive cholinoceptive neurons in the retinae of white leghorn chicks. In addition, selected sections were simultaneously labeled with antisera directed against glutamine synthetase (GS) or ChAT to classify the laminar distribution of the labeling of nAChR within the inner plexiform layer (IPL) and its distribution relative to ChAT-like staining, respectively.

The antibodies mAb 210 and 270 stain small to large-sized somata in the ganglion cell layer (GCL) and small to medium-sized cells in the inner half of the inner nuclear layer (INL). They also stain a population of large somata at the INL-IPL border. Labeled dendrites can be followed from the immunoreactive somata into two distinct laminae within the IPL. Double labeling with the GS antiserum reveals that the laminae seen with mAb 210 are centered in laminae 2 and 4. Each of these laminae receive arborizations from the immunoreactive cells in the INL and GCL. However, the relative contributions of the two populations cannot be readily discerned.

Double labeling with the ChAT antiserum reveals that the ChAT distribution within the IPL lies within the broader nAChr stained laminae. No evident co-occurrence of staining of ChAT and nAChR is found in the somata.

These data provide a preliminary identification of at least some of the presumptive cholinoceptive neurons in the chick retina. Since the small and medium-sized cells in the INL are confined to the inner half of the layer, it is probable that the majority of these neurons are amacrine cells. The large somata at the inner margin of the INL resemble the displaced ganglion cells in their morphology and distribution. The majority of the labeled somata in the ganglion cell layer can, on the basis of their size and morphology, be classified as ganglion cells.

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294.19 **DISTRIBUTIONS OF CHOLINE ACETYLTRANSFERASE AND ACETYLCHOLINESTERASE ACTIVITIES IN THE RETINAL LAYERS OF THREE AVIAN SPECIES.** L. E. White*, C. D. Ross, and D. A. Godfrey. Dept. of Physiology, Oral Roberts Univ. School of Medicine, Tulsa, OK 74171.

The activities of choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) were assayed in submicrogram samples from retinal layers of avian species using quantitative histochemical techniques. These quantitative data were compared to patterns of staining for ChAT-like immunoreactivity and AChE activity. Red-tailed hawk and road runner were sampled to determine these activities in species of different visually related behaviors. Red and yellow fields of pigeon were sampled to investigate the relationship between the enzymes of acetylcholine metabolism and the gradient of inner plexiform layer (IPL) complexity, increasing from the yellow field to the red. The results are summarized in the table below with enzyme activities expressed as mean ChAT activity (in µmol/kg dry wt/min)/mean AChE activity (in mmol/kg dry wt/min). Most standard errors are less than 15% of the means.

	Red-Tailed Hawk	Pigeon Red Field	Pigeon Yellow Field	Road Runner
OPL	3/163	16/6	4/3	5/3
Inner INL third	3080/72	1362/60	2374/77	547/72
Outer IPL peak	11,033/759	8120/599	9839/616	3000/916
Interpeak minimum	2141/416	557/256	986/399	233/335
Inner IPL peak	7343/687	4143/582	6957/672	1375/768
Ganglion cell layer	3034/166	3362/129	2328/135	917/244

ChAT and AChE activities were concentrated in and near the IPL. Within the IPL, two peaks of ChAT activity were obtained, with the activity of the outer peak exceeding that of the inner. Little ChAT activity was found superficial to the middle of the inner nuclear layer (INL). The distribution of AChE activity corresponded well to that of ChAT activity except in the outer plexiform layer (OPL) and the outer margin of the INL in hawk.

The distributions of enzyme activities suggest that populations of amacrine cells in these avian retinae are cholinergic. In addition to these same cells and presumably cholinoceptive ganglion cells, AChE activity was associated in hawk with a population of horizontal cells that should be unrelated to cholinergic neurotransmission. The quantitative similarities between the activities in red and yellow fields of pigeon indicate that the cholinergic system may not be specifically involved in the increase in IPL complexity across the pigeon retina. The four-fold range of ChAT activity between hawk and road runner suggests important differences in the densities and function of cholinergic elements in these species.

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295.1 MULTIMODAL CONVERGENCE OF SENSORY PATHWAYS ON MOTONEURONS OF FLIGHT MUSCLES IN THE FLY (*Calliphora*). K.Hausen* and R. Hengstenberg*. (SPON: C. Wehrhahn). Max-Planck-Institut für biologische Kybernetik, 74 Tübingen, FRG

The flight motor of flies consists of indirect flight muscles, which generate the basic wingbeat pattern during flight, and direct flight muscles, which control the flight course by altering differentially the pitch and beat amplitudes of the wings. The spike-activity in direct flight muscles is phase-coupled to the wing beat-cycle. Visual inputs modulating the activity of the muscles are gated by mechanosensory afferences of the wing-nerve and the halteres, both monitoring the wing-oscillations (Heide,G. in: Nachtigall,W (ed.) Biona-report 2, Fischer, Stuttgart, 1983, pp 35-52).

In order to analyze the neural circuit mediating these interactions, we studied the motoneurons of direct flight muscles and sensory projections in the thoracic ganglion using the cobalt-technique. We employed, in particular, cobalt-infusions of different durations in order to produce selectively first-order stainings, which are due to direct uptake of cobalt into injured neurons, and higher-order stainings of neurons, which are assumed to be caused by cobalt-diffusion through gap junctions from directly stained neurons and which, thus, can demonstrate functional pathways in the nervous system (Strausfeld,N.J. and Bassemir,U., J.Neurocytol., 12:971, 1983).

The motoneurons (Mn) of the direct flight muscles b1, b2, I1, III1, ps1, ps2 were identified and studied light microscopically. All of them show large dendritic domains in the dorsal part of the mesothoracic ganglion and additional dendrites in ventral layers of the ganglion. The axons of Mnb1, Mnb2, and Mn11 project through the anterior dorsal mesothoracic nerve, those of MnIII1 and Mnps1-2 run through the mesothoracic accessory nerve to their target muscles. The dendritic main trunks of Mnb1, Mnb2, and MnIII1 were found to be cobalt-coupled to large interneurons, which constitute a prominent commissure in the dorsal mesothoracic ganglion.

The mechanosensory projections of each haltere form a dense tract in the ipsilateral half of the thoracic ganglion. Prolonged cobalt-infusions into halteres resulted in higher order stainings of axonal bundles and single large interneurons, which terminate in the contralateral parts of the meso- and prothoracic ganglion, in impregnations of the above described neurons of the mesothoracic commissure and in stainings of the Mnb1, Mnb2 and MnIII1.

Impregnations of the wing-nerve revealed terminal arborizations mainly in the mesothoracic ganglion. Second order stainings after wing-nerve infusions did not occur. Dense appositions of wing-nerve terminals and the ventral dendrites of Mnb1 and Mnb2 were, however, frequently observed. Preliminary observations on descending neurons derived from the optic foci of the deutocerebrum and the antennal lobes indicate that these neurons terminate also in close vicinity to the dendrites of the described motoneurons.

The results indicate strongly that the motoneurons of the direct flight muscles are synaptically coupled to interneurons of the dorsal mesothoracic commissure and to different types of sensory interneurons.This suggests that multimodal convergence and gating as described above takes place directly at the dendrites of the motoneurons.

295.2 CHANGES OF THE FLIGHT MOTOR PATTERN DURING PHONOTACTIC STEERING OF THE CRICKET, *TELEOGRYLLUS OCEANICUS*. S. Wang* and R.M. Robertson (SPON: E.G. Gisel). Department of Biology, McGill University, 1205 Ave. Dr. Penfield, Montreal, PQ, H3A 1B1, Canada.

During flight crickets will turn towards conspecific calling songs (positive phonotaxis) and away from ultrasound (negative phonotaxis). Cricket phonotaxis has received a lot of attention in the literature, but how the flight muscles cooperate to achieve steering is still unknown. By making electromyographic (EMG) recordings from the wing muscles of tethered, minimally dissected crickets, *Teleogryllus oceanicus*, we determined the motor pattern during straight flight. Then, by playing either synthesized conspecific calling song (pulses of 5 kHz sound of 30ms duration repeated at 16 pulses/s) or pulses of ultrasound (30 kHz, 30ms duration, 2 pulses/s) to induce steering, we characterized the flight motor pattern underlying steering. Steering was monitored conventionally by observing the movements of the abdomen (the abdomen deflected in the direction of steering).

A photographic analysis of the wing movements showed that during straight flight the extent of elevation of the right and left hindwings was roughly equivalent. However when the cricket turned, the hindwing inside the turn showed a markedly greater elevation than did the hindwing outside the turn. EMG recordings showed that the presentation of sound induced more vigorous flight and a maintained increase in the wingbeat frequency. Also, elevator muscles of the hindwing (118, anterior tergocoxal and 119, posterior tergocoxal) inside a turn increased their level of activity while the same muscles of the hindwing outside a turn showed no change or reduced their level of activity for a few cycles after each sound pulse. The phase of elevator activity within each cycle of depressor activity was altered. Inside a turn the elevator phase decreased indicating that the elevators were active earlier in each cycle. Outside a turn the elevator phase increased but for only a few cycles immediately after each sound pulse. The evidence supports the ideas that steering during flight is accomplished in part by a greater elevation of the hindwing on the inside of a turn relative to that of the hindwing on the outside of a turn, and that this is brought about by an increase in the phase and strength of activity of elevator muscles of the hindwing on the inside of a turn and by a decrease of the same on the outside of a turn.

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295.3 EVALUATION OF A HYPOTHESIS FOR THE NEURONAL CONTROL OF JUMPING IN THE LOCUST. I.C. Gynther* and K.G. Pearson (SPON: J. Gillespie). Dept. of Physiology, U. of Alberta, Edmonton, Alberta, CANADA T6G 2H7.

In 1980 Pearson, Heitler and Steeves (J. Neurophysiol., 43:257) presented a hypothesis for explaining the neuronal mechanisms for triggering the jump in the locust, Locusta migratoria. The main features of this hypothesis were 1) the co-contraction phase of the jump is terminated by a burst of activity in a pair of identified interneurons (the M-neurons) which inhibit flexor motoneurons, 2) during the co-contraction phase the excitability of the M-neurons progressively increases due to proprioceptive feedback from hindleg receptors, and 3) burst activity in the M-neurons is generated either by proprioceptive input shifting the membrane potential beyond threshold or by additional excitatory input from the visual or auditory systems summing with the proprioceptive input. This hypothesis was based primarily on the known connectivity of neurons and the responses of the M-neurons to various types of sensory input. Recently we have examined this hypothesis by recording from the M-neurons in behaving animals capable of producing bilateral kicks of the hindlegs (the motor program for these kicks being similar to that for a jump). These recordings have confirmed that the M-neurons are involved in triggering the jump but they have failed to demonstrate other aspects predicted by the original hypothesis.

In support of the proposal that the M-neurons are involved in triggering the jump were the findings that these neurons produce high frequency bursts corresponding to the time of inhibition of flexor activity and that the injection of a brief depolarizing current pulse into an M-neuron could initiate a premature triggering of a kick in one leg. However, suppressing spike activity in an M-neuron by injecting hyperpolarizing currents did not prevent kicks from being triggered. Thus triggering, i.e. flexor inhibition, involves other neurons in addition to the M-neurons.

Findings which were not consistent with the hypothesis of Pearson et al. were that the M-neurons were hyperpolarized during the co-contraction phase and that visual and auditory input during co-contraction failed to trigger kicks. Thus the proposal that M-neuron excitability is increased progressively during co-contraction must be abandoned. The problem of how the M-neurons are excited, thereby helping to terminate co-contraction, remains to be solved.

295.4 DIFFERENT MODES OF INTERNEURONAL ACTIVITY DURING FICTIVE LOCOMOTION IN THE CRAYFISH, PROCAMBARUS CLARKII. A. Chrachri* and F. Clarac, Lab. de Neuro. Comp., 33120 Arcachon, France

Although the thoracic locomotor central generator (LCG) is not rhythmically active when isolated from sensory input, we have shown that application of the muscarinic agonists oxotremorine and pilocarpine induces rhythmic fictive locomotory output in isolated preparations (Neurosci. Lett., in press). We showed this activation results in part from the induction of active regenerative properties in some of the LCG motorneurons (MNs) themselves, and that certain of the MNs have access to the LCG, as current injection into them affects the rhythm's frequency. We have used this pharmacological activation to investigate the cellular and synaptic properties of the locomotory interneurons (INTs).

There are both spiking and nonspiking locomotory INTs; the spiking INTs synapse with fewer MNs than the nonspiking INTs,two types of spiking INTs synapse only with levator and depressor MNs;one type excites levator MNs and inhibits depressor MNs, the other inhibits levator MNs and excites depressor MNs. These INTs presumably reinforce the mutually inhibitory synapses made by these two sets of MNs. However, all spiking INTs that synapse onto promotor or remotor MNs also synapse onto both levator and depressor MNs. These INTs may thus play a limited role in coordinating movements of the two joints. Tonic depolarization of these INTs decreases the intensity of MN bursts, but does not affect cycle frequency. That they are not the rhythmic center of the LCG is further supported in that muscarinic agonists do not induce regenerative membrane properties in them.

All identified nonspiking INTs synapse onto the MNs of both joints, and divide into two classes on the basis of their pattern of synaptic connectivity. One class is appropriate for supporting forward walking (simultaneously exciting levator and promotor and inhibiting depressor and remotor MNs), the other for backward walking (simultaneously exciting levator and remotor MNs, inhibiting depressor and promotor MNs). Current injection into them does not affect pattern frequency, nor do they respond to muscarinic agonists; they are not members of the LCG rhythmic center but instead organize its output into either forward or backward walking.

We tentatively conclude: 1) The motoneurons of the locomotory CG are capable of exhibiting active regenerative membrane properties and have access to its rhythmic center. 2) The locomotory spiking interneurons serve as local coordinating elements in the network, particularly to reinforce the mutual inhibition of MNs innervating antagonistic muscles. 3) The nonspiking interneurons we have identified are appropriate for organizing the motor pattern into backward or forward walking.

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295.5 NEURAL MECHANISMS OF REFLEX REVERSAL IN THE CRAYFISH
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Experimental work over the last few years in both invertebrates and vertebrates has resulted in changes to the concept of the reflex. Most importantly it is now recognized that the neural circuits underlying reflexes are not hardwired and discrete, but may be substantially modified by centrally generated neural activity. The thoracic CNS of the crayfish maintained in vitro together with a basal limb proprioceptor (the thoracocoxal muscle receptor organ - TCMRO) provides an ideal model system for studying the cellular mechanisms of central reflex modulation.

The isolated thoracic ganglia can produce fictive locomotion, consisting of alternating bursts of activity in promotor motoneurones (MNs) (normally underlying the swing phase in walking) and remotor MNs (normally underlying the stance phase). In the absence of fictive locomotion stretch of the TCMRO elicits a negative feedback promotor stretch reflex mediated by the dynamically sensitive T fibre (one of the two afferent neurones of the TCMRO). During fictive locomotion, however, the reflex effect of the TCMRO reverses in a phase-dependent manner (Skorupski & Sillar, J. Neurophysiol. 55, 689, 1986), so that stretch of the TCMRO during the remotor phase of the rhythm elicits reflex excitation of remotor MNs and inhibition of promotor MNs. Like the promotor stretch reflex recorded in quiescent preparations, this reversed reflex is mediated by the T fibre.

The neural mechanisms underlying the reversal in the sign of reflex transmission from afferent to MN are currently being examined. In principle there are three possible levels at which such modulation could occur: 1) pre-synaptically at the central terminals of afferents, 2) post-synaptically upon interposed interneurones, and 3) post-synaptically upon MNs.

Experiments involving selective intracellular stimulation of the T fibre with simultaneous recording from different categories of post-synaptic neurones indicate that in the crayfish the flow of information from afferent to effector may be modulated at all three levels defined above. Particular attention, however, will be focused on the modulatory role of presynaptic effects on the central terminal of the T fibre.

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295.6 Intracellular recordings During Locomotion of Intact Ascaris lumbriciodes.
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The question of the control of nematode locomotion has been approached in a variety of ways. Those studying Caenorhabditis elegans have used genetic mutations and laser ablation of specific neurons in order to determine which cells may contribute to or control locomotion (Chalfie et al.,J. Neurosci., 5:4, 1985). Those studying Ascaris have made use of intracellular recordings in dissected preparations to answer the same question.

While results from both directions have been informative, conclusive results are still lacking. Part of the problem is the lack of in vivo experiments. Current studies are being performed on intact Ascaris in an attempt to resolve this question directly.

Intracellular recordings have been made in muscle and motor neurons in intact preparations. The experiments are carried out on an animal which has about 1 cm. dissected in the region just posterior to the pharynx in order to expose the first pair of commissures in the second neural repeating unit. The rest of the animal is free to move. The membrane oscillations seen in dissected pieces of Ascaris do not correlate directly with the locomotory waveform. Instead they seem to occur in bouts which have the same period as that of the body wave as it travels down the animal. It has also been found that mechanical positoning of the portions anterior or posterior to the dissection can change the activity in the dorsal and ventral muscle, suggesting that proprioception may indeed be important.

(Stretton,A.O.W.,et al.TINS,8:6, June '85)

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295.7 OCTOPAMINE INDUCES PROLONGED SPONTANEOUS SWIMMING ACTIVITY IN THE MEDICINAL LEECH, HIRUDO MEDICINALIS.
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Although spontaneous swimming activity occurs infrequently in preparations of the isolated leech nerve cord, such activity can be induced by adding 0.005-0.1 mM serotonin (5-HT) to the saline bathing the preparation [Willard, A.L., J. Neurosci. 1:936-944, (1981)]. To assess the potential role of other chemical agents for modulation of swimming activity and to analyze the neural mechanisms underlying swim oscillations in the leech, we tested a broad range of substances with leech nerve-cord preparations.

Bath-application of many drugs (transmitters, transmitter precursors, neuromodulators and activators of rhythmic activity in other systems) to leech nerve cords failed to induce swimming activity; namely, cGMP, ACh, NE, dopamine, L-DOPA, D-DOPA, DL-DOPA, histamine, glutamate, kainic acid, kynurenic acid, homosysteic acid, FMRFamide, proctolin, GABA, strychnine, and ethanol. However, bath application of octopamine (OA, 0.001-2 mM), elicited impulse activity in the dorsal posterior (DP) nerves, often as isolated impulse bursts, and promoted spontaneous swimming activity. At low concentrations (0.1 mM), OA induced a few swim episodes with a short latency followed in about 30 min by repeated swim episodes (2-3 per min). Addition of OA at high concentrations (0.1-1.0 mM) quickly induced a series of swimming episodes that continued with little interruption for 2-3 hours. Such spontaneous swimming activity continued when the nerve-cord preparation was reduced to a chain of three segments; moreover, episodes of swim-like bursts (period about 1 s) persisted with diminished regularity in isolated ganglia. The agonists, naphazoline (0.015 mM) and synephrine (0.03-0.3 mM), had effects similar to those observed for OA.

We found that bath application of agents that increase intracellular cAMP levels, including the phosphodiesterase inhibitors, theophylline (2 mM) and IBMX (0.4 mM), and cAMP (0.02-1 mM) and its analogs, 8 Br-cAMP and dibutryl cAMP (0.03-1 mM), reliably mimicked the effects of OA and 5-HT. We propose, therefore, that the induction of spontaneous swimming activity in the leech by OA and 5-HT is mediated by the intracellular accumulation of cyclic AMP. Surprisingly, bath-application of phentolamine (0.08-0.1 mM), an OA antagonist, also mimicked the actions of OA on swimming activity. Supported by NSF grant BNS84-14988.

295.8 TERMINATION OF SWIMMING ACTIVITY BY STIMULATION OF A NEURON IN THE SUBESOPHAGEAL GANGLION OF THE LEECH, HIRUDO MEDICINALIS.
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Locomotion in many animals is episodic, implying that control mechanisms exit for both the initiation and termination of the underlying neuronal activity patterns. In the leech, initiation of swimming activity in the isolated nerve cord is controlled in part by trigger neurons, Tr1 and Tr2, which, when transiently stimulated, elicit episodes of swimming oscillations (Brodfuehrer & Friesen, J. Comp. Physiol. A 159:489-502, 1986). Tr1 is the more effective trigger neuron with direct excitatory connections to swim-gating neurons (cells 204 and cells 21/61) that drive the central oscillator circuit for swimming activity. Tr2 appears to have no direct excitatory connections to the swim-gating neurons and moreover provides inhibitory input to many of the neurons of the swim oscillator. We have now found that Tr2 can function to terminate swimming activity in isolated nerve cord preparations (head ganglia through midbody ganglion 18).

When stimulated with brief (0.5 s) depolarizing pulses during an ongoing swim episode, Tr2 acts to delay the onset of the next burst and to reduce slightly the excitation of cells 204 (ibid.). Intense stimulation of Tr2 (3 s train at 50 Hz) terminates swim-related DP (dorsal posterior) nerve bursts and causes a repolarization of cell 204 to the resting level. Stimulation of Tr2 at intermediate intensities causes an interruption of swimming activity for several seconds followed by a gradual depolarization in cell 204 and the resumption of swimming. The onset of both swim termination and cell 204 repolarization follows the onset of Tr2 stimulation with a latency of less than 0.5s. The same stimulus parameters can lead either to swim initiation (when the preparation is quiescent) or to swim termination (during a swim episode), however the latency for swim initiation by Tr2 is about 4 to 9 s compared to about 0.5 s for termination. We propose that Tr2 may have as its primary role the termination of swimming activity in the leech and that swim-initiation by cell Tr2 may be the result of some process of postinhibitory rebound. Supported by NIH grant NS21778.

295.9 NEUROMUSCULAR ARRANGEMENT FOR TWO-SPEED SWIMMING IN A PTEROPOD MOLLUSC. R.A. Satterlie and G.E. Goslow Jr. Dept. of Zoology, Arizona State Univ., Tempe, AZ 85287; Dept. of Biology, Northern Arizona Univ. Flagstaff, AZ 86011.

Forward swimming in the pteropod mollusc Clione limacina features two distinct swimming speeds; slow/hovering and fast. In the former, wing beat frequencies range from 1 to 5 Hz resulting in hovering or slow forward displacement of the animal in the water column. During fast swimming, wing beat frequencies range from 5 to 10 Hz and produce rapid forward movement of the animal, up to 5 body lengths per second.

Two types of histochemically distinct muscle fiber types have been identified in the parapodia of Clione. One type exhibits heavy staining for oxidative enzymes and low myosin-ATPase activity. These fibers show mean peak-twitch times of 73.8 ms and low fatigability, and are thus referred to as slow twitch fatigue-resistant fibers. The second type of fiber shows complementary histochemical staining; low for oxidative enzyme activity and high myosin-ATPase activity. These fibers show peak-twitch times of 20 ms and significant fatigue following three to eight stimuli within the normal range of swimming frequencies (fast twitch fatigue-resistant fibers).

Slow twitch fibers are innervated by a population of small pedal motor neurons (20 - 30 in each pedal ganglion). Cell body diameters range from 15 to 25 μm, and each motor neuron has a restricted innervation field in the ipsilateral parapodium. In contrast, the fast twitch fibers are innervated by two large (cell body diameters - 50-80 μm) pedal motor neurons. One innervates the dorsal swimming musculature and the other the ventral muscles. The innervation field for each cell covers the entire parapodium. Preliminary evidence suggest that the large motor neurons (called general exciters) also innervate some slow twitch fibers, and centrally excite synergistic motor neurons. All neuromuscular contacts are monosynaptic.

During slow swimming, all small motor neurons appear to be active (spiking). The shift from slow to fast swimming involves recruitment of general exciter motor neurons (to spiking mode) and an increase in impulse frequency (per burst) in small motor neurons. The role of pedal interneurons and modulatory inputs to the motor neurons is being investigated.

295.10 CONTROL OF BUCCAL MOTOR PROGRAMS IN *APLYSIA* BY IDENTIFIED NEURONS IN THE CEREBRAL GANGLION. S.C. Rosen*, M.W. Miller*, K. R. Weiss, and I. Kupfermann. Cntr. for Neurobiol. & Behav., Columbia Univ. and New York State Psychiatric Inst., New York, NY 10032.

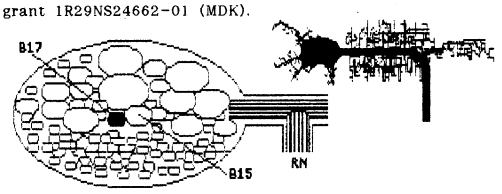
The motoneurons and pattern generator for consummatory phases of feeding behavior, including biting, swallowing, and rejection responses, appear to be located in the buccal ganglion. We have investigated whether specific components of consummatory behavior are controlled by command elements in the cerebral ganglion. Backfills of the cerebral-buccal (C-B) connectives stain approximately 20 cells in each cerebral hemi-ganglion. These include the neuromodulatory, serotonergic, metacerebral cell, 6-8 mechanoafferent (ICBM) neurons that synapse upon major, identified, buccal interneurons B_{4-5} (Rosen et al., *J. Neurophysiol.* 48:271, 1982), and about 12 other neurons, three of which we have now identified. Each of the three: (1) has a characteristic morphology, (2) receives either extensive mechanosensory or chemosensory input, and (3) makes powerful, convergent, synaptic connections to numerous buccal motoneurons and interneurons, including B_{4-5}, via an axon in the C-B connective. Cerebral-Buccal Interneuron 1 (CBI-1), receives monosynaptic EPSPs from primary cerebral mechanoafferent neurons. Firing the CBI-1 produces fast, rapidly decrementing, EPSPs in B_{4-5} and a single, short-latency, coordinated burst pattern involving many buccal cells. Cerebral-Buccal Interneuron 2 (CBI-2) receives excitatory chemosensory inputs from the lips and tentacles. Firing the CBI-2 produces complex, fast and slow, IPSPs and EPSPs in B_{4-5}, and can trigger multiple coordinated bursts from B_{4-5} and other buccal cells. Tonic depolarization of CBI-2 produces repetitive bursting patterns at a rate comparable to that which occurs during normal biting responses. The third interneuron (CBI-3) produces fast and slow IPSPs in B_{4-5} and in excitatory followers of B_{4-5}, but is incapable of producing coordinated buccal bursting. Two hypotheses are suggested by these findings: (1) each interneuron is involved in a separate circuit capable of triggering a different behavioral response, e.g. CBI-1 may trigger defensive withdrawal or rejection and CBI-2 may trigger biting; (2) the coordinated actions of all of three interneurons are necessary for the integration of chemosensory and mechanosensory inputs involved in the initiation of normal feeding.

295.11 IDENTIFICATION OF A BUCCAL NEURON IN *APLYSIA* WHOSE MOTOR OUTPUT CAN BE MODULATED BY SCPb AND 5-HT. M.D. Kirk and M.R. Plummer. Boston University, Dept. of Biology, 2 Cummington St., Boston, MA 02215.

The buccal ganglion of *Aplysia* contains neurons that mediate the patterned motor output underlying consummatory feeding. Several bilaterally paired neurons in this system are identifed and some of them are peptidergic and/or are modulated by peptides. We have identified a neuron unlike those identified previously in that it elicits cyclic patterned motor activity upon maintained depolarization; we have tentatively labelled this neuron B17. The soma of B17 is multipolar and is located on the rostral side of the buccal ganglion lateral to the identified motoneuron B15 (see sketch). The neuron arborizes extensively in the ipsilateral neuropil and in the buccal commissure before sending an axon out the radular nerve (RN).

B17 is normally silent, but bursts during spontaneous cyclic motor activity. It receives direct IPSPs from multifunction neurons B4/B5 and is electrically coupled to the peptidergic retractor motoneuron B15. Suprathreshold depolariztion of B17 can elicit activity in a central pattern generator (CPG) from which it receives synaptic feedback. B17 is electrically coupled to its contralateral homologue and fires in synchrony with it. In cases where cycling is not elicited, maintained depolarization still causes endogenous bursting in B17.

The motor pattern elicited by B17 can be modulated by the peptide SCPb and serotonin. Bath application of these transmitters reversibly altered the interburst intervals and burst durations of the neurons examined. We intend to evaluate more quantitatively the contribution of B17 to the CPG(s) for feeding and how its motor output is modulated by peptide and amine transmitters. Supported by NIH grant 1R29NS24662-01 (MDK).



296.1 CONTRIBUTION OF A CA^{2+} ACTIVATED K^+ CHANNEL TO THE DYNAMICS OF THE MUSCLE SPINDLE. Kruse, M.N. and R.E. Poppele. Laboratory of Neurophysiology, Dept. of Physiology, University of Minnesota. Minneapolis, MN 55455

Reflex tension is developed in phase with a sinusoidally varying muscle stretch. This behavior requires that the phase-lag behavior of muscle contraction be compensated by a (precisely timed) phase-lead in the reflex loop. The appropriate phase-lead occurs in the steps involved in the conversion of muscle stretch into afferent signals by the muscle spindle (Poppele and Terzuolo, Science 159:743,1968). The firing rate of spindles reaches a peak before peak stretch by an amount that depends on the frequency of the stretching sinusoid. The phase advance between 1 Hz and 4 Hz (the mid-frequency dynamics) is adequate compensation for the phase-lag characteristics of a muscle and may represent a "tuning" of the dynamic processes in the spindle which matches the dynamic processes involved in muscle contraction.

We have examined possible mechanisms responsible for the mid-frequency dynamics of the cat muscle spindle. We have shown that they are present when intrafusal muscle fibers are destroyed by mechanical transection or freezing (Kruse and Quick, 1986 Soc. Neurosci. Abst. 12:1084) which suggests that the sensory nerve terminals are responsible for the observed kinetics. This hypothesis was tested by blocking specific ion channels to observe their effect on the mid-frequency dynamics.

Spindles were isolated from cat tenuissimus muscle and their capsule was opened. The spindle's response to sinusoidal stretches of 0.3-4 Hz was recorded both before and after addition of a channel blocker to the bathing solution. TEA and 4-aminopyridine (K^+ channel blockers) and $ZnCl_2$ (a Ca^{2+} channel blocker) were all found to alter the dynamic response in a similar manner. The mid-frequency phase lead and the amplitude dependence on stretch frequency were both reduced. As the K^+ and Ca^{2+} channel blockers had similar effects on the mid-frequency dynamics, it was possible that they were acting via the same mechanism, i.e. a $K^+(Ca^{2+})$ channel. Apamin, a toxin which is specific for $K^+(Ca^{2+})$ channels, had the same effect on the spindle's dynamics as the previous drugs. In addition, the apamin effect was almost completely reversible.

These data support the hypothesis that ion channels in the sensory nerve are in part responsible for the spindle's mid-frequency dynamics, and furthermore specifically implicate a $K^+(Ca^{2+})$ conductance in the mechanism. Thus $K^+(Ca^{2+})$ channels may help "tune" the spindle's stretch response to compensate for muscle dynamics in the stretch reflex. Supported in part by NSF(BNS85-18714).

296.2 MECHANISM AND FUNCTION OF HETEROGENIC INHIBITION BETWEEN ANKLE FLEXORS AND SOLEUS MUSCLE IN THE DECEREBRATE CAT. T. R. Nichols and D. J. Koffler*. Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322.

It was shown previously (Nichols, T. R., Soc. Neurosci. Abstr. 15:213, 1985) that stretching the ankle flexor tibialis anterior (TA) or extensor digitorum longus (EDL) powerfully inhibited the response to stretch of soleus (SOL), and that heterogenic inhibition from SOL to TA (or EDL) was weak or absent. Postsynaptic, reciprocal inhibition is an obvious candidate to mediate the observed heterogenic inhibition, but the asymmetry of the reflex matches the asymmetry of presynaptic inhibition which is powerful from flexor afferents to extensor afferents but not the other way around. The studies reported here were designed to evaluate whether heterogenic inhibition from TA and EDL could be explained by postsynaptic inhibition, or whether a component of the inhibition might be explained by a change in the gain of the stretch reflex as would be expected from presynaptic inhibition. We made use of the technique of delivering mechanical inputs to both tendons of a pair of muscles in premammillary and intercollicular decerebrate cats (Nichols, ibid.). Heterogenic inhibition was evaluated either by stretching TA (or EDL) alone and measuring the change in force in the isometric SOL, or by stretching both flexor and extensor simultaneously and measuring the decrement in the stretch response of SOL. When both experiments were performed at the same initial force, the inhibition observed in the former experiment was usually much smaller than in the latter. This discrepancy was found to be due, however, to a dependence of the inhibition on force rather than to the heterogenic inhibition of autogenic reflex gain. The responses of isometric SOL varied with the amplitude of stretch applied to TA but less than proportionately. The responses to the stretch of TA and EDL together were approximately the sum of the responses to stretch of each flexor individually. Applying equal and opposite length changes of different amplitudes to SOL and to TA (or EDL) as occurs naturally showed that heterogenic inhibition significantly increases the stiffness of SOL even when TA and EDL are inactive. These results suggest that heterogenic inhibition from ankle flexors to SOL can be explained mainly by postsynaptic reciprocal inhibition and that its function is to enhance the mechanical stiffness of the in situ SOL. In support of the proposed mechanism, it was found that heterogenic inhibition was abolished by the intravenous administration of strychnine.

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296.3 MODULATION OF PRESYNAPTIC INHIBITION BY THE SKIN AND MUSCLE. M. Sabbahi, C.C. Luk*, J.E. Estrella*, and M. Sneath*. Texas Woman's University, School of Physical Therapy, Houston, TX.

Desensitization of the skin with topical anesthesia has been reported to increase the excitability of the H-reflex (Sabbahi & DeLuca, 1982). Desensitization of the muscle (afferents) resulted in similar modulation of the H-reflex (unpublished results). This report discusses the effect of desensitization of skin or muscle on the process of presynaptic inhibition.

Soleus H-reflexes of normal subjects were recorded after electrical stimulation of the posterior tibial nerve with unipolar pulses (1 msec, 0.2 pps at H-max.). The H-reflex was monitored by the M-response. Then an air-driven vibrator (80 Hz) was applied on the Achilles tendon for 60 sec. and the degree of H-reflex inhibition with vibration was calculated and used as a measure for presynaptic inhibition (PI) process (Hagbarth, 1973). Calf skin area was sprayed with topical anesthetic (10% xylocaine) or a placebo. A "phoresor" delivering 4-5 mA of DC for 30 minutes with either 2% Xylocaine with 1:100,00 epinephrine or a placebo was applied to the motor points of the soleus muscle. Previous reports showed that this treatment dose introduces the anesthetic about 2 cm under the treatment electrode. The PI measurements were tested at intervals up to 30 minutes post topical spray or after termination of iontophoresis. The percent change in H-reflex amplitude was averaged and normalized to those before anesthesia and a t-test measured the significance of the difference post-treatment.

Results showed significant inhibition ($p < 0.05$) of the H-reflex 30 min. after anesthetic iontophoresis. As was previously reported the peak-to-peak amplitude of the H-reflex was significantly increased post topical anesthetic. PI was significantly ($p < 0.05$) increased in all subjects after topical or iontophoretic anesthesia. This effect lasted for 30 min. post anesthetic. No measurable changes were recorded post placebo or in the M-response.

These results indicate an increased presynaptic inhibition after desensitization of cutaneous or muscle receptors regardless of the change in the excitability of the α - motoneuron. Cutaneous and muscle receptor afferents may decrease presynaptic inhibition in normal subjects.

296.4 EFFECTS OF CHLORALOSE-URETHANE ANESTHESIA ON RECIPROCAL Ia IPSPs S.-I. Sasaki*, C.S. Yuan*, R.M. Reinking*, A. Taylor and D.G. Stuart. Department of Physiology, University of Arizona Health Science Center, Tucson, AZ 85724.

Previously (J. Neurophysiol., 39: 1375, 1976), we reported that single-axon reciprocal Ia IPSPs in cat motoneurons can be measured by use of spike-triggered averaging (STA). Chloralose-urethane (C-U) anesthesia was used, which we now know has a depressant effect on single-axon recurrent IPSPs (J. Physiol. London, July, 1987, In Press). This and other findings (J. Physiol., London, 218: 495, 1971) suggested that C-U might have a depressant effect on reciprocal Ia IPSPs. We tested for this, by comparing the previous measurements to those obtained from preparations allowed to become unanesthetized following ischemic decapitation (I-D) under halothane anesthesia. In both studies, the reciprocal pathway tested was from Ia afferents in the medial gastrocnemius muscle to motoneurons supplying the extensor digitorum longus and tibialis anterior muscles.

Significantly more ($\chi^2=4.19$, $P<0.05$) single-axon reciprocal Ia IPSPs were recorded in cells from I-D preparations (26/46; 36%) than from C-U preparations (31/105; 23%). Comparisons of selected IPSP characteristics included:

Preparation	Amplitude (uV)	Latency (ms)	Rise-time (ms)	Half-width (ms)
C-U (n=31)	4.9 ± 2.5 (1.5-10.4)	1.8 ± 0.3 (1.2-2.4)	1.4 ± 0.5 (0.6-2.3)	2.8 ± 1.6 (0.9-6.6)
I-D (n=26)	17.5 ± 5.5 (6.4-26.3)	1.7 ± 0.6 (1.0-2.2)	1.8 ± 0.7 (0.9-3.0)	3.9 ± 1.7 (1.6-7.4)

In summary, C-U has as pronounced a depressant effect on the incidence and magnitude of single-axon reciprocal Ia IPSPs as it does on single-axon recurrent IPSPs. This finding invites the possibility of using STA to bring out functional and organizational features of the reciprocal pathway, not readily revealable by other means. (Supported by USPHS grants HL 07249 and NS 07888).

296.5 MONO- AND POLYSYNAPTIC EXCITATORY CONNECTIONS FROM THE GLOSSOPHARYNGEAL AFFERENTS TO THE HYPOGLOSSAL MOTONEURONS IN THE JAPANESE TOAD. T.Matsushima*, M.Satou* and K.Ueda* (SPON:A.Urano) Zool.Inst., Fac. of Sci., Univ. of Tokyo, Tokyo 113, Japan.

Anuran tongue movements are controlled by the visual stimuli for releasing prey-catching behavior ('snapping') and the tactile/gustatory/nociceptive stimuli for eliciting lingual reflexes, including the 'rejection' of unpalatable objects out of the mouth. As a step toward elucidating the neural basis of these movements, we analyzed the neuronal connections from the glossopharyngeal (IX) lingual afferents to the hypoglossal (XII) motoneurons innervating the tongue-protractor and retractor muscles (PMNs and RMNs, respectively), and examined the relationships between these reflex pathways and the polysynaptic excitatory pathways from the optic tectum (Satou,M.et al.,J.Comp.Physiol.A,157:717-737).

Electrical stimuli applied to the ipsilateral IX nerve elicited polysynaptic EPSPs in PMNs and mixed mono- and polysynaptic EPSPs in RMNs. The polysynaptic EPSPs were further spatially facilitated when the optic tectum was simultaneously stimulated. These results suggest that (i) some of the IX afferents have direct connections with RMNs but not with PMNs, and that (ii) some common excitatory interneurons, on which the tectal descending volleys and the IX afferent volleys converge, mediate polysynaptic activation of these motoneurons.

Further double labeling experiments using horseradish peroxidase (HRP) and cobaltic lysine (Co-lys) supported the result (i) of the neurophysiological analyses described above. The IX afferents and their terminals were transganglionically stained with Co-lys, and the XII motoneurons were retrogradely labeled with HRP. The IX axons descending through the fasciculus solitarius (fsol) gave off many terminal branches with beaded appearance in the gray matter ventral and ventrolateral to the fsol. On the other hand, the XII motoneurons had extensive spread of dendritic trees in the lateral, dorsal, medial and contralateral directions. Some of the IX nerve terminals had direct contacts with the dorsal dendrites, the lateral dendrites and the somata of the XII motoneurons, but not with their medial dendrites. These direct contacts mainly occurred in the rostral region of the dorsomedial XII nucleus where RMNs predominates, but not in the caudal region nor in the ventrolateral XII nucleus where PMNs and other XII motoneurons are distributed.

296.6 PERIODONTAL AFFERENTS ALONE DO NOT CAUSE AN ACTIVE JAW-OPENING REFLEX IN THE CAT. A. Taylor, D. Dessem* and O.D. Iyadurai*. Sherrington School of Physiology, UMDS, St. Thomas's Hospital, London SE1 7EH, U.K.

Periodontal mechano-receptors respond to forces applied to the teeth during mastication. They are sensitive and direction specific and have large fast afferents. The part they play in controlling jaw movements must depend primarily on their reflex connexions. They are known to exert a disynaptic inhibition on jaw-closer motoneurones and are commonly thought to excite opener motoneurones. However this may not be the case, because though low-strength electrical stimulation of alveolar nerves readily produces digastric contraction, adequate controls have not been used to ensure that stimulation is restricted to periodontal afferents.

In cats anaesthetised with chloralose the inferior alveolar nerve (IAN) has been stimulated whole or as its separate cutaneous (mental) or periodontal afferent-containing ("tooth") branches. The afferent volley was recorded at the brain-stem entry of the trigeminal nerve, and reflex responses recorded as the emg of temporalis and digastric muscles.

Strong stimulation of IAN caused temporalis inhibition and digastric excitation. Tooth nerve stimulation at just above threshold (T) gave an afferent volley with conduction velocity of 65m/s. Paired stimuli (1.7ms spacing) at 1.25T did not excite digastric, though single stimuli at this strength strongly inhibited temporalis. Full development of the early volley required 1.75T but a slower (Aδ) component appeared at 1.5T at which strength the digastric response occurred. Selective natural stimulation of periodontal afferents by tapping canine teeth inhibited temporalis but failed to excite digastric. Finally, local stimulation of the mesencephalic tract of the trigeminal nerve to excite periodontal afferents (as evidenced by antidromic conduction in tooth nerves but not mental nerves) also caused no jaw-opening reflex (JOR).

Thus we have found no evidence of a contribution of periodontal afferents to the active JOR though they may converge with other afferents on interneurones involved. Consequently, changes in the JOR elicited by IAN stimulation during mastication (Lund, J.P., Drew, T. & Rossignol, S., Brain Behav. Evol., 25; 146, 1984) cannot be taken to indicate changes in periodontal receptor-based reflexes. It remains reasonable to suppose that periodontal afferents can provide negative feedback control of active force applied to the teeth during mastication.

296.7 VARIANCE OF SINGLE-AXON RECURRENT IPSPS: IMPLICATIONS FOR TRANSMISSION IN THE RECURRENT RENSHAW PATHWAY. T.M. Hamm, C.-S. Yuan*, S.-I. Sasaki*, U. Windhorst* and D.G. Stuart. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013; and Dept. of Physiology, Univ. of Arizona Health Sci. Center, Tucson, AZ 85724.

We have shown recently that stimulation of single motor axons is effective in producing recurrent IPSPs in spinal motoneurons (Hamm et al., J. Physiol. (Lond.), July, 1987, In press). The characteristics of these single-axon recurrent IPSPs permits the analysis of several features of the recurrent Renshaw pathway, such as the distribution of recurrent inhibition between individual motoneurons, as indicated by the magnitude of single-axon recurrent IPSPs (Hamm et al., In press). In addition, the variance of these IPSPs provides additional information about transmission in this circuit, since this characteristic depends not only upon fluctuations in synaptic strength which occur at monosynaptic connections (Jack et al., J. Physiol. (Lond), 321: 65, 1980; Cope and Mendell, J. Neurophysiol., 47: 455, 1981), but also upon the probabilistic discharge of the interposed Renshaw cells. Determinations of the variance of single-axon recurrent IPSPs in motoneurons innervating medial gastrocnemius muscle were made for recordings obtained from cats allowed to become unanesthetized after ischemic decapitation (I.D.). As was found in a preliminary analysis (Hamm et al., Proc. IUPS XXX Congress XVI: 258, 1986), changes in variance associated with single-axon recurrent IPSPs could not be resolved in many instances. This lack of resolvability can be attributed to the small amplitude of the potentials (mean of 46 μV) and the large amount of background synaptic activity in the motoneurons of I.D. preparations. However, the variance of the IPSP could be resolved in some recordings, showing an increase above the level of background synaptic noise during the timecourse of the IPSP. In such instances, the ratio of the standard deviation to the mean amplitude of the recurrent IPSP was in the range of 1.7 to 2.8. Based upon a model of transmission through a disynaptic pathway, ratios of this magnitude suggest that: 1) the combined probability of Renshaw cell discharge and synaptic transmission at each synapse of Renshaw cell and motoneuron in response to motor-axon stimulation is small; 2) the magnitude of IPSPs produced in motoneurons by Renshaw cells is several times that of single-axon recurrent IPSPs (i.e., the amplitude of the former IPSPs should be 100-350 μV).

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296.8 PRESYNAPTIC AND POSTSYNAPTIC MECHANISMS CONTROLLING THE GASTROCNEMIUS-SOLEUS MONOSYNAPTIC REFLEXES DURING SCRATCHING. SH DUEMAS* R CUEVA*, J ZAVALA* Spon: William. B. Marks CUIB. Univ. de Colima, Apdo. Postal 199 Colima, Col Mexico & Depto. de Invest., Esc. de Med.,Univ. Autonoma de Chihuahua. Apdo. Postal 1090. Chihuahua, Chih. Mexico.

In decerebrate cats, the phasic gaiting of the monosynaptic reflex (MSR) could be enterly dependent on the excitability of the gastrocnemius-soleus motoneurons (GS-MN) since the excitability of GS group Ia afferent fibers ending in the motor nucleus (MNU) was similar in both phases of the scratch cycle (Baev KV and Kostyuk PG, Neurosci., 6: 205-215, 1981). In this work we studied the MSR and the excitability of group Ia afferent fibers and of the GS-MN in decorticated and in spinalized cats during fictive scratching. A sustained and phasic increase in amplitude of the GS MSR occurred during fictive scratching. Maximal amplituded occurred during activation of GS-MN and minimal amplitud during the flexion (F) phase. There was a sustained increase in excitability in a population of Ia afferent fibers in both decerebrated and spinalized cats. In spinal cats the excitability of group Ia fibers was maximal at the end of the (F) phase and minimal in the third bin of the F-phase. In decorticated cats, the phasic changes in excitability in group Ia ending in the motor nucleus were not observed but antidromic activity (AA) in GS group I afferent fibers occurred during the entire F-phase. Parallel to the episodes of scratching extracellular negative potential shifts (ECP) with a duration of several second were observed in motor nuclei. Phasic potentials with a frequency of 4-6 Hs were superimposed on the ECPs. Intracellular recording of GS-MN showed a depolarization shift in the membrane potential (MPD) related to the GS motoneuron burst activity. A membrane potential hyperpolarization of 1-2 mv occurred between the MPD. Occasionally this MPD changed its frequency of oscillation from 6 Hz to 3 Hz. Sometimes scratch-like activity occurred in the GS nerve at the same time that locomotor-like activity was observed in the tibial anterior nerve.

A primary afferent depolarization (PAD) in GS Ia afferents and a MPD in GS-MN seems to be responsable for the modulation of the GS MSR. The ECPs suggest that this PAD may be potassium mediated. The appearance of different rhythms in antagonist muscular nerves support the hypotesisis of the unit burst generator.

296.9 REVERSIBLE ALTERATIONS OF SPINAL MOTOR OUTPUT IN DECEREBRATE CAT BY BLOCKADE OF TRANSMISSION IN DORSOLATERAL FUNICULI WITH COLD OR LIDOCAINE.
J. S. Carp, R. K. Powers and W. Z. Rymer. Department of Physiology, Northwestern Univ., Chicago, IL 60611.

Several features of partial spinal lesion in humans can be reproduced in dorsolateral funiculi (DLF) lesioned decerebrate cats, including diminished ability to generate force, decreased motoneuron discharge rate, and clasp-knife reactions. This disruption in motor output may result at least in part from a lesion-induced loss of descending modulatory input to spinal neurons. In order to develop a more accessible model of spinal cord injury, we have investigated the possibility that interruption of pathways descending in the DLF using reversible methods can be used to mimic the effects of a surgical lesion.

Cats were decerebrated under halothane/nitrous oxide anesthesia and then allowed to respire room air. Medial gastrocnemius muscle (MG) force, length and EMG were recorded and stored on computer during muscle stretch or manipulation of the contralateral hindlimb to evoke the crossed extensor reflex. Laminectomies were performed at T12 and L5 to allow placement of stimulating and recording electrodes, respectively, for determination of the effects of reversible blockade on a descending volley in the contralateral DLF. Reversible DLF block was instituted at the site of the rostral laminectomy caudal to the stimulating electrode. Cold block was performed by bilateral application of small thermoelectric cooling devices over the DLF. For lidocaine block, a shallow pool was formed with agar to restrict the lidocaine (4%) to this region of the dorsal spinal cord. After recovery from one of these methods of DLF interruption, a surgical lesion of the DLF was performed in some experiments.

During application of either lidocaine or cold block for 20-30 minutes, the amplitude of the descending volley was reduced. Alterations were seen in motor output which were similar to those seen after surgical lesion. Specifically, MG force production was reduced during cold or lidocaine block with respect to that seen at comparable levels of EMG prior to DLF interruption. The power of the MG force spectrum was increased under these conditions. Upon removal of lidocaine or cold block, the effects on MG force-EMG relationships, stretch response and force spectrum showed good recovery. These data suggest that the motor abnormalities induced by surgical lesion can be mimicked by reversible blocking techniques. Experiments are currently being performed to record intracellularly from motoneurons before and during reversible blockade of the DLF to determine if these descending systems modulate motoneuron behavior on a moment-to-moment basis. (Supported by VA and SCRF awards to WZR)

296.10 CHANGES IN Ia EPSP SHAPES IN CHRONIC SPINAL CATS ARE NOT EXPLAINED BY CHANGES IN MOTONEURON MEMBRANE PROPERTIES.
S. Hochman*, D. McCrea & B. Gustafsson*, Depts. of Physiology, Univ. of Manitoba, Winnipeg, R3E 0W3, Canada and Univ. of Göteborg, Göteborg, S-400 33, Sweden.

We previously demonstrated that the rise times and half widths of triceps surae composite Ia EPSPs in 6 week spinalized (L1-2) cats were decreased compared to unlesioned controls (Neurosci. Abst 12:186.12, 1986). The present investigation explores the possible contribution of changes in the electrical properties of motoneurons to the demonstrated changes in EPSP shape. Intracellular injection of current pulses was used to determine electrical properties of triceps surae motoneurons. Measurements from each motoneuron in both chronic spinal and unlesioned animals were used to obtain compartmental parameters in a "Rall model" of the motoneuron. For each motoneuron, model "EPSP" rise time and half width were calculated (Gustafsson and Pinter; Neurosci. Lett.51:67- 72, 1984). In addition, composite EPSPs following low threshold electrical stimulation were recorded from these same motoneurons. A 10 compartment model was used and "EPSP conductance" placed in various compartments. The relative distribution of the conductances was -> 0, 0, 0, .15, .35, .35, .15, 0, 0, 0. Mean values for modelled and experimentally measured EPSPs were:

	Rise time (ms)		Half Width (ms)	
	Measured	Modelled	Measured	Modelled
CONTROL	.67	.71	5.30	4.56
SPINAL	.53	.70	3.76	4.32

For unlesioned animals, this distribution produced modelled EPSPs with a similar rise time but somewhat shorter half width than the measured ones. On the other hand, measured EPSPs in chronic spinal animals had considerably faster rise times and faster decays. Moving the above distribution of conductances more proximal (about one compartment), gave modelled EPSPs that more closely approximated those in the chronic spinal animals. It is clear from the present results that changes in EPSPs following chronic spinalization cannot be explained simply on the basis of changes in passive electrical properties of motoneurons. EPSP shape changes are better explained by a change in the distribution of Ia afferent fiber synapses to a more proximal location on the motoneuron.

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296.11 DIFFERENTIAL CONTRIBUTIONS OF SOLEUS AND MEDIAL GASTROCNEMIUS TO THE CROSSED EXTENSION REFLEX IN DECEREBRATE CAT.
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An earlier study in this laboratory (Hannon et al., Soc. Neurosci. Abs. 11:700, 1985) demonstrated that vestibular and proprioceptive inputs differentially affect excitability of crossed extension reflex (CER) recorded from rectus femoris (RF) and vastus medialis (VM). RF appears to contribute more to postural reflexes than to dynamic reflexes, suggesting it is "slower" than VM. However, limited anatomical evidence suggests that RF may in fact be "faster" than VM. In the present study we tested the hypothesis that differences between RF and VM are not due to a difference in fast- and slow-twitch units, but rather to the fact that RF is biarticular (strong knee extensor and weak hip flexor) whereas VM is a uniarticular knee extensor.

We recorded CER from two ankle extensors: soleus (SOL) and medial gastrocnemius (MG). SOL is slow and uniarticular. MG is faster and is biarticular (strong ankle extensor and weak knee flexor). Thus, if characteristics of RF reflect its biarticular actions, descending inputs should affect MG and RF similarly. Conversely, if RF does act like a "slow" muscle, then such inputs should affect SOL and RF similarly.

The CER was monitored by recording isometric tension from right SOL and MG in 8 decerebrate cats. Reflexes were evoked by stimulating left sciatic nerve via indwelling electrodes.

The CER from SOL consistently had a lower threshold to stimulation of sciatic nerve than did that from MG. However, threshold for SOL was not appreciably higher when animal was placed on left vs. right body side as is the case for rectus femoris.

Reflexes from both had greater amplitudes when the animal was placed on the right vs. left body side. However, SOL consistently showed a greater sensitivity to body position, much as does RF.

Rotation of chin to right consistently facilitated CER from both SOL and MG. Rotation of chin to left consistently inhibited CER from SOL but had equivocal effects on CER from MG.

In summary, effects of descending inputs on SOL were much more like those on RF than on VM. Neuron pools of SOL and RF share some common integrative substrates which are different in some respects than those impinging on neuron pools of MG and VM. This would suggest that differences between RF and VM are not attributed to the number of joints spanned by each. We propose that, contrary to anatomical evidence, RF is functionally "slower" than is VM.

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296.12 DEPRESSION AND FACILITATION OF SPINAL CORD REFLEXES FOLLOWING PHYSOSTIGMINE.
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Physostigmine is a reversible acetylcholinesterase inhibitor that has been shown to alter the activity of central nervous system cholinergic neurons. The actions of physostigmine on spinal cord reflexes were studied in spinal cord transected cats (C1 section). Supramaximal stimulation of the cut L_7 dorsal root evoked a dorsal root reflex (DRR) in the cut L_6 dorsal root and a monosynaptic reflex (MSR) in the cut L_7 ventral root. Time course experiments revealed that the control DRR tended to increase with time while the control MSR showed little or no change over time. A single injection of physostigmine (0.8 mg/kg, i.v.) depressed the time-dependent increase of the DRR by 20%, 12% and 22% of the control at 5, 60 and 180 min after the injection. However, at higher doses (2 mg/kg, i.v.), physostigmine was found to depress the time-dependent increase of the DRR by 55%, 37% and 61% of the control at similar time intervals following administration. The injection of 0.8 mg/kg physostigmine markedly facilitated the MSR with time. The facilitation of the MSR began shortly after the injection reaching a maximum of 113% above the pre-physostigmine control by 10 min. A slight decrease to 87% above the pre-physostigmine control MSR was observed at 20 min; which was maintained for 3 hr. Conversely, when administered in a dose of 2 mg/kg physostigmine depressed the MSR by 45% of the pre-drug value within the first 5 min of injection. Beyond this period of time, the MSR gradually increased with a maximum value of 165% above the pre-physostigmine control obtained 3 hr after the injection.

Neither atropine nor mecamylamine pretreatment (1 mg/kg, i.v.) prevented significantly the depression of the DRR caused by lower doses of physostigmine. On the other hand, atropine but not mecamylamine markedly antagonized the depressant action of the higher dose of physostigmine on the DRR and reversed the early facilitation of the MSR produced by 0.8 mg/kg physostigmine. The late facilitation observed in the MSR at the lower dose of physostigmine was depressed by both antagonists. At the higher dose of physostigmine (2 mg/kg), mecamylamine but not atropine reversed the early depression of the MSR and blocked the late facilitation. These data suggest that physostigmine modifies the spinal cord reflexes in a dose-dependent manner, probably through muscarinic receptors in the dorsal horn and through both muscarinic and nicotinic receptors in the ventral horn.
Supported by U.S. Army Contract DAMD 17-86-C-6007.

297.1 DIFFERENTIAL ROLES OF HIPPOCAMPUS AND CAUDATE NUCLEUS IN MEMORY: SELECTIVE MEDIATION OF "COGNITIVE" AND "ASSOCIATIVE" LEARNING. Mark Packard and Norman M. White, Department of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Quebec, H3A 1B1, Canada.

Learning has been described as the acquisition of associations between stimuli and other stimuli or responses. However it is very difficult to use such a model to explain some instances of learning, and it has therefore been suggested that learning is in fact a much more complicated cognitive process. We tested the hypothesis that the normal brain contains both "associative" and "cognitive" memory substrates in different brain areas.

Animals with bilateral lesions of caudate nucleus or fornix and a control group were tested 2 wk post-operatively on one of two versions of the 8 arm radial maze task. In the standard "cognitive" version, they were habituated to the maze for two days with no food present and then tested daily for 4 days with a food pellet at the end of each arm. The number of errors (revisits) was recorded. In agreement with previous reports, rats with fornix lesions were impaired on this task. The rats with caudate lesions showed no deficit compared to controls.

Different groups of rats with similar lesions were tested on a stimulus-response ("associative") version of the radial maze. Small lights were attached at the entrance to each arm and a tubing system was constructed to permit rapid, unobtrusive rebaiting of the arms. On each of 15 daily trials 4 randomly selected arms were lit and baited. After a rat had visited one of the lit arms it was rebaited. After a second visit to the same arm its light was turned off and no further food was placed there. By visiting each of the 4 lit, baited arms twice animals earned 8 pellets per trial. Rats with caudate lesions were impaired on this task while those with fornix lesions were superior to controls in choice accuracy. Probe trials, run on days 16-19, with only 1 arm lit and baited at a time, showed that the animals had in fact learned to select the lit arms at a level greater than chance, although caudate animals were less accurate than fornix or control rats. Reducing extramaze cues by hanging curtains around the maze significantly improved perfomance of control and fornix animals on this task, however caudate animals were unable to benefit from the removal of these cues and remained significantly impaired.

These results demonstrate a double dissociation of the function of the caudate nucleus and the hippocampus with respect to simple, stimulus-response learning versus more complex, cognitive learning. They are consistent with the hypothesis that neural substrates for both kinds of learning exist in the normal brain: the caudate nucelus is selectively involved in processing associative learning, while the hippocampus selectively mediates cognitive tasks. Ordinarily, the two systems may act synergistically to produce learned behaviors, but the supernormal performance of the fornix animals on the S-R task shows that the two systems can also conflict with each other in some instances.

297.2 RECOVERY OF PERFORMANCE OF SPATIAL NAVIGATION BUT NOT SPATIAL WORKING MEMORY IN WATER MAZE TASKS FOLLOWING ENTORHINAL CORTEX LESIONS. C.R. Goodlett, J.M. Nichols*, R.W. Halloran* and J.R. West, Dept. of Anatomy, Univ. of Iowa, Iowa City, IA 52242.

Entorhinal cortex (ERC) lesions in rats impair spatial navigation (Schenk and Morris, Exp. Brain Res., 58:11, 1985) and spatial working memory processes in appetitive maze tasks (Olton et al., Brain Res., 233:241, 1982). Since the extent of recovery of spatial navigation relative to spatial working memory processes has not been examined using comparable procedures, the effects of ERC lesions were compared in adult male Sprague-Dawley rats given extended testing on the Morris water maze task or on a conditional alternation swim-escape task.

The Morris water maze training required rats to escape from swimming in a water tank by locating a small platform hidden underwater. Eight days of training (56 trials) were given preoperatively, including a probe trial (30 sec with the platform removed). Rats then were given either bilateral ERC lesions or sham surgery and trained postoperatively for 18 (sham) or 25 (lesion) consecutive days, beginning 4 days after surgery, with probe trials interspersed every 4 days. Dependent measures included latencies and path lengths to reach the platform and the percent of time in the target quadrant on the probe trials.

Conditional alternation training used a T-maze that was partially immersed in the water tank. Eight trials were given each day, each consisting of an information run (forced escape with one arm blocked), followed 6 sec later by a choice run (escape ladder in the arm previously blocked). Preoperatively the rats were trained to a performance criterion, and surgery (ERC lesions or sham) was performed on the next day. Postoperative testing began 4 days after surgery and continued for 16 (sham) or 40 (lesion) consecutive days. Horizontal sections stained with cresyl violet were used to confirm the lesions histologically.

The rats trained in the Morris maze learned the spatial navigation problem rapidly (mean latencies < 5 sec in the last 4 days). Sham operations initially resulted in moderate deficits in performance (initial mean latencies $\sim$ 12 sec) that returned to preoperative levels within 5 days. Bilateral ERC lesions severely impaired navigation performance relative to shams (initial mean latencies > 30 sec). However, substantial improvement in performance occurred over days as indicated by all dependent measures, with mean latencies below 9 sec by the 21st postoperative day. In contrast, the ERC lesions reduced performance in the conditional alternation problem to chance, and no improvement in error scores was observed over the 40 days of training. Thus, spatial navigation may be dissociated from spatial working memory on the basis of recovery following ERC lesions. (Supported by NIAAA grant AA06192 to JRW).

297.3 BEHAVIORAL CONSEQUENCES OF N-METHYL ASPARTATE-INDUCED DESTRUCTION OF BASAL FOREBRAIN CHOLINERGIC NEURONS. G.R. Stewart, D.F. Wozniak*, S. Finger* J.W. Olney and C. Cozzari[1]*. Depts. of Psychiatry and Psychology, Washington University, St. Louis, MO 63110, [1]Istituto Di Biologia Cellulare, Rome, Italy.

The basal forebrain cholinergic system (BFCS) is believed to play an important role in cognitive processes, and BFCS dysfunction may be responsible, in part, for cognitive disturbances in Alzheimer's Disease. Several investigators have shown that an excitotoxin lesion destroying BFCS neurons results in significant learning impairment in rats. The excitotoxins used in such studies were either kainate or ibotenate and the primary behavioral focus was on visually guided spatial learning. In the present study a different excitotoxin, N-methyl-D,L-aspartate (NMA), was used to destroy BFCS neurons and behavioral tests included a tactile discrimination (non visual, non spatial) task.

Adult male rats (n=8), injected with NMA bilaterally into the basal forebrain, were tested after a 1 month recovery period. In neurological tests for sensorimotor integrity the rats appeared to be "hyperemotional" but were not otherwise different from unoperated, age-matched controls. When both groups were trained on a rough/smooth tactile discrimination in a T maze the rats with lesions took significantly longer to reach criterion in the acquisition of this task and most did not learn (2 of 8 reached criterion compared to 7 of 8 controls). The same rats were then trained on a radial arm maze to test "working memory". Rats with lesions were significantly impaired in performing this task, but 7 out of 8 reached criterion.

Histological analysis revealed that rats with lesions were largely depleted of neurons immunoreactive for cholineacetyltransferase in peripallidal portions of the BFCS which comprise the rodent homolog to the primate nucleus basalis. There was also a decrease in acetylcholinesterase staining in cerebrocortical terminal fields to which these neurons project.

Our findings support the conclusion that NMA-induced lesions of the BFCS result in the same kind of behavioral deficits associated with other excitotoxin lesions, i.e., deficits in working memory dependent on spatial and visual cues. It is now possible to add that this type of lesion can impair tactile discrimination learning. Because many BFCS neurons destroyed by the excitotoxin projected to the somatosensory cortex, defective somatosensory information processing might have contributed to some of the deficits in the tactile discrimination learning. Supported by RSA MH 38894 (JWO), AG05681 and NS 07057.

297.4 COMPARISON OF BEHAVIORAL EFFECTS OF NUCLEUS BASALIS LESIONS VERSUS SENSORIMOTOR CORTEX ABLATION. D.F. Wozniak*, G.R. Stewart, S. Finger* and J.W. Olney (SPON: B. Hartman). Depts. of Psychiatry and Psychology, Washington University Medical School, St. Louis, MO 63110.

Excitotoxin lesions of the nucleus basalis magnocellularis (NBM) cause deficits in working memory. In addition, we have observed (Stewart et al., this meeting) that rats with such lesions appear hyperemotional (irritable) and are severely impaired in performing a tactile discrimination. Since NBM lesions destroy cholinergic neurons that densely innervate the sensorimotor cortex, the behavioral findings might be explained in terms of an impairment in sensory processing rather than memory. To explore this, we divided rats into 3 groups. In group 1 (n=9), neurons were destroyed by injection of N-methyl-DL-aspartate bilaterally into the NBM; group 2 (n=10) received bilateral ablation (aspiration) of the sensorimotor (SM) cortex; group 3 (n=10) were given sham operations (needle lowered to a point just dorsal to the NBM with no injection). Each group was assessed on a neurological battery to evaluate sensorimotor integrity and on a learning task conducted in a T maze which consisted of a series of five different two-choice tactile discriminations which were graded in difficulty.

The neurological battery revealed that the SM ablated rats showed significantly attenuated tactile hopping and placing responses and, consistent with our prior observation, NBM lesioned rats showed more agitation (irritability) during a reactivity to handling test. Also they spent less time on an elevated perch (platform test), an apparent reflection of hyperemotionality. It should be noted, however, that no significant differences were found among the three groups on 10 other tests of sensorimotor function. In the tactile discrimination test, the NBM lesioned and SM ablated groups did not differ significantly from each other on the series of discriminations but both were significantly impaired compared to the sham operated group.

These findings suggest that the deficit displayed by rats with NBM lesions in the tactile discrimination paradigm might be due, in part, to a loss of NBM fiber inputs to the SM cortex and consequent disruption of SM information processing. Our findings also suggest that NBM lesions may be associated with hyperemotionality, which may additionally confound interpretation of performance in memory/learning tests. Supported by RSA MH 38894 (JWO), AG 05681 and NS 07057.

297.5 THE PREFRONTAL CORTICAL SYSTEM: ALLOCENTRIC VERSUS EGOCENTRIC RESPONDING. M. Rasmussen*, C.A. Barnes and B.L. McNaughton. Behavioral Neuroscience Program, Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Deficits in spatial delayed response tasks have been found after lesions to the prefrontal cortex both in primates (Goldman and Rosvold, 1970) and in rats (Kolb, Nonneman and Singh, 1974). While the deficit appears to be one of spatial memory, it is impossible to know from these studies whether the subjects use an allocentric or an egocentric response strategy.

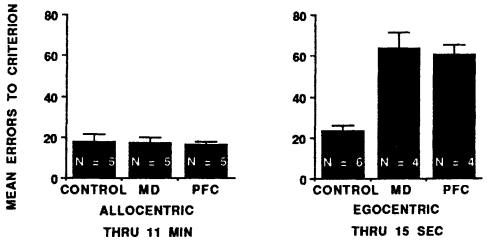
The purpose of the current study is to determine the nature of the spatial memory deficit after injury to the prefrontal cortex and medial dorsal thalamus.

Animals from these two lesion groups were compared with controls on two tests of spatial memory (5 trials per day). The allocentric version, in which the solution depended upon locating alternate places using distal cues, consisted of a cross-maze. Half of the time the Subject must turn in the opposite direction to alternate place and half of the time the direction of the turn is the same on forced and choice trials. The egocentric version requires the Subject to alternate body turns on a Y-maze - half of the time going to a different place on the choice trial and half of the time going to the same place in order to execute the correct turn.

No deficits were found on the allocentric version. All animals were able to meet the test criterion with delays between information and choice trials up to and including 1 minute.

Both prefrontal and medial dorsal thalamic lesions produced severe deficits on the egocentric version relative to controls. Control animals were able to sustain the 1 minute delays while most of the lesioned animals took many more days to master the zero-delay condition and were unable to perform better than chance at the 30 second delay.

Further studies are being conducted to determine whether the deficit is due to inability to remember a previous body turn or inability to select the correct response strategy in the face of damage to the prefrontal system. Supported by AG03376 and NS20331.



297.6 ANTEROGRADE AND RETROGRADE EFFECTS ON PLACE MEMORY AFTER LIMBIC OR DIENCEPHALIC DAMAGE. R. J. Sutherland, K. A. Arnold and A. R. Rodriguez. The University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4.

Damage to the hippocampal formation (HPC) produces a long lasting behavioural impairment in some memory tasks, particularly those involving conditional associations (e.g., place learning). We address two questions: 1) do subcortical structures connected with the HPC via the fornix differentially contribute to place learning, and 2) are recent and remote place memories differentially affected by damage to the HPC or its subcortical targets?

In Experiment 1 we assessed the effects of damage in several limbic and diencephalic structures on place navigation by rats in the Morris water task. Testing was conducted over ten consecutive days (8 trials per day) and included tests with visible and hidden platforms in a fixed location in the pool. Half of the operated controls and half of the brain damaged rats were trained before surgery, using the same procedures as during testing. All lesions were placed electrolytically, except for HPC damage which was made by multiple, bilateral microinjections of the neurotoxin, colchicine (2.5 µg/0.5 µl saline at 3 sites).

The rats of all lesion groups were able to swim as well as controls to the visible platform. Acquisition of accurate place navigation was achieved by rats with medial septal lesions, incomplete fornix/fimbria transections, and mammillary nuclei, albeit after more trials than controls. Damage to anterior thalamus, medial nucleus accumbens, fornix/fimbria, and HPC prevented acquisition of accurate place navigation. Only complete fornix/fimbria transection or HPC damage abolished preoperatively acquired place navigation.

In Experiment 2 we systematically varied the interval between the end of preoperative place navigation training and colchicine-induced HPC damage (1-12 weeks). Place navigation by all HPC groups was worse than that of their operated control groups during the initial trials of retention testing. However, there was a significant improvement in place navigation performance associated with longer training-surgery intervals. Unlike the other HPC damage groups, by the end of retention testing, the 12-wk group was not significantly different from controls.

The results are consistent with the theory that the HPC, in concert with posterior cortical areas, represents and initially stores conditional associations among events necessary for place memory and over a period of weeks participates in "consolidating" representations stored elsewhere (Sutherland and Rudy, 1987). Relevant subcortical areas only facilitate performance during initial acquisition of information.

297.7 **HIPPOCAMPAL DENERVATION FACILITATES OLFACTORY LEARNING-SET FORMATION AND DOES NOT IMPAIR MEMORY IN A SUCCESSIVE-CUE GO, NO-GO TASK.** T.A. Otto, F. Schottler*, U. Staubli, and G. Lynch. Center for the Neurobiology of Learning and Memory, Univ. of California, Irvine, CA 92717.

In an olfactory discrimination task using simultaneous odor presentation, hippocampal denervation by lesions of the entorhinal cortex produces an 'anterograde amnesia' syndrome in rats which is characterized by unimpaired acquisition of new discriminations (given short intertrial intervals) but deficits in retention of those discriminations when tested 1 hour later (Staubli et al., Proc. Natl. Acad. Sci., 81:5885, 1984). Eichenbaum et al. (Behav. Neurosci., in press) have recently reported that lesions to the fornix either facilitate or impair olfactory learning-set acquisition depending on whether the olfactory cues are presented sequentially or simultaneously, respectively. In the present experiments, we investigated the effects of a more specific hippocampal denervation on two aspects of olfactory learning using sequential odor presentation: 1) the formation of learning sets, and 2) the retention of individual cues.

Ten male Sprague-Dawley rats, 250-280 g, served as subjects. Five received bilateral electrolytic lesions to the entorhinal cortex. The remaining five rats served as sham-lesion controls. During daily odor training sessions, these water-deprived rats were trained to discriminate a single pair of odors which were ejected randomly and successively into the cage by constant-flow air pressure. Nosepoke responses to the arbitrarily-designated 'positive' odor resulted in access to a 0.05 ml water reinforcer; responses to the 'negative' odor went unreinforced. Sessions were terminated when the subject reached a criterion of 18 correct responses in 20 consecutive trials, or at 400 trials maximum. Five such sessions, with session-unique odor pairs, were conducted.

Both groups exhibited olfactory learning set acquisition, evidenced by a marked decrease in the number of trials to criterion across sessions. Hippocampally denervated subjects, however, outperformed their sham-lesioned counterparts in the number of correct responses during the first 20 trials of a session ($p<.05$) and in overall accuracy within a session ($p<.01$). In contrast to the results obtained using simultaneous odor presentation, the experimental animals exhibited no deficit in subsequent tests of retention (reversal). These data are consistent with the notion that the hippocampus is not required for 'procedural' types of memory and suggest that its role in the encoding of specific cues is task, or 'strategy', dependent.

This research was supported by ONR grant N00014-86-K-0333 to G. Lynch and by PHS grant 1 F32 NS08136-01 BNS-1 to T. Otto.

297.8 NORMALIZATION OF HIPPOCAMPAL LESION-INDUCED IMPAIRMENTS BY AN ATYPICAL NEUROLEPTIC. T.L. Steele & L.D. Devenport. Department of Psychology, University of Oklahoma, Norman, OK 73019.

Lesions of the hippocampus produce impairments in many learning tasks, especially those requiring a shift from one behavior to another, as in extinction (1). Similar deficits occur in rats given dopamine (DA) agonists. In both cases, the impairments may be due to stereotypy, increased activity, and decreased exploration (2). Neuroleptics, which block DA receptors, reduce the lesion-induced (3) and drug-induced behaviors (4). Considering that many of the behaviors found in hippocampal-lesioned (HIPP) animals mimic those seen in animals with high levels of DA activity, it is possible that some impairments observed in HIPP animals may not be a direct result of the lesion, but instead are due to increased DA activity. If true, manipulations which suppress DA transmission should normalize performance in HIPP animals. The present experiment tested this assumption by administering an atypical neuroleptic, sulpiride, to sham-operated (SHAM) and HIPP rats.

SHAM and HIPP rats were given daily injections of sulpiride (0, 15, 30, or 60 mg/kg) for 16 consecutive days. On each of these days, rats were placed in a straight alley containing reward and in a second alley (of different color) containing no reward. Animals were given 16 days of extinction (with continued drug administration) in which neither alley contained reward. Running time, exploration, and stereotypy were recorded across sessions.

Sulpiride had little effect on the behavior of SHAM rats, which learned the discrimination readily. HIPP rats receiving saline or low doses of sulpiride ran equally fast in both baited and non-baited alleys. Stereotypy was higher and exploration lower in these rats. However, stereotypy decreased and exploration increased in HIPP rats receiving the higher doses of sulpiride, and these rats were able to acquire the discrimination. During extinctino, sulpiride normalized behavior in HIPP rats, leaving that of SHAM rats unaffected.

The normalization of behavior in HIPP rats implies that hippocampal lesions may be only indirectly responsible for some of their impairments. Since these impairments were corrected by a DA antagonist, it is possible that the hippocampus exerts its effects through the DA system.

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2. Soc. Neurosci. Abstr., 1986, 12, 743.
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4. Neuropharm., 1981, 20, 1279.

297.9 PATHOLOGICAL ALTERATIONS IN THE BASOLATERAL AMYGDALA IN ALZHEIMER'S DISEASE. L.J. Kromer*, B.T. Hyman, G.W. Van Hoesen and A.R. Damasio. Depts. of Anatomy and Neurology, University of Iowa College of Medicine, Iowa City, IA 52242.

The basolateral complex of the amygdala in the human and non-human primate brain accounts for much of the mass of this structure and resembles the cortex in terms of its neural connectivity. For example, it sends projections to the striatum, parts of it receive a sizeable cholinergic input from the nucleus basalis of Meynert and it is interconnected strongly with frontal and temporal cortical areas. Connections with the dorsomedial thalamic nucleus, the nucleus basalis of Meynert, and with the hippocampus and entorhinal cortex, place the basolateral complex of the amygdala in a pivotal position to influence nearly all of the known memory-related neural systems of the forebrain. It is known that the amygdala is targeted for pathology in Alzheimer's disease (AD), but the extent of its involvement has not been delineated or analyzed in terms of its known connectivity. Therefore, we have studied the patterns of pathology in the amygdala of 10 cases of confirmed AD and 5 age-compatible controls using conventional pathological stains such as thionin, Congo red and thioflavin S and immunostaining with the monoclonal antibody Alz-50. Of the four major nuclei that form the basolateral complex, the accessory basal has consistently contained the highest degree of pathological involvement in terms of neuritic plaques and neurofibrillary tangles, and the lateral nucleus, the least. The laterobasal nucleus which receives a powerful cholinergic input typically contains few neuritic plaques, whereas these are found in abundance in the mediobasal nucleus. The heavy pathological involvement of the accessory basal and mediobasal nuclei is of interest, since these nuclei in non-human primates send projections to and receive projections from the subicular and CA1 fields of the hippocampal formation, structures damaged heavily by pathology in AD. Moreover, both nuclei contribute afferents to the dorsomedial thalamic nucleus. Thus, there seems good reason to believe that pathological alterations in the amygdala may contribute to some aspects of the memory impairment that characterizes AD. The selective nature of these changes further highlights the fact that pathological changes in AD target and dissect key structures of the forebrain that contribute to neural systems implicated in memory. (Supported by NS14944, PO NS 19632 and the Mathers Foundation).

297.10 **EFFECTS OF LESIONS OF THE DORSAL CORTEX AND MEDIAL CORTEX ON THE REVERSAL OF A GO/NO-GO DISCRIMINATION IN TURTLES.** **(Spon. O. B. Ward). W. Grisham* and A. S. Powers. Rosemont College, Rosemont, PA 19010 and Department of Psychology, St. John's University, Jamaica, NY 11439.**

Previous research has shown that turtles with lesions of the dorsal cortex (cd) show a deficit in the reversal of a simultaneous pattern discrimination (Cranney & Powers, 1983). The present experiment examined the effects of cd lesions on the retention and reversal of a go/no-go pattern discrimination. Lesions of the medial cortex (cm) were included as a control.

All turtles were trained on a go/no-go pattern discrimination until the difference in the mean latency to respond to the S+ and S- was at least 50 sec. across 4 consecutive sessions of 20 trials each. After this, the turtles were either given suction ablations aimed at the cd (n=4), electrolytic lesions aimed at the cm (n=5), or were shams (n=6). The turtles were given retention testing to criterion after which the discrimination contingencies were reversed. If the animals failed to respond to either the S+ or S- for at least three consecutive days or if they failed to discriminate between the S+ and S- for many days, they were given remedial training. In remedial training, the turtles were given only S+ trials until they responded with a mean latency of 10 sec. or less for a single session.

As previously reported, lesions of either the cd or the cm did not have any effect on retention (Grisham and Powers, 1985). Nonetheless, brain lesions did produce effects in reversal. A significantly greater proportion of brain-lesioned turtles than shams required remedial training ($p < .05$). The number of days in remedial training required was significantly correlated with bilateral cd damage (Spearman's rho=.49, $p < .05$) but was not correlated with bilateral cm damage (rho=.15, $p > .05$). Apart from the number of days required in remedial training, no other measures showed a difference between lesioned and sham animals.

The results of this experiment suggest that lesions of the cd produce deficits in the reversal of a go/no-go pattern discrimination. The results also suggest that the basis of this deficit is a retardation in the association of excitatory properties with the S+ in cd lesioned animals. This result is consistent with previous results which showed that cd lesioned animals are impaired in the acquisition of a go/no-go discrimination.

Cranney, J., & Powers, A. S., 1983, Physiological Psychology, 11, 103-111.
Grisham, W., & Powers, A. S., 1985, Society for Neuroscience Abstracts, 11, 1113.

297.11 MEDIODORSAL THALAMIC LESIONS IN RATS ALTER REACTIVITY TO ENVIRONMENTAL CUE CHANGES. K. A. Stokes* and P. J. Best. Department of Psychology, University of Virginia, Charlottesville, VA, 22901.

Rats with mediodorsal (MD) thalamic lesions present a number of deficits in learning tasks. MD animals do not perform well on radial maze tasks and have impaired memory for serial position (Stokes and Best, Neurosci. Abstr., 11, 833, 1985; 12, 747, 1986). These deficits imply that MD animals have compromised ability to utilize environmental stimuli to guide behaviour.

The present study examines the effects of electrolytic and ibotenic acid lesions of MD on reactivity to changes in environmental stimuli. The simple behavioural task employed requires the animals merely to move from a start box to a goal box for food reward. Start and goal boxes (each 30 cm long, 15.5 cm wide and 15 cm high) contained one of three sets of visual and tactile cues: 1) white walls and grid floor, 2) black walls and smooth floor or 3) black and white striped walls and carpet floor.

Initially, animals were trained for 5 trials a day in one start box connected to an identical goal box, and for 5 additional trials in a second start box also connected to an identical goal box, until they attained stable latencies to capture the food (40 trials, 4 days). At this point, the goal boxes were changed. For one group, the goal boxes were simply switched. These animals, then, still experienced familiar goal boxes, but in the wrong place. For the other group, both goal boxes were replaced by boxes containing the third set of cues. So, these animals experienced totally novel cues in both goal boxes. The particular cues in each condition were counterbalanced.

Intact animals demonstrate awareness of the goal box switch by arresting, briefly exploring the goal box, and, thus, showing longer latencies to approach the food. They show this reaction not only when the goal box is totally novel, but even when a familiar goal box appears in an inappropriate context. In contrast, MD lesioned animals do not show behavioural arrest and longer latencies when familiar goal boxes are switched. Either they do not pay attention to the goal box cues or they do not recognize their occurrence in an inappropriate or unusual context. However, when goal boxes are changed to novel cues, MD animals do orient to, or "notice", this change. Thus, MD animals can pay attention to environmental cues, but do not process familiar cues in the same way intact animals do, i.e., as specific to particular contexts.

297.12 SPATIAL DELAYED NONMATCHING-TO-SAMPLE AFTER FIMBRIA-FORNIX OR CINGULATE CORTEX LESIONS: A COMPARATIVE STUDY IN RATS AND MONKEYS. A.L.Markowska*, D.S.Olton, E.A.Murray and D. Gaffan. Dept. Psychology, Johns Hopkins Univ.,Baltimore, MD 21218, Lab. Neuropsychology, NIMH, Bethesda, MD 20892, Dept. Expt. Psychology, Univ. Oxford, Oxford, England.

Although experiments with rats and monkeys both provide animal models to examine the brain mechanisms involved in different types of memory, the experimental procedures used for the two species often differ, complicating a direct comparisons of the experimental results. The present experiment tested rats in a spatial delayed nonmatching-to-sample procedure identical to that used by Murray et al for monkeys (Society for Neuroscience Abstract, 1986, p. 976) in order to compare the effect of fornix lesions and cingulate lesions on performance in this task in the two species.

The apparatus was a T maze. Each trial consisted of two runs. During the force run, the entrance to one arm was blocked and the rat entered the other arm to receive food in the box. Following a one-minute interrun interval, the rat had a choice run with both arms open; food was available only in the arm not entered during the previous force run. Four trials were given each day with an intertrial interval of 1 & 1/2 hours. After the rats reached a criterion of 36 correct responses in 40 consecutive trials, they were tested with longer interrun delays (5 mins and 15 mins).

Preoperatively, the rats learned the task more quickly than the monkeys, taking a mean of 70 trials and 11 errors to complete criterion. This rapid acquisition took place in spite of the unusual procedure: only 4 trials per day, a long interrun interval even during initial acquisition, and a long intertrial interval. Postoperatively, control rats continued to choose accurately, and reached criterion in a mean of 46 trials and 4 errors. Both lesions produced a severe impairment of choice accuracy: rats with lesions had a mean choice accuracy of less than 70% after three weeks of testing, and showed no signs of improvement. The magnitude and duration of this impairment was greater for the rats than for the monkeys.

These results demonstrate that when rats and monkeys are tested in similar experimental procedures, lesions of the fimbria-fornix and the cingulate cortex produce similar behavioral impairments. However, the magnitude of the impairment differed in the two species, as did the performance of normal animals. These results provide an important funtional comparison of the behavioral roles of these brain structures, and emphasize the difficulties in obtaining tasks that are equivalent not only in procedure but also in difficulty for two different species.

297.13 CENTRAL BUT NOT BASOLATERAL AMYGDALA MEDIATES MEMORY FOR POSITIVE AFFECTIVE EXPERIENCES. R. D. Walser*, F. P. Kesner, and G. Winzenried*. Department of Psychology, University of Utah, Salt Lake City, Utah 84112.

It has been suggested that the amygdala is involved in mediating memory to the extent that the memory requires the coding of positive affect information. In order to provide support for this idea, rats were trained on a task in which memory for magnitude of reinforcement was tested. It is assumed that memory for magnitude of reinforcement is a function of the activation of affective experiences. To be more specific, rats were first acclimated to a radial arm maze. They then received a single trial per day consisting of a study phase and a test phase. In the study phase, the animals received 1 or 7 pieces (1/4 pieces of Froot Loop cereal) of food on different arms of the maze. After the study phase, the rats were delayed for 10 seconds, 5 or 15 minutes. After the delay during the test phase the animals were allowed to choose between the two arms presented in the study phase. The correct response, leading to an additional Froot Loop reinforcement, was to select the arm in which the animal had received the 7 pieces of food. Animals learned this task to a criterion of 80% or better performance on blocks of 10 trials for each delay within an average of 138 trials. They then received electrolytic lesions of the basolateral or central amygdala. After recovery from surgery animals were tested at each delay. The results indicate that there were no deficits at any delay with basolateral amygdala lesions. In contrast, lesions of the central amygdala produced a marked deficit at the 5 and 15 minute delays, but no deficit at the 10 second delay. Based on the assumption that memory for magnitude of reinforcement is a function of the activation of affective experiences, it is concluded that the central but not basolateral amygdala is involved in the coding of positive affect information.

297.14 REVERSIBLE COLD LESIONS OF THE PARAHIPPOCAMPAL GYRUS RESULT IN DEFICITS ON THE DELAYED MATCH-TO-SAMPLE TASK. George, P.J.[1]*, Horel, J.A.[2], and Cirillo, R.A.[1]*. (SPON: G. Keating) 1. Dept. of Psychology, Syracuse University, Syracuse, NY 13244. 2. Dept. of Anatomy and Cell Biology, SUNY Health Science Center at Syracuse, Syracuse, NY 13210.

In a previous experiment we found that reversible cold lesions of the anterior portion of the inferotemporal gyrus (a. itg.) produced deficits on a delayed match-to-sample (DMS) task, especially at the longer delays (45 seconds). Lesions of posterior itg or any part of the middle temporal gyrus (mtg) did not have any effect on DMS performance at any delay (0-45 seconds). (Horel et al., Society for Neuroscience Abstracts, 15: 324, 1985). This left the question of how a. itg gets its visual input necessary for the DMS task. Martin-Elkins (personal communication) injected horseradish-peroxidase into anterior itg in monkeys and found cells labelled in the posterior parahippocampal gyrus, suggesting that this may be a source of visual input into anterior itg.

In the present experiment, we placed 2 cryodes bilaterally over the mediventral temporal cortex in cynamologous monkeys (Macacca fascicularis). One cryode covered the posterior extent of the posterior parahippocampal gyrus (pg), and one covered the tissue lying lateral to pg, the posterior infertemporal gyrus (p. itg). Both cryodes consisted of 23-ga stainless steel tubing shape to fit over the gyri. Both measured 11 mm long X 5 mm wide and were placed over the dura mater.

Animals were trained preoperatively on a variable DMS task with 0-45 second delays. Stimuli consisted of 560 colored photographs of objects and were presented on rear projection screens that faced the animals who were restrained in chairs. During experimental trials, cooled methanol was pumped through the tubing to bring the temperature of the probes to -5°C; behavioral effects of the lesions were completely reversible within 3 minutes of removing the cold.

Results show a deficit for DMS at the longest delay (45 seconds) with reversible lesions of the pg. Performance on DMS during lesions of p. itg was similar to that of control. The monkeys were also tested on a concurrent visual discriminations. Eight object pairs were presented randomly and in succession and trials-to-criterion were measured under control and the two lesion conditions. Trials-to-criterion during both p. itg and pg lesions were much greater than that of control, suggesting a learning deficit for this task with both cortical lesions. (Supported by NINCDS grant NS 1829-05).

297.15 BEHAVIORAL RESPONSES PARALLEL THE RESPONSES OF HIPPOCAMPAL PLACE CELLS TO ENVIRONMENTAL MANIPULATIONS. J. L. Kubie, R. U. Muller*, S. Davani* and D. Morgenstern*. Depts of Physiol. and Anat. and Cell Biol., S.U.N.Y. Health Sci. Center at Brooklyn, Brooklyn, NY 11203.

The water maze developed by Richard Morris has been of great value in studying the spatial abilities of rats. Moreover, the finding that rats with damaged parts of the hippocampal formation perform poorly in the water maze (Morris & Garrard, *Behav. Br. Res.* 1980) strongly supports the idea that the hippocampus is important in spatial processing, as does the existence of hippocampal place cells (O'Keefe and Nadel, *The Hippocampus as a Cognitive Map*, 1978). Unfortunately, it is extremely difficult to record from or study place cells in the water maze. Our aim was, therefore, to develop a "dry" version of the water maze. This would permit study of place cell activity and a very interesting form of spatial problem solving under similar circumstances, or at the same time.

The dry maze is a 6 ft diameter cylinder. The goal is a sealed food cup buried below 4 inches of wood chips; this replaces the submerged platform of the water maze. The two goals are equivalent in that neither provides stimulus information, so that the location of each can only be efficiently found from spatial relations to distant cues. In the dry maze the major salient cue is a white card that covers 100° of the cylinder wall; other cues are hidden by a circular curtain. Use of the card permits direct comparison with our place cell studies, which employed similar cards. We found that rotating the cue card causes equal rotations of place cell firing fields; halving the size of the cue card causes, at most, rotations of the firing fields, and removing the cue card causes fields to rotate to an unpredictable angular location, but to retain their integrity (Muller & Kubie, *J. Neurosci.* 1987). Our aim was to test the idea that cue manipulations in the dry maze will lead to similar rotations in spatial behavior.

Rats are first permitted to find food on the surface of the wood chips. During formal training, rats start from one of 3 randomly chosen positions. Group 1 rats are subsequently trained to find food buried in a fixed location on each trial. Group 2 rats are trained to find buried food in a different location on each trial. For group 2, each trial is divided into a first half, in which the food location was marked with a dowel, and a second half, in which the food is buried at the same location but is unmarked.

Both groups learned to find the food accurately within thirty trials. Rats took direct paths and usually found the buried food within 15 sec. On trials when no food was buried, rats ran directly to the last rewarded location and dug there, demonstrating the use of spatial cues. On probe trials with food buried randomly with respect to the fixed (group 1) or 1st half of the trial (group 2) locations, rats never found the food, indicating stimuli from the food dishes do not guide the behavior.

When the cue card was rotated and no food was buried, the behavior of rats in both groups was to first dig at a spot consistent with rotation, and then to choose a second spot consistent with no rotation. The results obtained to date demonstrate that the cue card controls spatial behavior in the same way it controls place cell firing. Further studies are needed to determine whether place cell activity and spatial behavior always respond in parallel. The methods described here ought to be of use in a variety of studies on the neurobiology of spatial behavior. (Supported by N.I.H. grant NS20686)

297.16 EFFECTS OF PERINATAL COPPER DEFICIENCY ON RADIAL MAZE PERFORMANCE OF REHABILITATED ADULT RATS. E.S. Halas. USDA, ARS Human Nutrition Research Center, Grand Forks, North Dakota 58202.

Dietary trace elements, including zinc, copper, and iron, have been found to be essential for the proper development of the brain. For example, rehabilitated adult rats who suffered zinc deficiency during gestation and/or lactation were found to have an impaired learning ability and working (short-term) memory. Histological abnormalities were found in the hippocampi of these rats. Interestingly, the hippocampus has the highest concentration of zinc while the locus coeruleus has the highest concentration of copper. Thus, we decided to examine the importance of copper for learning and memory. Starting on the day of conception, and ending on the day of delivery, one group of rat dams was fed a diet containing 1.2 ppm copper; a second group of rats was fed a diet containing 1.7 ppm copper. These two groups were fed a diet containing 0.5 ppm Cu from the day of delivery until the pups were weaned at 23 days of age. A third group of dams was fed a 5.0 ppm Cu diet throughout gestation and lactation. After weaning, all three groups were maintained on the 5.0 ppm Cu diet until they were 120 days old; they were then reduced to 80% of their normal weight and trained on a 17-arm radial maze. There were 8 female rats selected from each of the three groups for a total of 24 rats. Each rat was given 1 trial per day for 25 days. All 17 arms were baited with a single 190 mg food pellet. A trial was terminated when the rat retrieved all 17 pellets or 15 min. had elapsed. The data were analyzed in blocks of 5 trials. During the first 5 trials, no significant differences in percent of correct responses were found among the 3 groups. In the second block, (trials 6-10), the 1.2 ppm group had a significantly lower percent of correct responses than the 1.7 and 5.0 ppm groups. When compared to the other 2 groups, the 1.2 ppm group continued to be significantly inferior in performance during block three (trials 11-15). In the remaining blocks of trials, no significant differences in performance were found among the three groups. No significant differences were found between the 1.7 and 5.00 ppm groups during any of the blocks of trials. These findings suggest that learning was retarded in the 1.2 ppm group but working memory was not impaired. These findings contrasts with those of zinc deficiency, which impaired both learning and working memory. Thus, different trace element deficiencies might injure different structures of the brain resulting in different types of behavioral impairment.

297.17 SEPTO-HIPPOCAMPAL SYSTEM AND PRELIMBIC CORTEX: A NEUROPSYCHOLOGICAL BATTERY ANALYSIS IN THE RAT. G.N.O. BRITO and L.S.O.Brito*. Setor de Neurociencias, Inst. Biom., Univ.Fed.Fluminense, Niteroi, RJ 24210, Brasil.

Research in the neuropsychology of animal memory suggests that lesions in the septo-hippocampal system or medial frontal cortex disrupt working-memory mechanisms but leave intact reference-memory processes. However, most studies use a limited number of behavioral tasks. Also, work on the frontal cortex has usually disregarded the different divisions of the medial frontal area. In the present study, we investigated the functions of the septo-hippocampal system and a specific sector of the medial frontal cortex, the prelimbic area, using a neuropsychological test battery. Procedures for surgery and coordinates for electrolytic lesions in the posterodorsal septum (SEP, N=9), prelimbic cortex (PRE, N=9) and control operations (CON, N=8) were as described in Brito et al (Exp.Brain Res., 46: 52, 1982). After a 3-wk postoperative recovery period, rats were tested in a neuropsychological battery comprised of the following tasks in the order administered: time-spent-eating in a strange box; time-to-emerge from home cage into an open field; activity box; adaptation to a T-maze, continuous alternation, visual discrimination, discrete alternation, and olfactory discrimination all in the same maze; adaptation to a straight runway, response patterning under a single alternation schedule of rewarded and nonrewarded trials, a tactile Go-No Go discrimination, and an approach-avoidance conflict task all in the same runway. SEP and PRE rats were hyperactive compared to CON rats. SEP rats performed the visual, olfactory and tactile discrimination tasks as well as CON rats. However, SEP rats could not perform the T-maze and runway alternation tasks. PRE rats performed as well as CON rats in the T-maze visual and olfactory discrimination tasks. However, PRE rats performed worse than CON rats in the alternation and tactile Go-No Go discrimination tasks. PRE rats performed the alternation tasks better than SEP rats, whereas SEP rats performed the tactile discrimination task better than PRE rats. There were no differences between groups in the performance of the approach-avoidance conflict test. These results support the hypothesis that the septo-hippocampal circuitry and, to a lesser extent, the prelimbic cortex are part of a system involved in working-memory processes.

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297.18 CHRONIC ETHANOL CONSUMPTION AND MAMMILLARY BODY LESIONS PRODUCED SIMILAR SPATIAL MEMORY RETRIEVAL DEFICIT IN MICE. D.J. Béracochéa, A.N. Tako and R. Jaffard, Lab. Psychophysiologie, Univ. Bordeaux I, 33405 Talence Cedex.

Prolonged ethanol consumption in mice has been found to result in spatial memory deficits (Béracochéa and Jaffard, Behav. Br. Res.,15:15,198) associated with marked neuronal loss in the median mammillary nucleus (MM)(Lescaudron et al., Neurosc. Lett., 50:151,1984). Other structures generally considered to play a key role in memory processes were also found to exhibit some degree of neuronal loss (see Béracochéa et al., Neurosc. Lett., 73:81, 1987). Consequently the purpose of the present experiment was to determine to what extent, an experimental lesion of the MM alone would produce the same deficit as alcohol treatment. Spontaneous alternation behavior (S.A.) in a T-maze and delayed non matching to sample task in an automated 8-arm radial maze were used. For S.A. two procedures were employed : a sequential and a discrete alternation procedures respectively aimed at evaluating proactive interference (P.I.) and the rate of forgetting.

Results showed that both 6 months of ethanol consumption and experimental MM lesions (electrolytic or cytotoxic) result in an accelerated rate of decay of S.A. (discrete alternation procedure) experimental subjects exhibiting normal S.A. rate at the 5 mn retention interval (R.I.) but reaching chance level performance at 6 hrs as compared to 48 hrs for controls. In the sequential test procedure, both experimental groups exhibited a progressive impairment of performance as the number of trials increased; thus, while their S.A. rate was normal for the 3 first trials of the series, additional trials (4th to 6th) revealed a marked impairment of S.A. as compared to controls. A similar but lesser deficit were observed in the radial maze suggesting that both MM lesioned and alcohol treated mice suffered from an exaggerated vulnerability to P.I. and from an abnormally rapid rate of forgetting.

The second part of the experiment was aimed at testing whether these impairments might result from frequent use of the same material (familiarity). Accordingly, tests were conducted under different conditions varying the intramaze context by placing (ON) or not (OFF: usual test condition) a white cardboard in front of the stem between the two goal arms. Results showed that the deficit observed in both alcohol-treated and MM lesioned animals was totaly alleviated by this change of context, most importantly when it occured on the retention test trial alone.

These results show that both alcohol-treated and MM lesioned animals suffered from a retrieval spatial memory deficit which closely depends on the testing condition and emphasize the key role of mamillary body lesions in this form of alcohol-induced amnesia.

297.19 THE ROLE OF GENDER IN THE BEHAVIORAL EFFECTS OF HIPPOCAMPAL SYMPATHETIC INGROWTH. L. Harrell and D. Parsons*. Dept. of Neurology, VAMC and Univ. Alabama Med. Ctr., Birmingham, AL 35294.

Following cholinergic denervation of the hippocampal formation, peripheral sympathetic nerves originating from the superior cervical ganglia grow into the hippocampus. Multiple studies from our laboratory suggest that hippocampal sympathetic ingrowth (HSI) is functional and can alter behavior. Since gender is known to alter the anatomy of HSI, the present study was performed to assess the effect of this variable on behavioral recovery following HSI.

Sixty-one female and 33 adult male Sprague-Dawley rats were trained on a standard version of the radial 8-arm maze task until they reached a specific learning criterion (visiting 8 baited arms in the first 10 selections over 5 days). Animals from each sex then under 1 of 3 surgical procedures: CON (sham surgeries): MS + Gx (medial septal lesions + ganglionectomy); MS + SGx (MS lesion + sham Gx). Reacquisition of the maze was then assessed.

Twenty-nine female and 17 male rats were excluded from data analysis due to lesion placement or surgical death. Prior to surgery acquisition of the task was significantly faster in male (7.43 $\pm$ .97, (SEM) trials) than female (14.0 $\pm$ 3.4) rats ($p < .01$; ANOVA). No differences were observed by within each gender group. Following surgery male and female rats recovered over-all performance at similar rates. However, marked group differences were observed ($p < .001$; ANOVA). In males, the CON (n=7; 6.0 $\pm$ 6.5) group recovered faster than the MS + Gx (n=5; 19.2 $\pm$ 7.0) group , which recovered faster than the MS + SGx (n=4; 35.7 $\pm$ 7) group ($p < .05$). In females, the CON group (n=12; 12.6 $\pm$ 2.7) recovered faster than the MS + SGx group (n=9; 23.7 $\pm$ 4.5), which inturn recovered faster than the MS + Gx group (n=11; 42.0 $\pm$ 9.1).

The results of this study clearly demonstrate that gender can influence the behavioral effects of HSI. As expected from our previous work, HSI in male rats produced a determintal effect, with slowing of behavioral recovery. In females, however, HSI produced exactly the opposite effect with facilitation of behavior. We believe that this is the first instance in which gender has been shown to alter the behavioral effect of a neuronal reorganization. This work may eventually have important implications in Alzheimer's disease, an illness found in both sexes and associated with cholinergic degeneration and sympathetic ingrowth.

298.1 IDENTIFICATION AND CHARACTERIZATION OF THE OPIATE RECEPTOR IN THE CILIATED PROTOZOAN, TETRAHYMENA. J.B. O'Neill*, B. Zipser*, M.R. Ruff, C.C. Smith, W.J. Higgins and C.B. Pert. Section on Brain Biochemistry, Clinical Neuroscience Branch, National Institute of Mental Health, ADAMHA, Bethesda, Maryland 20892 and Department of Zoology, University of Maryland, College Park, Maryland 20742.

Tetrahymena, a ciliated protozoan, has a low phylogenetic order, yet is a highly specialized, differentiated organism. It is known to secrete many informational substances, including peptides such as β-endorphin. We wished to investigate the possibility that this organism possesses a functional opiate receptor which might be similar to the well characterized opiate receptor in the rat brain. Binding assays using both living cells and membrane preparations, verified saturable stereospecific, [^{125}I]-β-endorphin binding which was displaceable by various opiates chosen for their specificity for the putative opiate subtypes. SDS-PAGE of a DSS cross-linked receptor-[^{125}I]-β-endorphin complex revealed a pattern of bands which consistently included bands at 110, 58-55, and 29 kD. These bands were all displaceable by the classical antagonist, naloxone, as well as by other opiates thought to be prototypic for various opiate receptor subtypes. Limited proteolysis in SDS-PAGE showed that the 110 kD band could be fragmented into 58-55 and 29 kD bands and that the 58 kD band could generate a 29 kD fragment. The limited digest fragments of the 110, 58-55 doublet and 29 kD bands were remarkably similar to those generated from the rat brain receptor. Analytical isoelectric focusing of digitonin solubilized [^{125}I]-β-endorphin-receptor complexes showed that the isoelectric points from both the rat and Tetrahymena were identical (pI 4.6).

Chemotactic experiments with intact Tetrahymena, demonstrated that these unicellular animals migrated toward a 10^{-9}M β-endorphin gradient. Chemotaxis was blocked by (-) naloxone but not (+) naloxone, suggesting a stereospecific opiate receptor-mediated response. We conclude that Tetrahymena possesses a functional opiate receptor (recognition molecule) similar to the opiate receptor of the rat brain.

298.2 The Effects of FMRFamide on Longitudinal Muscle in the Medicinal Leech. B.J. Norris, J.A. Fiez*, and R.L. Calabrese. Dept. of Biology. Emory Univ. Atlanta, GA 30322

We have previously shown immunocytochemically that a FMRFamide-like peptide is localized in cholinergic motor neurons innervating longitudinal muscle in the medicinal leech. Further, bath applied FMRFamide caused a tonic contraction in longitudinal muscle and occasionally triggered a weak myogenic rhythm (Norris and Calabrese, Neurosci. Abstr. 11:942, 1985). We have gone on to study further the effects of FMRFamide on longitudinal muscle and to characterize the FMRFamide receptors.

Although brief applications of FMRFamide do not potentiate the response of longitudinal muscle to bath applied ACh, we found that extended exposure (30 mins) of longitudinal muscle to FMRFamide potentiates the muscle's response to ACh. This potentiation was manifested as an increase in both the peak tension and the total tension generated with little or no change in the rate of contraction or relaxation. It persisted long after exposure to FMRFamide was terminated. Exposure to FMRFamide also resulted in a potentiation of the response of longitudinal muscle to motor nerve stimulation. The potentiation of the neuromuscular response consisted of little or no increase in the peak tension but a large decline in the rate of relaxation and an increase in the total amount of tension generated. Further experiments will be aimed at determining FMRFamide's effect on the membrane potential of longitudinal muscle and its effect on neuromuscular transmission.

The effects of bath applied FMRFamide analogs on leech longitudinal muscle were compared to those of FMRFamide. The analogs FMRF-OH, RFamide, FMdRFamide and LWMRFA caused no discernible contraction. FLRFamide and YMRFamide caused effects similar to FMRFamide, while Helix FMRFamide-like peptide (pQDPFLRFamide) caused a contraction 2 to 4 times as strong as equimolar concentrations of FMRFamide. The blocked N-terminus of pQDPFLRFamide could protect the molecule from breakdown by proteolytic enzymes, and thus increase its potency. In preliminary experiments, FMRFamide bath applied with protease inhibitors (Trypsin II-T, 1.25 mgs/ml; L-Phenylalanine-L-Alanine 10 mgs/ml; Phenylmethylsulfonyl Fluoride, 1.5 mgs/ml) caused a 3 fold increase in the contraction produced by FMRFamide alone. Further experiments will attempt to characterize the nature of the proteases present and their role in normal peptidergic transmission.
This work is supported by NIH grant no. NS24072-03.

298.3 ELECTROPHYSIOLOGICAL & BIOCHEMICAL EVIDENCE FOR GABA AS A NEUROTRANSMITTER IN HELISOMA TRIVOLVIS. J.E. Richmond, A.G.M. Bulloch and K.D. Lukowiak. Neuroscience Research Group. University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Several aspects of the neural basis of feeding in the pond snail, Helisoma have been described. We have found that the feeding patterned motor activity (PMA) can be modulated by several neurotransmitters including the amino acid, gamma-aminobutyric acid (GABA). GABA has been implicated in synaptic transmission in both vertebrates and invertebrates and is generally considered to be an inhibitory neurotransmitter. This has prompted us to test the hypothesis that GABA may act as a neurotransmitter on some aspect(s) of the rhythm generation which underlies fictive feeding in this snail. Several lines of evidence have been pursued in order to establish a neurotransmitter role for GABA in the CNS and to identify candidate GABAergic neurons.

As reported previously bath application of GABA at concentrations from 5 x 10^{-6}M brings about the onset of PMA in the isolated CNS. Furthermore, iontophoresis of GABA onto key neurons in the buccal ganglia involved in PMA produces both depolarizing and hyperpolarizing responses. Using a GABA antibody, we have mapped candidate GABA neurons in several ganglia of the Helisoma CNS including the buccal ganglia. Almost exclusively these neurons do not have peripheral projections and thus can be considered interneuronal. The specificity of the immunoreactivity was tested by preabsorbing the antibody with a GABA-BSA conjugate. This abolished all staining. HPLC analysis of the CNS using two different analytical systems has confirmed the presence for GABA in the CNS, whereas there is no evidence of GABA in the hemolymph of this snail. In a further investigation in which the CNS was preincubated with ^{3}H glutamate, we were able to demonstrate the incorporation of tritium into the GABA peak which suggests the de novo synthesis of GABA takes place in the CNS of Helisoma.

Taken together this evidence strongly supports the hypothesis that GABA acts as a neurotransmitter in Helisoma. Current experiments are aimed at establishing whether there is a specific uptake mechanism for GABA using autoradiographic techniques and whether GABA is released during excitation of the CNS.

298.4 SIZE AND NUMBER OF SEROTONIN IMMUNOREACTIVE CELLS CHANGE WITH AGE IN ADULTS OF THE SNAIL MELAMPUS. R.H. May, R.L. Ridgway, and S.B. Moffett. Dept. of Zoology, Washington State University, Pullman, WA 99164-4220.

Snails are favored for study by many neurobiologists because of the relative simplicity of their nervous systems, which often contain individually identifiable large cells. The presence of such cells has fostered an assumption that molluscan neurons, while increasing in size via polyploidy, remain constant in number and position during adulthood. We have tested this assumption by determining the size, number, and position of serotonergic cells within the CNS of adult Melampus bidentatus. This pulmonate species is slow-growing and has a long lifespan (> 4 yrs). Shell lengths of reproductive Melampus range from 5-12 mm. An indirect immunofluorescence method, employing a commercial antiserum to serotonin (ImmunoNuclear Corp.), was used to visualize serotonin-containing cells within whole-mounted ganglia of 5, 8, and 10 mm snails. Cell counts and diameters were determined using image analysis (digitizing morphometry: Bioquant II software).

Cells having serotonin-like immunoreactivity (SIR) occurred in 8 of 11 central ganglia of Melampus, the exceptions being the left pleural and paired buccal ganglia. The right pleural ganglion contained only 1-3 SIR cells. Base cell counts (derived from 5 mm snails, n=5) within the other ganglia were as follows: left cerebral, $\bar{X}$=32.4 ± 1.6 (SE); right cerebral, 32.3 ± 3.8; left parietal, 11.8 ± 2.7; right parietal, 29.4 ± 2.7; visceral, 29.2 ± 4.0; left pedal, 74.0 ± 8.5; and right pedal, 89.8 ± 10.6. Comparison of 8 mm animals (n=5) to these baseline values showed significantly ($P < 0.05$) more SIR cells in the right parietal ($\bar{X}$=40.6 ± 2.8 SE) and visceral ($\bar{X}$=34.6 ± 2.7 SE) ganglia, but not in other ganglia. Similar results were obtained when 10 mm (n=5) animals were compared to the baseline values. When values for 10 mm and 8 mm animals were compared only the visceral ganglion ($\bar{X}$=46.5 ± 4.9 SE) showed significantly more SIR cells. The increases in cell number appear to be restricted to clusters of small (8-12 μm diam.) SIR cells. Clusters of larger cells and solitary large SIR cells did not increase in number although anomalies (e.g. duplications) were occasionally seen. All SIR clusters and most solitary large cells showed a significant ($P < 0.05$) increase in average cell diameter with age when 5 mm animals were compared with 8 or 10 mm animals.

We conclude that addition of serotonergic cells can occur into adulthood in Melampus. The data suggest that mitotically competent stem cells or post-mitotic undifferentiated cells exist within the CNS of adults which may contribute to the remarkable ability of this species to replace lost neurons (see Moffett and Austin, J. Comp. Neurol. 207:177-182, 1982). Supported by a Sigma Xi grant to RLR and NIH grant RO1 NS 22896 to SBM.

298.5 SUBSTANCE P-LIKE AND SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE CNS OF THE SNAIL MELAMPUS. R.L. Ridgway and S.B. Moffett, Dept. of Zoology, Washington State Univ., Pullman, WA 99164-4220.

The primitive pulmonate gastropod Melampus bidentatus exhibits a striking ability to regenerate neural tissues in response to injury that can include the replacement of lost neurons (Moffett and Austin, J. Comp. Neurol. 207:177-182, 1982). Our interest is in elucidating the roles played by various neuropeptides and transmitters in Melampus CNS regeneration. Somatostatin-like peptides have recently been shown to induce neuritic outgrowth of axotomized gastropod neurons (Grimm-Jorgenson, Brain Res. 403:121-126, 1987; Bulloch, Brain Res., 1987 (in press)) while substance P and related molecules are known to be potent mitogens in a number of preparations, including the blastema of regenerating flatworms (Salo and Baguna, J. Exp. Zool. 237:129-135, 1986). The present study documents the presence of somatostatin-like and substance P-like molecules in the CNS of Melampus as assayed by whole mount indirect immunofluorescence techniques using commercially prepared antisera (ImmunoNuclear Corp.).

Substance P-like immunoreactive (SP-IR) cells are found in the cerebral, parietal, visceral, and pedal ganglia of Melampus but not in the buccal or pleural ganglia. The SP-IR cells occur in clusters containing 5-30 neurons and are of small to medium size (7-35 μm diam.), the largest being in the right parietal and visceral ganglia. One cluster of smaller SP-IR cells is located within the lateral lobe of each cerebral ganglion. Processes from these cells terminate at an epithelial element within the lobe, the cerebral gland, the lumen of which is also immunoreactive.

Antisera to both somatostatin (SOM) and somatostatin-28 Tyr 4-14 (SOM-28) were employed. As found in the snail Helisoma (Bulloch, see above), only the antiserum to SOM-28 stained neuronal somata; staining by the antiserum to SOM appeared diffuse and non-specific. SOM-28 immunoreactive cells primarily occur in the cerebral ganglia in clusters along the mediodorsal surface. This location corresponds to a group staining light green by Alcian blue/Alcian yellow histochemistry (Ridgway, Comp. Biochem. Physiol., 1987 (in press)). A smaller cluster, including 1-3 cells, occurs in the lateral lobe and, like the SP-IR cells of this region, are associated with the cerebral gland. Substance P-like and somatostatin-28-like immunoreactive neurites are present in virtually all nerves and connectives of Melampus but are especially prevalent in the labial and tentacle nerves. Many of these neurites may be afferent, originating from somata located in peripheral tissues. This study was supported by a Sigma Xi and WSU grants to RLR and by NIH grant R01 NS 22896 to SBM.

298.6 GONADOTROPIC HORMONE-LIKE IMMUNOREACTIVITY IN THE CNS AND THE REPRODUCTIVE TRACTS OF SNAILS AND RATS. J. van Minnen*, R.H.M. Ebberink* and E. Vreugdenhil* (SPON: P.G. Sokolove). Department of Biology, Vrije Universiteit, 1007 MC Amsterdam, The Netherlands

The Caudo-Dorsal Cells (CDC) of the hermaphrodite snail *Lymnaea stagnalis* play a central role in the control of egg laying and associated stereotyped egg-laying behaviour. The CDC produce at least 9 neuropeptides. For 3 of them a function has been demonstrated. One of them is the ovulation hormone (CDCH); calfluxin (CaF1) controls calcium fluxes in the albumen gland and the autotransmitter has an excitatory effect on the CDC. These 3 peptides are all derived from a polyprotein peptide precursor molecule encoded on the CDCH-gene.

With a monoclonal antibody to a synthetic fragment of CDCH (m-aCDCH) and with a c-DNA probe encoding CDCH, the CDC, ectopic CDC, and another type of (small) neurons were labelled in the central nervous system (CNS). Outside the CNS neurons and exocrine epithelial cells reacted with m-aCDCH and the c-DNA probe. Most of the neurons were found in the female part of the reproductive tract. The neurons form numerous immunoreactive fibertracts with varicosities on secretory cells and muscle fibers. Furthermore a few immunoreactive neurons were found in the epithelium of the skin (these are probably sensory neurons). The exocrine cells that reacted with m-aCDCH and the c-DNA probe were only observed in the male part of the reproductive tract. Their secretory material is transferred during a copulation.

The results indicate that there exists a morphological basis for the control of egg-laying and associated behaviour by neurons expressing the CDC-gene(s). Whether the CDCH-like material of the male accessory sex glands has a function as a (pheromone-like?) messenger remains to be established.

In analogy to the situation in *Lymnaea*, we have investigated with immunocytochemistry the male reproductive tracts of rats on the occurrence of gonadotropic hormone-like immunoreactivity. Of the antibodies tried only an antibody to LHRH strongly reacted with most, but not all epithelial cells lining the epididymus, vas deferens, seminal vesicles and coagulation gland. Furthermore, the cells of Leydig showed a weak reaction to the antibody.

298.7 NEUROPEPTIDES IN THE NERVOUS SYSTEM OF THE MARINE SNAIL, BULLA GOULDIANA. J.C. Speh*, M.H. Roberts and R.Y. Moore (SPON: M.F. Bernstein), Depts. of Neurology and Neurobiology and Behavior, SUNY-Stony Brook, Stony Brook, NY 11794.

In this study we report the immunohistochemical identification of several neuropeptides in the central nervous system (CNS) of the marine snail, Bulla gouldiana. The Bulla CNS consists of a series of circumesophageal ganglia which were removed, immersion fixed in Bouin's solution, and imbedded in paraffin. Mounted sections were stained with cresyl violet or prepared for the immunohistochemical visualization of neuropeptides using the Sternberger peroxidase-anti-peroxidase (PAP) technique. Antisera to vasopressin (VP), vasoactive intestinal polypeptide (VIP), met-enkephalin (MENK), luteinizing hormone releasing hormone (LHRH), and cholecystokinin (CCK) were obtained from ImmunoNuclear Corp. Antisera to calcitonin gene related peptide (CGRP) was kindly provided by Dr. N. Brecha, FMRF by E. Weber and synapsin by C. Ouimet. Galanin (GAL) was obtained from Peninsula Labs. All antisera were diluted 1:1000.

The circumesophageal NS of Bulla is comprised of three bilaterally paired ganglia (cerebral:CG, pleural:PLG and pedal:PG) and one unpaired ganglion on the right side (pallial:PAG). VP-like immunoreactivity (VP-LI) is found in a few small cells in the CG and in some larger cells in the PG which also display LHRH-LI. LHRH fibers are found scattered throughout the CNS but are concentrated in the CG. The PAG contains cells that display LHRH-LI and CGRP-LI. In addition to that described above, CGRP-LI also occurs as fiber plexuses in the CG and PLG, and in cells of the PG that also display FMRF-LI. VIP-LI colocalizes with CGRP-LI in some cells of the PAG as well as being present in the neuropil of the CG and PAG. Synapsin containing cells, which also display FMRF-LI, are found in the CG. Some synapsin fibers are present in the neuropil of the CG and PAG. FMRF-LI is the most dense in the Bulla CNS, with cells and fibers found in all ganglia. Some cells in the PG approach 100um in diameter. MENK-LI consists of fibers in the CG and PG and cells in the PG that also contain CCK. CCK-LI is denser than most immunoreactivity, but less so than CGRP or FMRF, with reactive cells and fibers found in all ganglia. GAL-LI appears as a single cell in the CG that also displays CGRP-LI.

In this study we observed cells that contain two or more peptides. Many of these cells are large and identifiable, allowing the possible investigation of the physiology of differential release of colocalized neuroactive substances. In addition, similarities exist between the patterns of immunoreactivity in Bulla and that observed previously in the distantly related pond snail, Lymnaea stagnalis, suggesting that specific neuroanatomical and neurochemical patterns may have been conserved during gastropod evolution. Supported by NIH NS16304.

298.8 REGULATION OF ISOLATED BAG CELL NEURONS OF APLYSIA BY ALPHA-, BETA-, AND GAMMA-BAG CELL PEPTIDES. K.J. Loechner and L.K. Kaczmarek, Depts. of Pharmacology and Physiology, Yale University School of Medicine, New Haven, CT 06510.

Bag cell neurons of Aplysia are a homogeneous group of cells which control egg-laying behavior. Upon electrical stimulation or elevation of cAMP levels, these normally silent neurons generate a discharge during which several neuroactive bag cell peptides (BCPs) are released, including α-, β-, and γ-BCP. We have examined the effects of these peptides on cAMP levels in intact clusters of bag cells, as well as on the delayed voltage-dependent K^+-current (IK(v)) and on action potentials in isolated bag cell neurons.

β-BCP caused an elevation in cAMP levels in intact clusters of bag cell neurons (1uMβ-BCP: 58% increase, $p<.01$; 100uMβ-BCP: 95% increase, $p<.005$). The effect of β-BCP on IK(v) in isolated bag cell neurons was then studied using the whole cell patch-clamp technique. The intracellular solution contained EGTA to eliminate Ca^{++}-dependent K^+-currents. Current was measured during depolarizing steps from a holding potential of -40mv before and after application of peptide (≤10uM). β-BCP caused a decrease in IK(v) (mean decrease=33% ± 7%; n=10). The effect of β-BCP on action potentials in isolated bag cell neurons penetrated with microelectrodes was also examined. Action potentials were evoked with depolarizing pulses before and after application of peptide (≤10uM). β-BCP consistently increased spike amplitude. It is likely that these effects are mediated through the changes in cAMP levels, as similar effects on the delayed outward currents and spike amplitude have previously been described for cAMP analogs and for the adenylate cyclase activator, forskolin.

In contrast to β-BCP, γ-BCP applied to intact clusters resulted in a significant decrease in cAMP levels (1uMγ-BCP: 56% decrease, $p<.005$; 100uMγ-BCP: 49% decrease, $p<.01$). When applied to isolated neurons, γ-BCP (≤10uM) caused a small increase in IK(v) (mean increase= 14 ± 5%; n=6). γ-BCP, however, increased the amplitude of action potentials in the majority of isolated neurons impaled with microelectrodes, suggesting that additional conductances are influenced by this peptide.

Previous work has shown that α-BCP decreased basal and forskolin-stimulated cAMP levels. We have now shown that α-BCP (≤10uM) caused a small increase in IK(v) (mean increase= 16 ± 8%; n=3) in internally dialysed cells. In isolated neurons penetrated with a microelectrode, α-BCP either decreased or had no effect on action potential amplitude. Both of these inhibitory actions of α-BCP are consistent with a decrease in cAMP levels.

In conclusion, it is likely that the actions of these peptides on the neurons from which they are released play a role in setting the threshold and duration of discharges in the bag cell neurons.

298.9 **MYOMODULIN: A POSSIBLE COTRANSMITTER OF THE CHOLINERGIC NEURON L10 OF APLYSIA.** **A. Alevizos, K.R. Weiss, & I. Koester. Center for Neurobiology and Behavior and Departments of Psychiatry and Physiology, and NY State Psychiatric Institute, Columbia University, N.Y., N.Y. 10032**

The L10 neuron of Aplysia californica is a well studied cholinergic cell, having a variety of effects on several identified and non-identified cells of the abdominal ganglion. Kehoe (J. Physiol., 1972) has described some non-cholinergic effects of L10 on cells of the left upper quadrant neurons (cells L1 to L6), R15 and some unidentified cells of the abdominal ganglion. These effects cannot be blocked by acetylcholine blockers and cannot be mimicked by acetylcholine application. We investigated the possibility that some of these actions may be mediated by a peptide cotransmitter.

L10 neurons were labeled with ^{35}S methionine, and the chromatographic properties of radiolabeled peptides were compared to those of neuropeptides that are known to exist in the CNS of Aplysia. One peak of radioactivity coeluted with a recently sequenced Aplysia neuropeptide (Cropper et al, PNAS in press), myomodulin, through three steps of sequential reverse phase high pressure liquid chromatography in which 3 different counterions were used (TFA, HFBA and TEA acetate). This result provides strong evidence that L10 contains myomodulin, a candidate cotransmitter for the cholinergic neuron L10.

We studied the effects of the synthetic peptide on several of the L10 followers. Myomodulin, at concentrations as low as 10^{-7} M produces a long lasting inhibition of some of the RB cells. Similarly, the peptide produces hyperpolarization of some of the LUQ cells and an excitatory effect on unidentified cells of the right lower quadrant of the abdominal ganglion. In addition, bath application of myomodulin reduces the frequency of both spontaneous and evoked Interneuron II bursts by hyperpolarizing cells of the R25 cluster which mediate the bursts, and the R20 cells which modulate burst frequency (Koester, Neurosci. Absts, 1983).

Recently, it has been demonstrated that L10 innervates the renal pore of Aplysia (Koester et al, these abstracts). Thus L10 offers an advantageous model for studying the process of cotransmission both in the CNS and in the periphery.

298.10 CHEMICAL CHARACTERIZATION OF FACTORS RELEASED FROM APLYSIA BODY WALL BY TRAUMATIC STIMULATION. C.-Y. Lin*, J.K Krontiris-Litowitz, B.F. Cooper*, E.T. Walters, and D.J. McAdoo (SPON: W. Weems). Marine Biomed.Inst., U.Texas Med.Branch, Galveston, TX 30322, & Dept.Physiol. U.Texas Med.Sch. Houston, TX 77225.

Intense electrical or mechanical stimulation of isolated body wall of Aplysia releases factors that, if washed out of the stimulated body wall, cause persistent contraction when applied to body wall, cardioacceleration when applied to the heart, and suppression of defensive reflexes when applied to the CNS (Cooper et al., Soc. Neurosci. Abstr. 12:861, 1986; and Krontiris-Litowitz et al., this volume). It seems likely that some of the factors in stimulated body wall wash (SBW) are paracrine and endocrine signals involved in natural responses to traumatic stress. Contractile activity was measured with a strain gauge during perfusion of 0.1-0.5 ml samples through a cannula implanted between the skin and muscle layers of an isolated section of tail body wall. Fractionation of SBW by HPLC in a reverse phase column produced 3 major peaks of activity: one at the beginning of the chromatogram, one at the retention time of the gastropod neuropeptide SCP_B, and one 3 minutes after SCP_B. The first fraction contained a variety of amino acids, including glutamate (ca $5x10^{-6}$M), aspartate (ca 10^{-5}M), and taurine (ca $3x10^{-5}$M). Glutamate and taurine by themselves caused contractions with thresholds between 10^{-5} and 10^{-6}M, indicating that part of the activity in the first fraction is due to amino acids. The neurotransmitters ACh and 5-HT cause relaxation rather than contraction, and thus probably do not contribute to the activity. Dopamine causes strong contractions at concentrations of 10^{-6}-10^{-7}M, but HPLC analysis utilizing electrochemical detection demonstrated that less than $2x10^{-11}$M dopamine is present in SBW, ruling out dopamine as a mediator of SBW-induced contraction. The molluscan neuropeptide FMRFamide causes biphasic responses (brief contraction followed by prolonged relaxation), and thus may contribute to the early phase of SBW-induced contraction, but we have not yet tested for the presence of FMRFamide in SBW. SCP_B causes strong contractions with a threshold of about 10^{-8}M. Because SCP_B has the same retention time as one of the peaks of activity in SBW this peptide is likely to be one of the "trauma factors" released from body wall during noxious stimulation. Contractile activity of SBW has multiple sources, including amino acids, probably SCP_B, and other factors that we have yet to identify. We still need to determine which factors are responsible for an unusual property of SBW (one suggesting a role in wound closure) - its ability to produce maintained, non-desensitizing contractions during prolonged application.

298.11 EFFECTS OF 5,7-DIHYDROXYTRYPTAMINE ON SEROTONIN AND DOPAMINE CONTENTS OF APLYSIA NERVOUS SYSTEM. B. Jahan-Parwar, R.F. Seegal and D.O. Carpenter. Wadsworth Laboratories, NYS Department of Health and School of Public Health, University at Albany, Albany, NY 12201.

The neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) selectively labels serotonin-containing neurons in Aplysia without discernable long-term alteration of the neuronal physiology and total organismic behavior (Jahan-Parwar et al., Soc. Neurosci. Abst., 16:855,1986). This technique has opened avenues for the study of cellular and synaptic organization of the serotonin (5-HT) system and role of 5-HT transmission in mediation and modulation of behavior. As the first step in this study, we have analyzed the time course of 5,7-DHT effects on 5-HT and dopamine (DA) content using high performance liquid chromatography and electrochemical detection. Animals (n=15) were injected with 5,7-DHT creatinine sulfate (10mg/kg) in a 1 ml/kg vehicle solution containing 0.5 mg/ml L-ascorbic acid (as antioxidant) into the hemocoel, and the individual ganglia analyzed for 5-HT, DA and metabolites at days 1, 7 and 21, following injection. Controls (n=6) received vehicle only. The control ganglia contained 5-HT (ng/mg wet weight, mean $\pm$ SE): pedal ganglion, 5-HT = 2.17$\pm$.18, DA = 13.9$\pm$2.4; abdominal ganglion, 5-HT = 1.02$\pm$.09, DA = 7.5$\pm$.9; cerebral ganglion, 5-HT = .47$\pm$.03, DA = 3.57$\pm$.17; buccal ganglion, 5-HT = .41$\pm$.04, DA = 1.78$\pm$.2; pleural ganglion, 5-HT = .02$\pm$.02, DA = .29$\pm$.09. The 5-HT-content of individual ganglia was positively correlated with the number of 5-HT-containing neurons as revealed by the 5,7-DHT labeling and immunohistochemical methods. DA also had a similar distribution in the central ganglia. The 5-HT content of ganglia of 5,7-DHT treated animals was reduced to 90% in day 1, 50% in day 7, and recovered to 110% of the control levels in day 21 following treatment. There was no significant change in the DA content. The recovery of 5-HT content in Aplysia ganglia following treatment with 5,7-DHT contrasts to results obtained in mammalian serotonergic systems. However, the absence of active monoamine oxidase (MAO) in Aplysia (McCaman and Dewhurst, 1972), evidenced in our studies by an absence of MAO catalyzed biogenic amine metabolites (e.g. homovanillic acid or 5-hydroxyindoleacetic acid) in normal or 5,7-DHT treated ganglia, may provide a clue to the species differences in 5,7-DHT neurotoxicity. In mammalian preparations, both 5,6- and 5,7-DHT are readily oxidized by MAO to form reactive intermediates including 5,6 and 5,7-HTAA (Klemm and Baumgarten, 1978), 5,7 dihydroxyindoleacetaldehydes and free radicals. Furthermore, MAO inhibitors prevent the neurotoxic actions of 5,7-DHT. Thus the lack of 5,7-DHT induced 5-HT neurotoxicity in Aplysia may be due to the absence of MAO and hence the formation of reactive intermediate products. (Supported by NSF Grant BNS8313061 to B. J-P)

298.12 CORRELATION OF A SPECIFIC SEROTONIN-INDUCED MEMBRANE CURRENT WITH ^{125}I-LSD BINDING SITES ON IDENTIFIED APLYSIA NEURONS. M.L. Evans, M.J. Kadan, P.R. Hartig, and D.O. Carpenter. Wadsworth Laboratories, NYS Department of Health, School of Public Health, University at Albany, Albany, NY 12201 and School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD 21205.

The serotonergic ligand ^{125}I-LSD binds to sites in the Aplysia CNS which display regional distribution, pharmacological binding properties, and evidence of coupling to a G-protein consistent with labeling a class of functional serotonin (5-HT) receptors (Kadan and Hartig, Soc. Neurosci. Abstr. 11:994; Neurosci. in press, 1987). These receptors are located primarily within the neuropil, but also on a small subset of neuronal soma. We have identified a pair of upper quadrant neurons on the dorsal surface of the abdominal ganglion which are selectively and specifically labeled by ^{125}I-LSD. These neurons, based on their position, symmetry, and cytological properties, appear to be the functionally symmetrical neurons L1 and R1. In order to study the physiological role of the ^{125}I-LSD-labeled site on these neurons, we compared the electrophysiological and pharmacological properties of their 5-HT-evoked membrane ionic currents with those of neighboring neurons not labeled by ^{125}I-LSD.

Cells L1, L2, L3, L4, L6, and R1 were identified in desheathed ganglia by position, resting membrane potential, action potential firing pattern, synaptic input activity, and the effect of 50 μM acetylcholine (ACh). Voltage clamp currents in response to 50 μM 5-HT provided further criteria for identification. L3 and L6 respond to 5-HT with a slow outward current mediated by an increase in K^+ conductance. L2 and L4 respond to 5-HT with an early, rapidly desensitizating Na^+ conductance increase analogous to the A response described by Gerschenfeld and Paupardin-Tritsch (J. Physiol. 243:427, 1974) followed by a slow, non-desensitizing Ca^{2+} conductance of the type described by Pellmar (Fed. Proc. 40:2631, 1981). L1 and R1 have both components of the L2/L4 5-HT response but interposed between the two is a current due to an increase in Cl^- conductance. The serotonergic antagonists, cyproheptadine and mianserin, which inhibit ^{125}I-LSD binding in vitro, also inhibit the 5-HT evoked Na^+ and Cl^- currents, but have no effect on the K^+ and Ca^{2+} currents. The Na^+ current appears to result from a direct ionotropic effect of 5-HT on membrane conductance. The 5-HT Cl^- current of L1 and R1 has kinetics markedly slower than the rapid Cl^- current induced by ACh in these cells which may further implicate a second messenger involvement consistent with the in vivo evidence of G-protein coupling. We conclude that ^{125}I-LSD specifically labels a population of 5-HT receptors that gate a Cl^- conductance. (Supported by NIH Grant 18435)

298.13 DISTRIBUTION OF THREE MODULATORY TRANSMITTERS WITHIN THE PLEURAL GANGLION OF APLYSIA. L.-T. Lo, J.H. Byrne and L.J. Cleary. Department of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

Activation of neural circuits that modulate the tail withdrawal reflex can alter membrane conductances in sensory neurons in the pleural ganglion. Transmitters, including serotonin (5-HT), FMRFamide and small cardioactive peptide b (SCPb), produce changes similar to those produced by the modulatory circuits. For example, 5-HT and SCPb decrease outward currents, whereas FMRFamide increases them. We used immunoflourescence techniques to examine the distribution of these transmitters within the cluster of pleural sensory neurons.

The somata of most sensory neurons are enveloped by fibers with prominent varicosities that contain 5-HT-like immunoreactivity (IR). Immunopositive fibers are more abundant near somata that lie close to the neuropil, but a particularly dense plexus of 5-HT-like IR surrounds a few isolated somata. These fibers are extrinsic to the pleural ganglion, for we found no serotonergic cell bodies there.

FMRFamide-like IR is distributed within the cluster of sensory neurons in a pattern similar to that of 5-HT, although there are fewer processes. FMRFamide-like IR is also located close to the neuropil and densely envelops a few selected sensory neurons. On the other hand, some FMRFamide-like IR is intrinsic to the ganglion, for many somata within the ganglion are immunopositive. Among these are several smaller cells adjacent to the cluster of sensory neurons. We have found interneurons in this region that hyperpolarize sensory neurons.

SCPb has pharmacological effects on sensory neurons similar to those of 5-HT, but has a different distribution within the pleural ganglion. No SCPb-like IR is found around any cell bodies, although it does penetrate diffusely through the neuropil. Only one or two somata within the pleural ganglion contain SCPb-like IR.

The proximity of varicosities containing 5-HT- and FMRFamide-like IR to somata of sensory neurons suggests that these transmitters have multiple functions. In addition to short-term effects on membrane conductances, they may also have long-term effects on metabolic processes that are confined to the somata. For example, 5-HT is capable of inducing persistent cellular changes requiring protein synthesis (Montarolo et al, 1986). The lack of axosomatic contacts by fibers containing SCPb does not rule out a role for this transmitter, however, since the fibers may contact sensory neurons within the neuropil.

PEPTIDES: BIOSYNTHESIS, METABOLISM AND BIOCHEMICAL CHARACTERIZATION II

299.1 **Cholecystokinin Attenuates Dopamine-Mediated Glucose Utilization Changes in Selected Brain Areas After Nucleus Accumbens Injection**

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Several lines of evidence now indicate that cholecystokinin octapeptide (CCK-8) coexists with dopamine (DA) in some DA-containing mesencephalic neurons projecting to the nucleus accumbens (NA), amygdala, olfactory tubercles, and frontal cortex. Moreover, biochemical and electrophysiologic studies suggest interactions between CCK-8 and DA in these terminal areas. While the behavioral actions of DA system activation are well described, consequences of CCK-8 system perturbation are less firmly established. But, it has been shown that CCK-8, microinfused into the NA where CCK/DA neurons terminate, potentiates DA-induced hyperlocomotion and apomorphine-induced steretypy (Crawley, J Neurosci 5: 1972, 1985). The experiments reported here explore the neural pathway mediating the DA effect on locomotor behavior and the nature of the CCK-8 potentiation of this effect. To study this, we injected dopamine (20 g in 0.2 l), CCK-8 (10 ng in 0.2 l), dopamine + CCK-8 (above doses), or saline (0.2 l) bilaterally into the caudal medial NA in 20 male albino rats with chronically implanted guide canulae. Glucose utilization was measured in 54 different brain areas using quantitative 2-deoxyglucose (2DG) autoradiography, as previously described (Tamminga, Eur J Pharm, in Press). Results are expressed in moles glucose/100 gm brain tissue/min. Statistical analysis was done with analysis of variance and student's t test and Duncan test; results of principle component analysis will be reported.

Results show no effect of CCK-8 alone on local cerebral glucose utilization (LCGU) in the brain areas analyzed. Dopamine alone decreased LCGU in several areas related to motor function and sensory processing, including the superior olive, periaquaductal grey, medial geniculate, and auditory cortex; moreover, in all areas where suggestive changes occurred, the LCGU was reduced by DA alone. CCK-8 attenuated the effects of DA on LCGU changes in several brain areas, including the caudate nucleus, globus pallidus, subthalamic nucleus, lateral preoptic area, and the superficial and deep layers of superior colliculus. Multivariate analytic techniques may indicate key interactions between different motor areas responsible for behavioral potentiation.

299.2 LITHIUM AND PHORBOL ESTERS INCREASE RELEASE OF CHOLECYSTOKININ FROM BRAIN SLICES INCUBATED IN VITRO. L.R. Allard*, K. Gysling*, and M.C. Beinfeld* (SPON: N.A. Connors). Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

Previous studies in our laboratory have focused on identifying factors which regulate cholecystokinin (CCK) release from slices of rat caudato-putamen (cp) incubated in vitro, with CCK release elicited by potassium stimulation. Several lines of evidence suggested that the release is under predominantly negative control by an unknown substance (which we call "X") which is released along with CCK by a calcium-dependent mechanism. Further studies (described in adjacent poster) provide evidence that excitatory amino acids are potent inhibitors of CCK release. Studies described here were performed to determine whether the products of inositol phospholipid turnover might mediate the inhibitory action of substance X.

Lithium is known to interrupt the phosphoinositol cycle by inhibiting myo-inositol-1-phosphatase. Phorbol esters mimic the action of diacylglycerol (a product of inositol phospholipid turnover) and activate protein kinase C directly. Phorbols also have a negative "feedback" action which uncouples or desensitizes agonist receptors and can block inositol phospholipid turnover in the presence of agonists (Leeb-Lundberg et al., Proc. Natl. Acad. Sci. USA 82:5651, 1985).

Incubation of slices of cp and frontal cortex with lithium (10 mM) for 40 minutes prior to potassium stimulation elevated CCK release by about a factor of 2 relative to controls. Incubation of slices of cp, frontal cortex, and hippocampus with the active phorbols phorbol 12-13-dibutyrate and phorbol 12-myristate-13 acetate at 10^{-7} or 10^{-6}M for 15 minutes induced a 2 to 3 fold elevation of CCK release in the presence of either 40 or 60 mM potassium. The inactive 4 α-phorbol did not alter CCK release. The stimulatory effect of these compounds required preincubation.

The enhancement of CCK release by an agent which inhibits inositol phospholipid turnover (lithium) and by an agent which mimics the action of diacylglycerol (phorbols) can be best explained by proposing that the effect of phorbols on CCK release is related to their ability to inhibit inositol phospholipid turnover. These results suggest that the release of CCK in some brain regions is under inhibitory control by a substance or substances which use inositol phospholipid turnover as its mediator. The identity of these putative endogenous inhibitory substance(s) and their relationship to phosphoinositol turnover is still under investigation.

This work supported in part by NIH grant NS18667 and RR05388 (to St. Louis University).

299.3 CHOLECYSTOKININ RELEASE FROM SLICES OF RAT CAUDATO-PUTAMEN INDUCED BY POTASSIUM IS INHIBITED BY EXCITATORY AMINO ACIDS. J.M. Stone*, L.R. Allard*, and M.C. Beinfeld* (SPON: M.H. Cooper). Department of Pharmacology, St. Louis Univ. Med. Sch., St. Louis, MO 63104.

Previously this laboratory has shown that the release of cholecystokinin (CCK) from rat caudato-putamen (cp) slices is under strong inhibitory control by a substance (or substances) which we call "X" released from cp and cerebral cortical slices by potassium in a Ca^{++}-dependent manner. Further studies on the chemical nature of "X" have indicated that it is likely to be a small, polar substance not degradable by trypsin.

Cp is known to contain high levels of both excitatory and inhibitory amino acids. The possibility that "X" is an amino acid was investigated. The inhibitory amino acids, glycine (10^{-4} and 10^{-5}M) and GABA (10^{-4} and 10^{-5}M), did not alter CCK release, nor did the GABA agonist, muscimol (10^{-6}M) or the GABA antagonist, picrotoxin (10^{-4}M). However, at concentrations of 10^{-4}M the excitatory amino acids, glutamate and aspartate, and the excitatory dipeptide, N-acetyl-aspartyl-glutamate (NAAG), produced highly significant ($p < .001$) inhibition of CCK release. Percent inhibition of release of CCK as compared to controls ranged from 30% for aspartate, 41% for glutamate and 47% for NAAG. At a concentration of 10^{-5}M aspartate no longer produced a statistically significant inhibition while glutamate and NAAG still produced inhibitions ($p < .02$) of 22% and 16% respectively. The following excitatory amino acid analogs which have been used to define amino acid receptor subtypes had no effect on CCK release at 10^{-4}M: N-methyl-D-aspartic acid (NMDA), quisqualic acid, kainic acid, or ibotenic acid. Glutamate at 10^{-4}M did not inhibit CCK release from cortex or hippocampus.

Recently we have found that D-L-2-amino-4-phosphobutyric acid (APB) at $2x10^{-3}$M reverses the inhibition seen with glutamate at 10^{-4}M, while APB alone had no effect on CCK release. The inhibition of CCK release by these excitatory amino acid analogs does not appear to be mediated by NMDA, quisquate, or kainate-preferring receptors but may be mediated by an APB-sensitive presynaptic side.

The possibility that "X" may be an excitatory amino acid is still under investigation. The observation that glutamate increases phosphoinositol turnover in culture striatal neurons (Slaceczek, et al., Nature 317:717, 1985) and our observation (see adjacent poster) that lithium treatment increases CCK release is consistent with "X" being glutamate. These results suggest a possible mechanism whereby one cortico-striatal excitatory neurotransmitter (glutamate or aspartate) can regulate the activity of another excitatory neurotransmitter (CCK) by modulating its release.

This work supported in part by NIH grants NS18667 (to M.C.B.) and RR05388 (to St. Louis Univ.).

299.4 NUCLEUS BASALIS MAGNOCELLULARIS (nBM) LESIONS INDUCE ABNORMAL CORTICAL LEVELS OF SOMATOSTATIN, NEUROPEPTIDE Y (NPY), CORTICOTROPIN-RELEASING HORMONE (CRH), AND GLUTAMINE.
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Senile Dementia of the Alzheimer's type (SDAT) is characterized by marked cholinergic hypofunction in the cerebral cortex, apparently due to loss or dysfunction of cholinergic neurons arising from the nucleus basalis of Meynert (NBM). However, other cortical transmitter systems have also been shown to be affected in SDAT. In this regard, reductions in cortical levels of somatostatin, NPY, and CRH have recently been documented, suggesting involvement of these neuropeptides in the disease process. We have previously found that bilateral lesions of the rat nucleus basalis magnocellular (nBM), which mimic the spontaneous destruction of the NBM-to-cortex cholinergic pathway in SDAT, induce a profound elevation in cortical levels of somatostatin at 10 months after lesioning. The present study investigated the effects of both unilateral and bilateral excitotoxic lesions of the nBM on cortical levels of somatostatin, NPY, CRH, and neuroactive amino acids at several time points.

Adult male Sprague-Dawley rats were given unilateral or bilateral infusions of ibotenic acid (5 ug/1 ul) into the nBM and sacrificed 2, 10, 14, or 20 months later. At both 10 and 14 months following lesions, significant reductions in all presynaptic cholinergic markers were seen in the frontal cortex compared to sham-lesioned controls. In these same lesioned animals, parietal cortex levels of somatostatin, NPY, and CRH were markedly elevated (by 2-3 fold) compared to controls. At 2 months after unilateral nBM lesioning, however, a significant cortical cholinergic hypofunction on the lesion side was associated with reduced cortical levels of somatostatin and NPY, but no effect on cortical CRH, compared to the unlesioned side. Furthermore, the amino acid glutamine (but not aspertate or glutamate) was reduced in frontal cortex and entorhinal cortex at 20 months after bilateral nBM lesioning; a marked cortical cholinergic hypofunction was present at this time point as well.

These results suggest that, in SDAT, a dysfunction of the cortical neuropeptide transmitters somatostatin, NPY, and CRH may be related to a loss or dysfunction of cholinergic innervation to the cortex from the NBM region. The opposite effects of unilateral and bilateral nBM lesions on cortical neuropeptide levels may be related to a lesion-induced loss or dysfunction of corticocortical connections. Several lines of evidence suggest that a breakdown of such corticocortical connections occurs in SDAT.

299.5 **EFFECT OF CYSTEAMINE ADMINISTRATION ON THE IN VIVO BIOSYNTHESIS OF SOMATOSTATIN, OXYTOCIN AND VASOPRESSIN IN RAT HYPOTHALAMUS.** R.P.S. Kwok*, J.L. Cameron, and J.D. Fernstrom. Departments of Psychiatry and Behavioral Neuroscience and the Center for Neuroscience, University of Pittsburgh, Pittsburgh PA 15213.

Cysteamine (CSH) injection causes a rapid (1-4 hr), longlasting (>72 hr) depletion of immunoreactive (IR) somatostatin (SRIF) levels in the rat hypothalamus. IR-Vasopressin (AVP) and IR-oxytocin (OXT) levels are unaffected. We have previously shown that (^{35}S)cysteine incorporation into somatostatin-14 (SRIF-14) and somatostatin-28 (SRIF-28) also falls within 1-4 hr of CSH injection, but is normal 1 wk later (Cameron & Fernstrom, Endocrinology 119:1292,1986). Such results suggest that CSH injection inhibits SRIF biosynthesis, but do not give a complete picture of the role of synthesis inhibition in the prolonged depression of hypothalamic IR-SRIF levels. To gain further insight into the mechanisms by which CSH depletes hypothalamic SRIF, we have performed a more thorough temporal analysis of CSH's effects on the in vivo synthesis and IR levels of SRIF (and AVP and OXT) in the rat hypothalamus.

Male rats received CSH (300 mg/kg, sc) 1,4,6,8,10,12, & 24 hr before the injection of 50 microCi (^{35}S)cysteine into the third ventricle under urethane anesthesia. Four hr later, hypothalami were removed, pooled into groups of 3-4, and immediately homogenized and prepared for HPLC to separate labeled SRIF-14, SRIF-28, OXT and AVP. Other rats received CSH, but not label, and were killed at intervals ranging from ½ to 72 hr later for measurements of IR-SRIF, IR-AVP and IR-OXT in hypothalamus and posterior pituitary.

As previously observed, hypothalamic IR-SRIF levels fell within 2-4 hr to values 50-60% below normal, and remained low for 72 hr (the duration of the IR studies). In contrast, hypothalamic and posterior pituitary IR-AVP and IR-OXT levels remained normal throughout. Label incorporation into HPLC-isolated SRIF-14, SRIF-28 and OXT in hypothalamus was negligible 1 hr after CSH injection, remained low at 4 hr, but was essentially normal 8-10 hr post-CSH administration. In the same animals, label incorporation into AVP was stimulated by CSH injection (a 7-fold increase was noted at 4 hr), but returned to control levels by 8 hr.

The data suggest that SRIF synthesis returns to normal much more rapidly than total endogenous stores. Perhaps the rapid restoration of SRIF synthesis contributes to the longer-term replenishment of peptide stores, though other mechanisms may also (or instead) be responsible. The chemical mechanism(s) by which CSH alters the synthesis rates of these peptides continues to be elusive. However, because AVP labeling is stimulated by CSH injection, it seems unlikely that the drug produces it effects simply by a sulfhydryl-mediated chemical reaction that eliminates all cysteine-containing peptides from hypothalamus.

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299.6 CHARACTERIZATION OF HYPOTHALAMIC PEPTIDES IN OVERFED AND AD LIBITUM FED RATS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. M. G. Hulsey* and R. J. Martin. Department of Foods and Nutrition, University of Georgia, Athens, Georgia 30602

Sulfated cholecystokinin octapeptide, cholecystokinin tetrapeptide, growth hormone releasing hormone, insulin-like growth factor, somatostatin-14, thyrotropin releasing hormone, and ß-endorphin are thought to be involved in the regulation of food intake. In this study, we attempted to determine if overfeeding would produce changes in the endogenous hypothalamic levels of these (or other) peptides that would be detectable by HPLC.

Nine pairs of Sprague-Dawley rats were housed under a 12 h photoperiod. Initial body weights were 267 ± 2 g and 266 ± 3 g for the control and overfed rats, respectively. A powdered diet was prepared containing (by weight) 60% dextrose, 20% casein, 10% corn oil, 3% cellulose, and 7% AIN vitamin and mineral mix. For the overfed animals, a liquid diet was prepared by mixing four parts of the powdered diet with three parts tap water. Control rats were provided powdered diet ad libitum, and consumed an average of 15 g $daily^{-1}$ Overfed rats were tube-fed meals at 0530, 1330 and 2130 hours daily. The overfed rats were given meals increasing from 5 g to 12.9 g (powder equivalent) over a period of 8 days, and 12.9 g thereafter. After an additional 24 days, body weights were 290 ± 5 g and 501 ± 8 g for the control and overfed rats, respectively.

Animals were sacrificed by decapitation. Hypothalamic sections were immediately dissected on ice, weighed, and frozen on dry ice. Pooled tissues from overfed and ad libitum fed rats were homogenized and extracted with a reverse phase cartridge according to Bennett et al. (Biochem. J. 175:1139-1141). Extracts were chromatographed on a 4.6 mm by 125 mm 5 micron C_{18} column (Whatman Partisphere) using He_2 sparged H_3PO_4 at pH 2.1 with a linear gradient of 0 to 70% (v/v) Acetonitrile over 180 minutes. Detection was by absorbance at 212 nanometers. We obtained several unidentified peaks that had substantially different areas in overfed and control extracts. These peaks of interest do not co-elute with any of the above mentioned peptides. Work in progress will attempt to identify the unknown peaks in extracts from overfed and ad libitum fed rats.

299.7 PARTIAL PURIFICATION OF PEPTIDES WHICH CAUSE CONTRACTION OF APLYSIA MUSCLES. S.L. Knock and D.J. McAdoo. Marine Biomedical Institute and Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas 77550.

The left efferent vein of the marine gastropod, Aplysia, provides an ideal site from which neurons might release messengers into the circulatory system for actions on distant targets. It has been shown that neurons in the abdominal ganglion synthesize and transport peptides from the ganglion down the branchial nerve and store these peptides in terminals within the efferent vein. Substances have been partially purified from the efferent vein and have been tested for their ability to cause contractions of the gastroesophageal artery. These substances are susceptible to proteases, which indicates that they are peptides. Peptides were extracted in acetic acid/acetone and precipitated using excess cold acetone. Precipitates were dissolved in acetic acid/HCl buffer and chromatographed on a Sephadex G50 column. The active peptides eluted with retention volumes that correspond to molecular weights between 2000 and 5500 kilodaltons. The peptides were then separated using reversed phase high performance liquid chromatography. Separations were carried out on both C4 and C8 columns and peptides were eluted using a gradient of 0.1% trifluoroacetic acid (TFA) with acetonitrile as an organic modifier. Two distinct active peptide fractions can be separated by these means, eluting at 19% and 21.5% acetonitrile on the C4 column and 23.5% and 26% on the C8 column. Both peptide fractions cause a series of rhythmic contractions on top of a tonic contraction of the gastroesophageal artery and cause contractions of body wall muscle. These peptide fractions have been shown to elute at distinctly different retentions times from known molluscan cardioactive peptides, Small Cardioactive Peptide b (SCPb) and FMRFamide. SCPa co-elutes with the first active peptide fraction, but does not cause contractions of the gastroesophageal artery.

299.8 COMPARISON OF [^{125}I]AIII AND [^{125}I]AII BINDING TO RAT BRAIN MEMBRANES. R.H. Abhold, J.M. Hanesworth* and J.W. Harding. Dept. Vet. Comp. Anat. Pharmacol. Physiol., Washington State University, Pullman, WA 99164-6520.

Intracerebroventricularly (ICV) applied angiotensin III (AIII) has often been shown to be much less potent than angiotensin II (AII) in stimulating CNS-mediated pressor and dipsogenic responses. In contrast, AII and AIII appear to be equipotent in their ability to competitively displace [^{125}I]AII from CNS binding sites. Conclusions based on competitive displacement studies may be misleading, however, since they do not address the possibility of separate AII and AIII binding sites or activities.

In this study, the binding of [^{125}I]AIII to rat brain membranes was examined and compared to that of [^{125}I]AII. Degradation of each ligand, as monitored by HPLC, was effectively inhibited using fragments of AII and AIII known to have little affinity for angiotensin binding sites. Three classes of [^{125}I]AIII binding sites were observed based on affinity (K_d = 0.13, 1.83, and 10.16 nM) and capacity (B_{max} = 1.30, 18.41, and 67.2 fmol/mg protein, respectively). Two classes of [^{125}I]AII-binding sites of high affinity (K_d = 0.11 and 1.76 nM) and low capacity (B_{max} = 1.03 and 18.86 fmol/mg protein, respectively) were also identified. Cross-displacement studies confirmed that the two highest affinity [^{125}I]AIII-binding sites and the [^{125}I]AII-binding sites were the same. On the other hand, the binding of [^{125}I]AIII to the low affinity [^{125}I]AIII-binding site could not be inhibited with AII. These data imply that previously measured differences in the biological potency of ICV-applied AII and AIII probably do not result from differential binding of these peptides to central angiotensin receptors. It is possible, however, that such differences may result from differential metabolism by tissue angiotensinases.

299.9 SIMILARITIES IN SOLUBILIZED BRAIN AND ADRENAL ANGIOTENSIN RECEPTORS. I. Rogulja and J.W. Harding. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Previous studies from our laboratory comparing the binding properties of ^{125}I-angiotensin II (AII) and ^{125}I Sar^1,Ile^8-AII (SI-AII) in the rat brain and bovine adrenal cortex consistently indicate a 3-5 times higher number of binding sites for SI-AII as compared with AII. To more rigorously examine the binding properties of these ligands, we chose to use solubilized membrane preparations. ^{125}I-AII, ^{125}I- angiotensin III (AIII) and ^{125}I-SI-AII were bound to angiotensin binding sites from bovine adrenals and/or rat brain solubilized with CHAPS, and chromatographed on a TSK 3000 SW size exclusion column. Multiple binding peaks were detected for each radioligand with similar profiles in brain and adrenals. The elution pattern of bound ^{125}I-SI-AII in both bovine adrenal and rat brain was dramatically different from the patterns seen with ^{125}I-AII and ^{125}I-AIII. Specific ^{125}I-SI-AII binding was detected as 3-5 peaks while ^{125}I-AII and ^{125}I-AIII were found as 2-3 peaks. At least one peak in the adrenals appeared to be common to all these ligands, with selective peaks observable for SI-AII and AIII. Many of the individual peaks could be defined as "specific" based on the ability of cold ligand to displace binding. These results demonstrate that ^{125}I-SI-AII specifically binds to multiple proteins (or different conformations of the same protein) while AII and AIII exhibit binding to a more limited number of sites. The data encourage speculation that the additional binding sites for SI-AII may represent specific aminopeptidases which cannot be visualized with the more labile forms of angiotensin.

299.10 CHARACTERIZATION OF FMRF-NH_2 LIKE MATERIAL IN RAT SPINAL CORD USING ANTIBODIES TO THREE RELATED PEPTIDES IN RADIOIMMUNOASSAY AND HPLC. E.A. Majane and H.-Y.T. Yang. Laboratory of Preclinical Pharmacology, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Two neuropeptides, AGEGLSSPFTSLAAPQRF-NH_2 (A-18-F-NH_2) and FLFQPQRF-NH_2 (F-8-F-NH_2) which were originally detected by FMRF-NH_2 antisera have been isolated from bovine brain, characterized and synthesized. These two peptides can decrease rat tail flick latencies; F-8-F-NH_2 can also attenuate morphine induced analgesia. Using highly sensitive radioimmunoassays (RIAs) developed for F-8-F-NH_2 and A-18-F-NH_2, it has been shown that the highest concentration of these peptides in bovine CNS is found in dorsal spinal cord. A high concentration of F-8-F-NH_2 and A-18-F-NH_2 immunoreactivity (IR) (0.43 and 0.24 pmol/mg protein, respectively) is also found in rat spinal cord. However, HPLC analysis reveals that the rat peptides elute with higher retention times suggesting that they are not identical to the bovine A-18-F-NH_2 and F-8-F-NH_2. Some immunohistochemical studies have shown that FMRF-NH_2-IR and NPY-IR are found in the same neurons in rat CNS suggesting that mammalian FMRF-NH_2-like (LI) and NPY-LI are chemically identical or they are co-localized. In order to clarify this point, we have fractionated rat spinal cord extracts by reverse phase HPLC and radioimmunoassayed each fraction with antisera against the following peptides: FMRF-NH_2, A-18-F-NH_2, F-8-F-NH_2 and NPY (#1 and #2 with differing specificities). Analysis of these fractions with F-8-F-NH_2 antisera detects one major immunoreactive peak (F-8-F-NH_2-LI) eluting with a higher retention time than F-8-F-NH_2 while RIA analysis with A-18-F-NH_2 antisera detects two major peaks. The first peak elutes in the postion of F-8-F-NH_2-LI. Our specificity study indicates that A-18-F-NH_2 antisera cross-reacts considerably with F-8-F-NH_2 thus the first peak is identical to the F-8-F-NH_2-LI. The second peak detected by A-18-F-NH_2 antisera is poorly detected by F-8-F-NH_2 antisera suggesting that this represents rat A-18-F-NH_2-LI. Analysis with FMRF-NH_2 antisera detects at least three immunoreactive peaks; two of these have a much higher affinity for F-8-F-NH_2 and A-18-F-NH_2 antisera. NPY antisera #1 (not C-teriminal directed) detects one main immunoreactive peak eluting in the position of human (rat) NPY, while the C-terminal directed NPY antisera (#2) reveals an additional major cross-reactivity which elutes in the position of A-18-F-NH_2-LI. In conclusion, in rat CNS there are F-8-F-NH_2-LI and A-18-F-NH_2-LI peptides which are distinct from NPY. It is possible that these peptides (FMRF-NH_2-LI) may be detected as NPY-IR immunohistochemically by some NPY antisera.

299.11 THE DISTRIBUTION OF FMRFamide-RELATED PEPTIDES IN THE GASTROPODS. D.A. Price, K.E. Doble*, T.D. Lee*# and M.J. Greenberg. Whitney Lab, Rt. 1, Box 121, St. Augustine, FL 32086. #Div. of Immunol., Beckman Res. Inst. of the City of Hope, 1450 E. Duarte Rd., Duarte, CA 91010.

We have used HPLC, radioimmunoassay and FAB-mass spectroscopy to determine the FMRFamide-like peptides in whole animal extracts of several gastropod species. For two represenative species, Helisoma and Helix, we have compared whole animal extracts to ganglionic extracts, and found no differneces in the peptides present. So we hypothesize that there are no exclusively non-neural members of the FMRFamide family present in molluscs. The number of species we have examined is still small, but a few tentative generalizations about the distribution of these peptides can be made. The tetrapeptide FMRFamide is the predominant representative of its family in most molluscs, but related peptides also occur in some species, especially among the gastropods. First, the tetrapeptide FLRFamide seems to be a ubiquitous minor component, but its ratio to FMRFamide is not constant from species to species. For example, a 1:28 ratio is predicted for the opisthobranch Aplysia based on its FMRFamide precursor, whereas the prosobranch Pomacea has a 1:6 ratio. The shelled opisthobranch, Bulla umbilicata, has a ratio like that of Aplysia, so a low level of FLRFamide may be a general opisthobranch characteristic. The pulmonates have FLRFamide levels more in line with the rest of the phylum, but they contain significant quantities of heptapeptides of the form XDPFLRFamide [where X is Gly (G), Ser (S), pGlu (pQ) or Asn (N)] which are not present in the non-pulmonate members of the phylum. Our earlier work on Helix, Lymnaea and Siphonaria had led us to hypothesize that each pulmonate species has two different heptapeptides, but we now have found that Helix has three different heptapeptides: the pQ, G and S analogs while Helisoma has only the G analog. We had also found that the pQ analog only occurs in stylommatophoran species and the G analog only in basommatophoran ones and had suggested that these were charactersitics of the two suborders. This generalization remains intact.

299.12 **INCREASED THYROTROPIN RELEASING HORMONE [TRH] CATABOLISM IN HUMAN SPINAL CORD OF AMYOTROPHIC LATERAL SCLEROSIS [ALS] PATIENTS IS NOT CAUSED BY INCREASED SPINAL CORD MANGANESE CONTENT**

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TRH content in the cervical spinal cord of some ALS patients is decreased [Am J Med Sci 287: 34, 1984; Neurology 36(suppl 1): 139, 1986; Neurology 36: 1218, 1986]. A possible TRH catabolite, histidyl-proline diketopiperazine [HP-DKP], may be increased in the spinal cord of ALS patients [Neurology 36: 1218, 1986]. We have studied by HPLC the catabolism of [^{3}H-pro]-TRH in homogenates of cerebral cortex [CTX], cerebellar cortex [CRB] and cervical, thoracic, and/or lumbar spinal cord [SC]. Autopsy samples were obtained 2-48 hours post mortem in 11 ALS patients and 13 control patients with multiple sclerosis, Huntington's disease, and stroke. TRH degradation is significantly slower in SC [11 $\pm$ 12 fmole/min/mg protein] than in CTX [84 $\pm$ 18] or CRB [85 $\pm$ 14] in 100 mM KPO4 buffer without dithiothreitol [DTT]. Addition of DTT [2 mM] increases only SC TRH catabolism significantly [41 $\pm$ 14 ; $p < 0.05$] with increases of HP-DKP [184 $\pm$ 79 to 876 $\pm$ 193] and HP [22 $\pm$ 34 to 134 $\pm$ 55]. DTT induced TRH catabolism is increased in ALS SC compared with control SC [56 $\pm$ 20; $p < 0.04$]. The proline formed in ALS SC is significantly increased [200 $\pm$ 53 to 321 $\pm$ 58; $p < 0.05$]. Leupeptin, a thiol protease inhibitor, does not inhibit TRH metabolism in control or ALS SC. Manganese is elevated 2X in ALS SC [J Neurol Sci 61: 283, 1983; J Neurol Neurosurg Psychiat 49: 211, 1986]. Addition of Mn to control SC homogenates at 2X and 10X did not lead to increased TRH catabolism. Therefore, [1] SC catabolism of TRH is specifically increased in ALS patients, [2] increased SC catabolism of TRH is not due to a thiol activated protease, [3] in vitro addition of manganese, which is increased in ALS SC and is an activator of some aminopeptidases, does not cause increased TRH catabolism in control SC homogenates.

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299.13 ULTRASTRUCTURAL EVIDENCE SUGGESTS VARIATIONS IN BIOSYNTHESIS AND PROCESSING WITHIN LHRH NEURONS AS A FUNCTION OF OVARIECTOMY. J. C. King and G. R. Seiler*. Dept. of Anatomy and Cellular Biology, Tufts Univ. Sch. of Med., Boston MA 02111.

We have reported previously changes in populations of LHRH-immunopositive cell bodies and neurovascular terminals following gonadectomy of rats. The purpose of this study was to investigate the subcellular localization of LHRH within individual perikarya from these populations in ovariectomized female rats. This investigation was directed specifically toward the study of those organelles involved in protein synthesis and processing. The goal was to determine ultrastructural changes in these organelles that might reflect changes in levels of biosynthetic activity. Regions within 50 micrometer Vibratome sections of preoptic-hypothalamus containing LHRH-immunopositive neuronal perikarya [previously used to reconstruct three-dimensional populations, Soc. for Neurosci. 1985 Abs. # 47.1] were removed, mounted on resin cylinders, thin-sectioned and viewed with a JEOL 100 B or Philips CM-10. Electron microscopic studies were restricted to LHRH cells in a mid-line region surrounding the OVLT in the rostral preoptic area. Immunopositive cells identified in toluidine blue stained semi-thin sections were then completely thin-sectioned. One day following ovariectomy, which was performed on metestrus, numerous free polysomes filled the cytoplasm, whereas little rough endoplasmic reticulum [RER] was evident. Six days post-ovariectomy cisternae of RER were abundant and dilated; in close proximity to the RER, were Golgi apparati, which were both numerous and extensive within the cytoplasm. In addition, reaction product was heavily concentrated in the medial saccules or dilated regions of the Golgi complex. By three weeks post-ovariectomy the Golgi lamellae were extensively dilated and the associated vesicles were great in number. These ultrastructural features are consistent with increased synthesis and secretion of LHRH following ovariectomy with increased synthesis of message one day post-ovariectomy, increased processing and secretion six days post-ovariectomy, and reaching a new increased steady state level of biosynthesis and secretion, three weeks after ovariectomy. Few synapses were detected directly contacting LHRH perikarya in these conditions, while synaptic profiles in the surrounding neuropil varied with a suggestion of greater activity one day post-ovariectomy. The synaptic arrangements were often complex with two or more axons contacting a single dendritic profile. These data suggest that the removal of gonadal steroids results in greater biosynthetic activity in LHRH neurons, which may be related to increases in afferent activity in the preoptic area. This work was supported by NSF grant DCB-8702388.

300.1 MK-801 PREVENTS THALAMIC DAMAGE INDUCED BY FOCAL CORTICAL SEIZURES. D.B. Clifford, A. Benz*, J.W. Olney and C.F. Zorumski. Washington University School of Medicine, St. Louis, MO 63110.

Prolonged seizure activity induced by various methods results in brain damage which resembles the excitotoxic type of cytopathology glutamate (Glu) is known to cause. Recently we demonstrated that phencyclidine (PCP) and ketamine, which are non-competitive antagonists of the N-methyl-D-aspartate (NMDA) subtype of Glu receptor, protect against brain damage induced by kainic acid seizures. Here we have explored the ability of MK-801 to protect against thalamic damage induced by sustained seizure activity in the glutamergic corticothalamic tract. MK-801 is an orally active agent that resembles PCP and ketamine in receptor binding and NMDA antagonist properties and is the most powerful anti-excitotoxin known (Price et al, this meeting).

Focal cortical seizures were induced in Sprague Dawley rats by instilling a solution of bicuculline methiodide (2 mM) or pilocarpine (200mM) in an epidural well over the right sensorimotor cortex as previously described (Science 218:177, 1982). Seizure activity was recorded from surface cortical leads. Three hours after induction of seizures the rats were sacrificed by perfusion with aldehyde fixatives and their brains examined for histopathological changes. Experimental rats received MK-801 (1 mg/kg ip) 30 minutes prior to the convulsant; controls received only the convulsant. The convulsant solutions rapidly induced focal status epilepticus in both control and experimental rats which persisted for three hours. Glu-like excitotoxic lesions were consistently present in the thalamus of control rats and consistently absent in MK-801 treated rats. Electrographic seizure activity was recordable from both the cortex and thalamus throughout the 3 hour observation period in both control and experimental rats but it was moderately attenuated in the latter.

We conclude that MK-801 has substantial protective properties against seizure induced brain damage at doses which do not eliminate electrographic seizures. The protection is independent of the chemoconvulsant used to drive focal cortical seizures and presumably is mediated at NMDA receptors where MK-801 is a powerful antagonist. Our findings are consistent with the hypothesis that NMDA receptors are important in the generation of seizure induced brain damage.

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300.2 ^{3}H-GLYCOGEN HYDROLYSIS IN THE CEREBRAL CORTEX OF TWO SPONTANEOUSLY EPILEPTIC MOUSE MUTANTS : NORADRENERGIC SUBSENSITIVITY IN THE *TOTTERING* MOUSE AND AGE-DEPENDENT SUPERSENSITIVE RESPONSE TO K^+ IN THE *QUAKING* MOUSE. P.J. Magistretti and P.R. Hof, Dept of Pharmacology, CMU, 1211 - GENEVA 4, Switzerland.

The *tottering* mutant (tg) mouse is characterized by spike-wave discharges accompanied by behavioral absence seizures that resemble petit mal. A cytological abnormality observed in homozygous mutants (tg/tg) is an increase in the number of noradrenergic axons in the terminal fields innervated by the locus coeruleus, including the hippocampus, cerebellum, dorsal lateral geniculate nucleus and neocortex. This increase in axon number is accompanied by enhanced NE levels, up to 200 %, in the corresponding CNS areas. In mouse cerebral cortical slices, NE promotes a ß-adrenergic-mediated hydrolysis of newly synthesized ^{3}H-glycogen in a concentration-dependent manner. In order to assess the sensitivity state of ß-adrenergic receptors chronically exposed to enhanced NE levels, we examined the glycogenolytic effect of NE in the cerebral cortex of tg/tg homozygous, tg/+ heterozygous and +/+ control mice. Our results indicate a shift to the right of the concentration-response curve in tg/tg mice when compared to tg/+, with EC_{50} of approximately 1.7 and 0.6 µM respectively. Comparison with +/+ mice shows an even greater degree of subsensitivity of NE in tg/tg (EC_{50} 1.7 µM in tg/tg vs 0.17 µM in +/+). The decrease in the potency of NE to elicit glycogenolysis is accompanied by a decreased efficacy as the maximal glycogenolytic effect is 52.5 ± 3.1 % of basal levels in tg/tg, 59.6 ± 2 % in tg/+ and 77.6 ± 0.5 % in +/+. These results indicate that a subsensitivity of adrenergic receptors of the ß type, mediating a metabolic action of NE, exists in tg/tg mutants when compared to both tg/+ and +/+ mice. We also examined the glycogenolytic effect of isoproterenol (ISO), a ß-adrenergic agonist which is not actively taken up by noradrenergic terminals. The glycogenolytic response to ISO at 0.1 and 1 µM is also considerably reduced in tg/tg mice when compared to both tg/+ and +/+. These results indicate that the decreased potency and efficacy of NE in promoting glycogenolysis in tg/tg mice is caused by a true subsensitivity of postsynaptic ß-adrenergic receptors. A developmental study has indicated that a considerable degree of sub-sensitivity is already present at post-natal days 1, 3, 7 and 14.

The *quaking* mutant (qk) is characterized by a severe myelin deficiency in the brain due to the impaired differentiation of oligodendrocytes. We have observed a supersensitive response to the glycogenolytic effect of low concentrations of K^+ in cerebral cortical slices prepared from homozygous qk/qk when compared to heterozygous littermates qk/+. Results were (^{3}H-glycogen hydrolysis in % of basal levels ± SEM) : K^+ 6 mM in qk/qk = 35.1 ± 2.2, in qk/+ = 14.1 ± 2.4; K^+ 8 mM in qk/qk = 51.5 ± 1.7, in qk/+ = 30.7 ± 3.2; K^+ 11 mM in qk/qk = 66.9 ± 1.9, in qk/+ = 48.1 ± 2.2. This supersensitive response to K^+ was only observed in mice older than 7 weeks, with differences between qk/qk and qk/+ littermates of 11 % at 8 weeks, 17 % at 15 weeks and 30 % at 30 weeks for 11 mM K^+. No significant differences were observed between qk/+ and control +/+ mice.

300.3 A CHEMICAL & HISTOCHEMICAL ARCHITECTURE OF THE HUMAN EPILEPTIC FOCUS. NS Nadi & AR Wyler*. NINCDS, Bethesda, MD 20892 and Univ Tenn Med Ctr, Memphis, TN 38103.

The chemical parameters in the spiking and non-spiking portions of the human brain have remained largely unknown. Recently we have been able to analyze spiking and non-spiking regions from the human temporal lobe as well as the hippocampus for catecholamines, amino acids, neuropeptides, enzymes and receptors. The brains used in these studies (n=25) were surgically removed under general anesthesia and frozen or fixed within one minute after resection. In each case the non-spiking region was compared to the spiking region from the same patient. The catecholamines were elevated in the spiking region when compared to the non-spiking region: norepinephrine +47.5%, dopamine +58.4% and DOPA +23.5%. Several putative neurotransmitter amino acids were also elevated in the spiking region: glutamate +96.7%, aspartate +209.1%, and glycine +75.6%. GABA, alanine, taurine, and leucine were unchanged in the spiking vs non-spiking regions. Of the neurotransmitter enzymes investigated the spiking cortex contained elevated tyrosine hydroxylase +60.2%, and choline acetyltransferase +57.1%. Glutamate decarboxylase was unchanged. The epileptic cortex had elevated somatostatin +313%, neuropeptide Y +128%, and atrial natriuretic factor +42.7%. The levels of βendorphin, met-enkephalin, cholecystokinin, substance P, and neurotensin were not different in the spiking vs non-spiking region of the cortex. Vasoactive intestinal polypeptide was decreased in the epileptic cortex by 25.7%. Of the receptors measured the spiking region had elevated NMDA receptors +145% but decreased muscarinic receptors -32.5%; βreceptors -51.4%, αreceptors -38.4%. GABA and benzodiazepine receptors were unchanged. The K_d measurements of the receptors showed no change. The hippocampus had glutamate, aspartate, glycine, somatostatin, and neuropeptide Y comparable to the spiking cortex. Since non-spiking hippocampus was not available no comparative studies could be conducted. The increase in somatostatin in the spiking region may have contributed to the excitability of the focus, since this molecule regulates the action of acetylcholine. Glycine by virtue of increasing the potency of glutamate at the NMDA sites may also have contributed to local excitability. The increase in catecholamines may be interpreted as a secondary phenomenon in response to the increased excitability of the region. The histochemical studies in human brain showed a diffuse distribution of tyrosine hydroxylase and a localization of somatostatin to layers II, III, and V. The distribution of neuropeptide Y was similar to that of somatostatin indicating possible colocalization of the peptides. Further histochemical and autoradiographic studies are underway to determine in which regions the increases occur and how these might explain the chemical pathophysiology of seizures.

300.4 OUABAIN BINDING AND ATPASE ACTIVITY IN THE HUMAN EPILEPTIC FOCUS. CL Devlin*, AR Wyler* & NS Nadi, (SPON: RJ Porter). NINCDS, Bethesda, MD 20892, & Univ of Tenn Med Ctr, Memphis, TN 28103.

The role of Na^+-K^+ ATPase in the maintenance of membrane potentials is well established. Several studies in the kindled rat brain as well as other seizure models have been equivocal as to changes in activity of ATPase. Recent studies in brain slices have clearly demonstrated that blocking of Na^+-K^+ ATPase altered the excitability of the membranes. Earlier studies in our laboratory have shown that in the kindled rat brain ouabain binding was significantly decreased (-55%) whereas the Na^+-K^+ ATPase activity was unaltered which implied that there may be fewer molecules of Na^+-K^+ ATPase which may be turning over at a faster rate. The activity of Na^+-K^+ ATPase and the binding of ouabain in 10 spiking and non-spiking foci were removed surgically from the temporal lobe of patients with intractable seizures. The tissues were frozen on dry ice within one minute of resection. Na^+-K^+ ATPase activity and ouabain binding were assayed in the spiking and non-spiking regions of 10 patients. Na^+-K^+ ATPase activity was (mean ± SD) 3.5 ± 0.2 pmol/min/mg protein in the spiking vs 4.0 ± 0.3 pmol/min mg protein in the non-spiking region. Ouabain binding was (mean ± SD) 50.2 ± 15.6 fmol/mg protein in the spiking vs 65.6 ± 10.2 fmol/mg protein in the non-spiking region. Although trends towards a decrease were observed in some patients there was no significant change in the data overall when the data were compared by a student t test or a paired t test. The lack of change in ouabain binding may be interpreted that the decrease in activity of Na^+-K^+ ATPase may be localized to a very small region of the spiking cortex and may not be detectable with the currently available binding and enzyme assay methodologies. Further autoradiographic studies are underway in our laboratory to rule out very localized alterations and will be discussed.

300.5 DECREASED N-METHYL-D-ASPARTATE RECEPTOR DENSITY IN HIPPOCAMPUS OF SEIZURE-SENSITIVE GERBILS. L. Miller*, T.R. Insel, R. Gelhard* and C. Harbaugh*.(SPON: U. Vaidya) Neuroscience Lab, Vet Adm Med Ctr, Wash. D.C. 20422 and Laboratory of Clinical Science, NIMH, Poolesville, MD. 20837.

The Mongolian gerbil, Meriones unguiculatus, is a convenient animal model for investigating neurochemical mechanisms underlying the phenomena of epilepsy. These animals exhibit generalized seizures in response to any unusual stimulus, such as being handled or placed in a novel environment. The seizures vary in severity while the seizure resistant animals can appropriately be used as controls. In the present study we have examined the density of the various glutamate receptor subtypes (N-methyl-D-aspartate, NMDA; Quisqualate, QA; kainate, KA) utilizing this animal model. Our study follows from numerous investigations by others suggesting a possible role of particular glutamate receptor subtypes in the propagation of generalized seizure.

Adult gerbils were screened for seizure susceptibility (SS) and seizure resistance (SR) using a scale of 0-5 to rate the seizures with 5 being the most severe. All animals were examined at least three separate times and only those exhibiting a rank of 4-5 were chosen as SS subjects. Following decapitation gerbil brains were removed and frozen on dry ice. Receptor autoradiography was performed on 15 micron sections of brain tissue. The various glutamate receptor subtypes were analyzed essentially as described by Monaghan et al. (Nature 306: 176, 1984). For the NMDA receptor binding of H(3)-Glutamate was determined in the presence of 5 uM Quisqualic Acid and 100 uM SIIS, for the quisqualate receptor binding of H(3)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) was determined and for the kainate receptor binding of H(3)-kainic acid was determined. Non-specific binding was determined in the presence of excess quantities of the respective unlabeled ligand. Our results (N=5) are:

Hippocampal Region	% decrease in SS animals		
	NMDA	QA	KA
CA1	17	9	nc
CA3	15	nc	nc
dentate gyrus	16	nc	nc

(nc=no change)

Thus the density of NMDA receptors are significantly decreased in brain tissue from SS gerbils compared to SR controls. Whether the present results for the NMDA receptors represent a generalized pattern throughout the brain or show regional specificity is currently being explored. How these results relate to the seizure susceptibility of these animals will be discussed.

300.6 SUPPRESSION OF CHEMICALLY-INDUCED BEHAVIORAL SEIZURES IN RATS BY A NOVEL SPIDER TOXIN. H. Jackson and T.N. Parks. Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT. 84132

Venom from an American grass spider contains at least two toxins that affect calcium currents. The smaller of the two toxins, tentatively designated AG2, has a molecular weight of less than 1000 daltons and seems to suppress calcium currents in an unusually borad spectrum of tissues. Neuronal calcium channels are thought to participate in epileptiform activity by mediating transmitter release and by their probable involvement in the characteristic neuronal bursting associated with seizures. For these reasons, we thought that AG2 might prove to be an effective anticonvulsant when administered peripherally. Male Sprague-Dawley rats (200-300 gm) were injected via the tail vein with one of three widely-used chemical convulsants dissolved in saline: 12 mg/kg kainic acid (KA), 3.6 mg/kg picrotoxin (PIC), or 0.5 mg/kg bicuculline (BIC). Five minutes later, control animals received an additional 0.5 ml intravenous injection of saline or of a gel filtration or high-performance liquid chromatography (HPLC) fraction of the venom known not to have calcium antagonist activity. Experimental animals received 0.5 ml injections of gel filtration or HPLC fractions containing AG2 that have been shown in electrophysiological experiments to have calcium antagonist activity; we estimate this dose to be about 2 μM/kg. For KA treated animals, the incidence of three seizure behaviors was scored for 1 min sampling periods at 5 min intervals for two hours; these behaviors were "wet-dog shakes", forelimb clonus with rearing, and whole-body clonic seizures. Scores on these measures were combined into a seizure index value for each observation period. For PIC and BIC treated animals, the percentage of animals surviving for more than 2 hrs was determined for control and treated goups. AG2 significantly suppresses seizure behavior in KA-treated animals ($p<.001$) and prevents convulsant-induced death in the PIC and BIC treated groups ($p<.005$); these effects are dose-dependent. Venom fractions lacking AG2 have no effect on chemically-induced seizures. Animals treated with convulsants and AG2 appear slightly sedated but retain postural control and responsiveness to external stimuli; they survive indefinitely in apparent good health. The results show that AG2 in low doses can suppress seizures induced by an excitatory amino acid agonist (KA) or two chemically-dissimilar GABA antagonists (PIC and BIC) and suggest that this compound represents a new class of CNS calcium-channel antagonist with potential uses as anticonvulsants.

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300.7 ALTERATIONS IN BRAIN NEUROTRANSMITTER LEVELS AFFECT SEIZURE SUSCEPTIBILITY IN RAT PUPS. James E. Olson and David Holtzman. Dept Emerg Med, Wright State Univ, Dayton, OH and National Magnet Laboratory, MIT, Cambridge, MA.

Rat pups which receive a unilateral hypoxic-ischemic cerebral insult at 2 days of age are more susceptible than controls to hyperthermia-induced seizures at 10-20 days of age (Olson et al., Epilepsia, 26:360-364, 1985). Since neurotransmitter deficits may contribute to this increased seizure susceptibility, we measured levels of norepinephrine (NE) and acetylcholine (AC) in ten different brain regions of ischemia-damaged and normal animals at 10 days of age.

AC levels were not affected in any brain region by hypoxic-ischemic treatment. Pilot studies suggested that NE levels were decreased in frontal cerebral cortex ipsilateral to the ischemic insult. To investigate the relationship of ischemia-induced damage of specific neurotransmitter systems to increased seizure susceptibility, we measured hyperthermia-induced seizure temperature thresholds (STT) in animals which had been treated with 6-hydroxydopamine (6OHD) or with atropine (AT) to eliminate catecholaminergic neurons or decrease cerebral AC levels, respectively.

Experimental animals were paired by weight with littermates and injected with 0.1 mg 6OHD/gram body weight on the second and third days of life. At 10 days of age, each animal was weighed and the basal body temperature and STT were determined using a rectal thermocouple. Animals in a separate series of litters were treated with 50 ug AT/gram body weight at 10 days of age. Body weights, basal temperatures and STT's were determined 3 hr later.

As shown below, rats treated with 6OHD had lower STT's at 10 days of age compared to control animals ($p<0.05$). The experimental animals also weighed less than controls; however, STT's and body weights were not correlated in control rats. In contrast, rats injected with AT had STT's and body weights which were not different from controls. The mean±SEM basal temperature fell from 35.0±0.2°C in control animals (N=13) to 34.0±0.3°C in AT-injected pups (N=12, $p<0.05$).

These data suggest that a selective decrease in the number or activity of catecholaminergic neurons in ischemia-damaged neonatal rats may increase hyperthermia-induced seizure susceptibility. These results may be important to our understanding of the pathophysiology and sequelae of ischemic brain damage in children.

Agent	STT °C	Basal Temp °C	Weight gm
6OHD	42.6±0.2* (11)	34.8±0.3 (8)	19.0±0.7* (11)
None	43.3±0.2 (9)	35.2±0.4 (8)	21.8±0.6 (9)

Supported by the Epilepsy Foundation of America (JEO).

300.8 INCREASE IN IBOTENATE-STIMULATED PHOSPHATIDYLINOSITOL HYDROLYSIS IN SLICES OF THE AMYGDALA/PYRIFORM CORTEX AND HIPPOCAMPUS OF RATS BY AMYGDALOID KINDLING. K. Akiyama, N. Yamada*, H. Ujike*, M. Sato† and S. Otsuki*. Dept. of Neuropsychiatry, Okayama University Medical School, Okayama 700, and Dept. of Psychiatry, † Tohoku University School of Medicine, Sendai 980, JAPAN.

Kindling is the process whereby repeated administration of an initially subconvulsive electrical stimulation in the limbic brain areas results in progressive and permanent intensification of electrical discharges culminating in generalized seizure. Although several lines of evidence have indicated that enhancement of neurotransmission of excitatory amino acid (EAA) may underlie induction and maintenance of kindling, neurochemical mechanism of EAA responsible for seizure susceptibility remains to be known. The present study examined ibotenate (IBO)-stimulated phosphoinositide (PI) hydrolysis in slices of the amygdala (AM)/pyriform cortex (PC) and hippocampus (HIPP) of AM kindled rats. Male Sprague Dawley rats were used. A bipolar electrode was stereotaxically implanted into the left AM (coordinate, AP: -0.6, L: 5.2, D: 8.0) under pentobarbital anesthesia. Experimental animals received a kindling stimulus once daily in a 1 sec train of 60 Hz sine waves at a current intensity of 400 μA. Kindling seizure development was assessed using Racine's classification (1975). Daily kindling stimulus was administered to the left AM until a final stage 5 seizure was elicited on 20 consecutive days. Animals with sham operation were used as controls. AM kindled rats and matched controls were decapitated either 24 hours or 7 days after the last AM seizure. PI hydrolysis was measured by accumulation of [^{3}H]inositol 1-phosphate ([^{3}H]IP_1) in the rat brain slices according to the method of Berridge et al.(1982). IBO (10^{-3}M)-stimulated accumulation of [^{3}H]IP_1 increased significantly by 195 % in the AM/PC ($p<0.01$) and by 59 % in the HIPP ($p< 0.05$) of AM kindled rats sacrificed 24 hours after the last seizure. 7 days after the last seizure, a similar magnitude of significant increase (by 171 %, $p<0.05$) was maintained in the AM/PC of AM kindled rats. In contrast, the increase in the HIPP had attenuated to the control level by this time. These results suggest that remarkable and lasting increase in IBO-stimulated PI hydrolysis coupled to EAA receptors in the AM/PC may be associated with development of kindling and long-term maintenance of kindled events.

300.9 **ALTERATIONS IN EXCITATORY AMINO ACID RECEPTORS IN HUMAN TEMPORAL LOBE EPILEPSY.** L.D. Cahan*[1], J.W. Geddes[2], B.H. Choi[3], and C.W. Cotman[2] (SPON: S.K.R. Pixley). Div. of Neurosurgery[1], Depts. of Psychobiology[2] and Pathology[3], University of California, Irvine, CA 92717.

Abnormalities in excitatory amino acid transmitter pathways are proposed to be involved in epilepsy and in the neuronal damage resulting from sustained seizure activity. Vulnerable regions possess a high density of the N-methyl-D-aspartate (NMDA) subclass of glutamate receptors, and NMDA antagonists are effective anticonvulsants in a variety of animal seizure models. Using *in vitro* autoradiography, we have examined the status of the NMDA receptor and of the two additional glutamate receptor subtypes, the kainic acid (KA), and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, in hippocampal sections obtained from surgical resections of temporal lobe epilepsy patients. The results were compared to control tissues obtained at autopsy. Comparable sections were also stained for acetylcholinesterase activity.

The receptor profile in the entorhinal cortex was clearly abnormal, with a striking increase in the density of KA receptors in one patient, and in KA, NMDA, and AMPA receptors in a second patient. Intensification of AChE staining was also apparent. These results would predict hyperactivity of the entorhinal cortex, a prediction supported by the pattern of neuronal loss and the receptor distribution within the hippocampal formation.

In the outer 2/3 of the molecular layer of the dentate gyrus, the terminal zone of the major entorhinal input, excitatory amino acid receptor binding was abolished. Loss of granule cells was also evident. NMDA, AMPA, and KA receptor density was, however, maintained in the inner 1/3 of the molecular layer which receives its input from pyramidal neurons in the hilus. Neuronal and receptor density was also reduced in the hilus and in CA3. In particular, the distinct zone of high density KA receptors and AChE staining in CA3 was absent, replaced by a much broader band of lower intensity. These alterations may reflect plastic changes in the commissural/associational fiber system following the degeneration of granule cells and their axons, the mossy fibers.

Neuronal loss in CA1 was particularly devastating, with almost no pyramidal neurons remaining. This region normally contains a high density of NMDA and AMPA receptors, located postsynaptically on pyramidal cell dendrites. In the epileptic patients, NMDA, AMPA and KA receptors in CA1 were virtually absent. Receptor and neuronal density was, however, maintained in the adjacent prosubiculum. In the subicular region of the parahippocampal gyrus, a wider distribution and increased density of excitatory amino acid receptors was observed. These changes were paralleled by changes in AChE intensity.

These results are consistent with excitotoxic-induced damage but also suggest that the remaining neurons undergo plastic changes which may contribute to the functional propagation of neuronal activity within the epileptic hippocampus.

300.10 PHORBOL ESTER PRODUCES TRANSIENT EPILEPTIFORM ACTIVITY IN HIPPOCAMPAL SLICE. W.A. Wilson and D.V. Lewis V.A. Medical Center and Duke Medical Center, Durham, N.C. 27710

Electrical stimulation of the hippocampal slice induces two different types of plastic change. Moderate tetanic stimulation induces long term potentiation of synaptic transmission or LTP. More intense stimulation with repeated trains induces long term spontaneous epileptiform bursting, a phenomenon termed stimulus train induced bursting or STIB. Recently, activation of protein kinase C by brief application of phorbol esters has been shown to induce LTP in the absence of tetanic stimulation (Malenka et al, Nature, 321:175,1986). Our experiments address the possibility that long term epileptiform bursting could also be induced by phorbol application.

Hippocampal slices (625 uM) were prepared from Sprague-Dawley rats 25-40 days old (100-200 gm), placed in a submersion chamber and perfused with artificial cerebrospinal fluid (ACSF) containing (in mM); NaCl 120, KCl 3.3, CaC12 1.8, Mg SO4 1.2, NaHCO3 25, NaH2PO4 1.23 and dextrose 10 at pH 7.4. 4B phorbol -12, 13-diacetate (PDAc) was added to the ACSF. Field potentials were recorded in the s. pyramidale and radiatum of CA3b. Monopolar stimuli (0.1 msec, 5 -10V) were delivered to s. radiatum of CA3a.

After determining that the amplitudes of the fields were stable, perfusion with 2 uM PDAc was begun. After 5-6 min in PDAc, the amplitude of the field potentials (both orthodromic population spike and slow wave) in s. pyramidale was doubled or tripled. After 7-10 min spontaneous bursting began in CA3. Bursts had the typical appearance of multiple population spikes riding on a long positive slow wave and occurred at rates of 0.1 to 0.5 Hz. In some slices, there were transient episodes where the bursts became more rapid and complex resembling electrographic seizures. Washing the slices after 15-20 min exposure to PDAc resulted in a gradual (10-20 min) disappearance of the bursting. However, the increased amplitude of the field potentials persisted for hours and was stable indicating LTP had occurred.

These results suggest that, in CA3, PDAc can induce epileptiform bursting as well as persistent LTP. However, under appropriate conditions the bursting is reversible, whereas the potentiation is not. Perhaps the mechanisms underlying STIB involve more than transient activation of protein kinase C.

300.11 ALL-OR-NONE ELECTROGRAPHIC SEIZURES IN THE RAT HIPPOCAMPAL SLICE IN PHYSIOLOGICAL MAGNESIUM MEDIUM: INDUCTION AND TRIGGERING BY STIMULUS TRAINS. W.W. Anderson, H.S. Swartzwelder and W.A. Wilson, V.A. Med. Ctr. and Duke Univ. Med. Ctr. Durham NC 27705.

Previous studies have shown that spontaneous electrographic seizures (EGSs) can occur in the hippocampal slice for several hours in Mg-free artificial cerebrospinal fluid (0-Mg ACSF) containing the GABAb agonist baclofen (Anderson et al. Brain Res. 398:215 1986; Swartzwelder et al. Brain Res. 1987). We report here that similar all-or-none EGSs can be triggered by stimulus trains in ACSF containing normal Mg (with or without baclofen) following induction by several stimulus trains or exposure to 0-Mg ACSF.

Hippocampal slices (625 uM) were obtained from male Sprague-Dawley rats (25-35 days old). Extracellular recording and stimulation were performed from s. pyramidale and s. radiatum of CA3, respectively. Normal ACSF included (in mM) 0.9 Mg and 1.3 Ca. Rat CSF contains 0.88 mM Mg (Chutkow and Meyers, Neurology 18:963).

When slices were not producing spontaneous EGSs in 0-Mg ACSF + 2-5 uM baclofen, EGSs could be triggered by either a single pulse or a weak stimulus train (2 s, 3-10 pulses). Stronger stimulus trains (2 s, 120 pulses) triggered virtually identical EGSs.

When Mg was raised to 0.6, 0.9 or 1.2 mM (with or without baclofen) following exposure to 0-Mg ACSF, stimulus trains could still trigger EGSs, and their durations were independent of the suprathreshold stimulus intensity. However, the EGSs were shorter (25 versus 50 s), and more (17-56) pulses/train were required for triggering. Thresholds were also shown by varying train duration.

Alternatively, after slices had been stimulated by 10 or more trains (2 s, 60 Hz; once every 10 min) in normal ACSF + 2-5 uM baclofen, stimulus trains (above a threshold 17-29 pulses/train) triggered similar all-or-none EGSs. The EGSs increased after each of the first few stimulus trains, indicating that EGS induction occurred as a result of the trains. Five of 10 slices from 10 animals produced unambiguous all-or-none EGSs in 0.9-1.2 mM Mg following induction by stimulus trains, 0-Mg ACSF, or stimulus trains followed by 0-Mg ACSF.

In contrast, when slices from older rats (50-75 days old) were stimulated as during STIB (Stasheff et al. Brain Res. 344:296 1985) (2 s, 60 Hz trains once every 5 min, in 1.2 Mg/1.8 Ca, no baclofen, and with spontaneous bursting), the afterdischarge duration was not all-or-none but was sigmoidally related to stimulus intensity.

The induction, stimulus threshold, and all-or-none nature of EGSs are phenomenologically similar to the induction and triggering of seizures in whole animals during kindling. The existence of a post-stimulus all-or-none EGS also suggests that the EGS cannot simply be produced by a long duration EPSP, but implies the involvement of one or more regenerative mechanisms. Supported by the VA and NIH grant NS-17771.

300.12 MK-801 AND PCP PROTECT AGAINST ISCHEMIC NEURONAL DEGENERATION IN THE GERBIL HIPPOCAMPUS. J.J. Lawrence*, T.A. Fuller and J.W. Olney (SPON: M.S. Shahid Salles). Dept. of Psychiatry, Washington University School of Medicine, St. Louis, Mo. 63110.

Recent evidence suggests that certain neurodegenerative conditions, including anoxic-ischemic brain damage, may be mediated by the neurotoxic (excitotoxic) action of excitatory amino acid (EAA) transmitters. The N-methyl-D-aspartate (NMA) subtype of EAA receptor, the most abundant EAA receptor in brain, is thought to play a major role in such degenerative processes. Phencyclidine (PCP) and MK-801, which have similar receptor binding and NMA antagonist properties (Wong et al., PNAS, 83, 7104, 1986), are of potential interest as neuroprotective agents as they freely penetrate blood brain barriers and, by in vitro tests, are the most powerful (MK-801 > PCP) antagonists of NMA neurotoxicity known (Price et al., this meeting). Foster et al., (Br J Pharm 90, 9p, 1987) recently found that MK-801 (1-10 mg/kg ip) protects against the delayed degeneration of hippocampal CA1 neurons induced by 5 min of forebrain ischemia in the gerbil. Using both PCP and MK-801, we have tested the reproducibility of these findings.

Young adult gerbils (50-60 gm) were either untreated or given PCP or MK-801 in various doses ip 15 min prior to 7.5 min bilateral occlusion of the carotid arteries (in our hands, it required 7.5 min to consistently produce delayed degeneration of CA1 hippocampal pyramids). Four days following the ischemic episode, the gerbils were sacrificed by perfusion fixation under anesthesia and the brains processed for light and electron microscopy. Of 16 control animals that received no pretreatment, all had moderate-to-severe CA1 pyramidal cell loss which was bilateral but often non-symmetrical with regard to severity. In gerbils pretreated with MK-801 at 10 mg/kg (n = 6), total protection against CA1 hippocampal loss was evident in 50% and partial protection was achieved in the remainder (damage less severe and restricted to one side). A similar protective effect was provided by MK-801 at 1 mg/kg (n=6), with 50% having no damage and 50% having damage of reduced severity. At 0.1 mg/kg, MK-801 exerted no appreciable protective effect. Findings were less clearly interpretable with PCP in that 3 of 6 animals treated at 10 mg/kg escaped brain damage but the other 50% were severely damaged bilaterally, as were 4 of 4 gerbils treated with 5 mg/kg PCP.

These preliminary findings are generally confirmatory of the observations of Foster et al. We obtained a definite protective effect with MK-801 in the 1-10 mg/kg dose range which is not quite as dramatic as they reported, but we were dealing with a more prolonged ischemic insult. Supported by RSA MH 38894 (JWO) and Washington Univ/Monsanto Biomed Res Fund. MK-801 was generously supplied by Merck, Sharp and Dohme.

300.13 ALTERATION OF A1 ADENOSINE RECEPTOR DENSITY FOLLOWING TRANSIENT FOREBRAIN ISCHEMIA AND FIMBRIA/FORNIX LESIONS.

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Ischemic injury to CA1 pyramidal neurons is attenuated by A1 adenosine receptor agonist treatment (Block,G. and Pulsinelli,W.A., J. Cereb. Blood Flow Metab. ,in press) and fimbria/fornix lesioning (Buchan,A.M. and Pulsinelli,W.A.,Soc. Neurosci. Abs,submitted) and may be related to alterations in A1 adenosine receptors (Lee,K. et al.,Brain Res. 380:155,1986). To test the hypothesis that changes in A1 receptors contribute to selective neuronal loss, striatal and hippocampal ^{3}H-cyclohexyladenosine receptor density and affinity were measured in striatal and hippocampal membrane fractions of: 1)control, 2)unilateral fimbria/fornix lesioned, and 3) 2 and 6 hr. postischemic rats following 30 min. of transient forebrain ischemia.

A1 RECEPTOR DENSITY

	CONTROL	POST-ISCHEMIA 2 HR	POST-ISCHEMIA 6 HR
HIPPOCAMPUS	283±36(7)	219*±10(5)	243*±16(5)
STRIATUM	186±41(6)	110*±23(6)	139*±17(6)

(All values in fmoles/mg protein)
*p<0.005 vs. control (ANOVA with Scheffe comparison)

Transient ischemia resulted in a significant reduction in A1 receptor density but not affinity (data not shown). Fimbria/fornix lesioning of septal input to the hippocampus significantly increased ipsilateral receptor density [lesioned side 354±65 (3) vs. contralateral side 223±39 (3) ,p<0.02 (paired t-test)].

Thus,decreased A1 receptor density may explain post-ischemic neuronal hyperexcitability and death. Attenuation of CA1 pyramidal cell death by fimbria/fornix lesioning in part be due to possible heterologous increase in A1 receptors in the hippocampus.

300.14 THE GLUTAMATE(GLU) ANTAGONIST MK-801 BLOCKS HYPOXIC-ISCHEMIC(HI) BRAIN NECROSIS IN IMMATURE RATS.

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We found that MK-801, a noncompetetive blocker of excitatory amino acid responses mediated by NMDA receptors, protects the immature brain from necrosis caused by hypoxia-ischemia. In 7 d.o. rat pups anesthesized with ether, the right carotid artery was ligated. 2 hrs later, they were placed in 8% oxygen, 92% nitrogen for 3 hrs. Pups received I.P. injection of MK-801(1 mg/kg) or diluent before &/or mid-way in hypoxia. Examination of diluent treated pups 5 days later showed reduction in mass of the HI cerebral hemisphere compared with the opposite side with prominent neuronal loss in hippocampus(HIP), cortex & basal ganglia. Two injections of MK-801, just before & midway during hypoxia reduced the disparity in hemisphere weights(untreated, -11±3%, N=19 vs. treated, -2±1%,N=24 P<.007). A single dose before hypoxia(N=10) or in mid hypoxia(N=5) was similarly effective. MK-801 reduced the incidence of moderate to severe hemisphere necrosis(>10% reduction) from 58% in the diluent group(N=19) to 20% in groups given a single injection(N=10). Protocols with 2 injections(N=9) or 1 dose midway through hypoxia(N=5) eliminated severe injury. The drug preserved regions in the HI hemispheres which were grossly destroyed in untreated pups. In contrast to MK-801, I.C.V. injection of the GLU receptor antagonists APH & kynurenic acid either failed to protect or worsened neuronal injury in the model. The neuroprotective effects of MK-801 may be related to blockade of effects of GLU receptors expressed in the neonatal brain(Greenamyre et al, J Neurosci 7:1022). Using in vitro autoradiography, we identified distinct patterns of quisqualate & NMDA preferring binding sites for ^{3}H-glutamate in vulnerable regions. ^{3}H-TCP binding to the PCP/NMDA cation channel complex blocked by MK-801(Maragos et al, Eur J Pharm 123:173), corresponded to areas of NMDA receptor binding in cortex, HIP & striatum. Furthermore, MK-801 blocked the prominent destructive effects of NMDA(25 nmoles) injected directly into the striatum at this age. The results support the hypothesis that GLU release mediates HI neuronal destruction in the neonatal brain by activating receptor operated cation channels.

300.15 SELECTIVE SPARING OF NADPH-DIAPHORASE REACTIVE NEURONS IN NEONATAL HYPOXIC-ISCHEMIC INJURY.

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In murine cortical cell cultures exposed to the endogenous excitatory neurotoxin quinolinate (an N-methyl-D-aspartate [NMDA] agonist), there is striking preservation of a subpopulation of neurons containing the enzyme NADPH-diaphorase (NADPH-d). In rats, ischemia of the forebrain produces lesions of selectively vulnerable hippocampal pyramidal cells which can be blocked by focal microinfusion of 2-amino-7-phosphonoheptanoic acid, an antagonist at the NMDA receptor. Since excitatory amino acid transmitters, acting through the NMDA receptor, seem to be implicated in neuronal death from ischemia, we hypothesized that NADPH-d reactive neurons should be selectively spared in neonatal hypoxic-ischemia (HIE).

In the neonatal Levine model of HIE, the left carotid artery is ligated in 7 day old rat pups and animals are then subjected to a hypoxic (8% O_2, 92% N_2) atmosphere for 2 hours. This injury results in a marked loss of cortical and striatal neurons on the affected side of the brain. We have previously shown histochemically that, despite this loss, there is selective sparing of the NADPH-d reactive neurons. We now report biochemical data supporting the sparing of NADPH-d reactive neurons. Concentrations of somatostatin (SLI) and neuropeptide (NPY), two peptides which co-localize with NADPH-d in cortical and striatal neurons, were measured by RIA after 10 days survival. Dynorphin (dyn) and leucine-enkephalin (leu-enk) concentrations were determined as biochemical markers of non-NADPH-d reactive neurons. In the cortex and striatum, there was no difference in SLI and NPY concentrations between ischemic and contralateral sides. In the ischemic cortex, dyn and leu-enk decreased to 12% and 34%, respectively, of concentrations found in contralateral hemisphere. There was no change in dyn or leu-enk concentrations between ischemic and contralateral striatum.

These results show that, in the damaged cortex of HIE rat pups, there is selective sparing of a class of neurons containing SLI, NPY and NADPH-d. Cell death is documented by the loss of cortical neurons containing leu-enk and dyn. The mechanism of the cell death may be through excitatory transmitters acting at the NMDA receptor; the NADPH-d reactive neurons appear to be resistant to such damage.

301.1 SIMULTANEOUS DETECTION OF LHRH, LH AND FSH IN THE CASTRATED MALE RAT USING PUSH-PULL PERFUSION OF THE ANTERIOR PITUITARY COUPLED WITH CONTINUOUS EXCHANGE TRANSFUSION. R.L. Pickle,* V.D. Ramirez and H.F. Urbanski (SPON: S. Balagura). Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801 and Neuroscience Division, Oregon Regional Primate Research Center, Beaverton, OR 97006.

Push-pull perfusion (PPP) has earned the reputation of being a useful technique for the in vivo determination of the release rate of neurochemicals in several species. The recent application of the push-pull cannula (PPC) to the anterior pituitary (AP) has proven to be a valuable innovation for the examination of neuroendocrine control of the hypophysis. Previous studies using PPP to examine neuroendocrine control of gonadotrophin secretion were limited to placement of the PPC in the hypothalamus. Moreover, complications associated with sequential bleeding for a protracted period of time restricted these investigations to the use of larger animals. In an attempt to circumvent these limitations the current study combines PPP of the AP with exchange transfusion blood sampling, facilitating the simultaneous measurement of LHRH and gonadotrophin levels in the rat. Young adult male rats (90 days of age) were stereotaxically implanted with a PPC (24ga) in the AP and orchidectomized 7-10 d prior to sampling. One day prior to sampling the animals were fitted with indwelling jugular and femoral catheters. On the day of sampling the stylette of the outter cannula assembly was replaced with an inner cannula assembly (33ga). The AP was perfused with modified KRP (pH 7.4) containing 0.1mM bacitracin at a flow rate of 20 ul/min for 5-7 hrs. Continuous 10-min perfusate fractions were collected for subsequent LHRH assay. At least 15 min prior to the start of the blood sampling period the jugular and femoral catheters were flushed with heparinized saline and connected to the sampling and replacement tubing respectively. Continuous 5-min blood samples were withdrawn from the jugular vein at a rate of 30 ul/min using a peristaltic pump. A second channel of the same pump continuously infused a blood replacement mixture into the femoral vein, thereby maintaining a relatively constant blood volume. The samples were centrifuged and the plasmas stored until assayed for LH and FSH. Results were analyzed using the Pulsar program by Merriam & Wachter. The means of the release rate, frequency and amplitude of LHRH pulses were: 0.243 pg/min; 47 min; and 3.80 pg respectively (n=4). Of a total of 28 LHRH pulses observed 21% were not associated with a significantly detectable gonadotrophin pulse. However, of the 23 LH pulses observed, 78% were temporally associated with a LHRH pulse and of 9 FSH pulses observed, 88% exhibited this temporal relationship. These findings suggest that LHRH appears to orchestrate the pulsatile secretion of not only LH but also FSH, giving further support for the idea that LHRH is in fact, GnRH.

301.2 STUDIES OF THE ROLE OF THE NMDA RECEPTOR IN THE REGULATION OF HYPOTHALAMIC GONADOTROPIN RELEASING HORMONE RELEASE IN THE RAT M. Arslan*, C. R. Pohl*, and T. M. Plant (SPON: F. J. Huff). Department of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

When administered in subtoxic doses, the neuroexcitatory amino acid analog, N-methyl-DL-aspartic acid (NMA), acutely stimulates the release of luteinizing hormone (LH) in rodents and primates by driving a suprapituitary mechanism (Shainker, B.A. and Cicero, T.J., Brain Res., 184:425, 1980; Gay, V.L. and Plant, T.M. Endocrinology, in press, 1987). Neuroinhibitory amino acids, GABA and taurine, have been shown to be effective in blocking the NMA-induced LH release, but have been regarded as nonspecific antagonists since they exert their action at loci other than those of the NMA receptors (Olney, J.W. and Price, M.T., Brain Res. Bull., 5 Suppl. 2:361, 1980). Recent electrophysiological studies indicate that 2-amino-5-phosphonopentanoic acid (AP5) is a potent and specific antagonist of the NMA receptor (Olverman, H.J. et al., Nature, 307:460, 1984). In the present investigation, the antagonistic effect of AP5 on NMA-evoked GnRH release from hypothalamic neurons of the rat was examined indirectly by monitoring moment-to-moment changes in circulating LH concentrations.

Adult male Sprague-Dawley rats weighing between 250-300 g were used in the study. The animals were implanted with an indwelling cardiac catheter via the right internal jugular vein for withdrawal of sequential blood samples and drug infusion. Blood samples were collected at -5, 0,5,10,15,25, and 35 min relative to drug or vehicle (V) administration at 0 min. Plasma LH was estimated by RIA using NIH-LH-RP-2 as the standard. The animals received AP5+NMA, V+NMA, or V alone. AP5 (30 mg/kg BW) was injected 1 min prior to the administration of NMA (15 mg/kg BW). Following NMA injection, mean plasma LH concentrations rose from <0.3 to 1.4 ng/ml at 5 min, and reached a peak of ≈2.0 ng/ml at 10 min. The gonadotropin concentrations then declined and fell to levels of <0.4 ng/ml at 35 min post-injection. Treatment with AP5 prior to NMA administration completely blocked the LH releasing action of the neuroexcitatory amino acid. In AP5+NMA treated animals, the plasma LH concentrations were indistinguishable from the vehicle treated controls. These data indicate that AP5 is an effective and potent antagonist of NMA-dependent LH release in the adult male rat and apparently lacks the weak agonistic effect described for α-aminoadipate (αAA), which also specifically antagonizes the LH releasing action of NMA (Olney, J.W. and Price, M.T., Brain Res. Bull., 5 Suppl. 2:361, 1980). Whether AP5 can by itself alter the drive of the neuroregulatory system responsible for gonadotropin secretion, is yet to be determined.

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301.3 QUANTITATIVE HYBRIDIZATION HISTOCHEMISTRY FOR LHRH mRNA IN ORGANOTYPIC CULTURES. S. Wray, T. Zoeller[1] and H. Gainer, NINCDS and [1]NIMH, Bethesda,MD 20892.

We have developed an *in vitro* organotypic culturing system in which LHRH neurons express their peptide hormone and maintain an organization reminiscent of that seen *in vivo* (Wray et al., Soc.Neuroscience Abstr., 12,1177,1986). Using this technique, we have found that the number of immunoreactive LHRH neurons in cultured sections containing the OVLT (with or without the anterior pituitary (AP)) significantly decreases in the presence of estradiol (E_2 ,10^{-7}M) for 18-21 days (Wray et al., in prep). This effect is not seen in cultured sections containing LHRH neurons from areas rostral to, or caudal to, the OVLT. A decrease in immunoreactive cells could represent either an increase in peptide turnover or release and/or a decrease in peptide synthesis. To address this question, *in situ* hybridization on cultured sections was performed.

400 μm diencephalon sections from 4 day old rats were plated on coverslips with or without a section of the AP. Half of the coverslips received E_2 (10^{-7}M) in their media. The cultures were maintained at 36° for 17-20 days and then fixed in 4% paraformaldehyde/0.2% picric acid in 0.1M sodium cacodylate buffer (pH 7.2) for 20 min. The sections were rinsed in phosphate buffered saline, treated with acetic anhydride, dehydrated in alcohol, and delipidated in chloroform. After rehydration, the sections were air dried.The coverslips were attached, culture side up, to glass slides with at least one untreated and one treated culture/slide. Cultures were hybridized overnight at 37° with an ^{35}S-labeled 48-base oligonucleotide probe complementary to the mRNA region encoding LHRH. Following post-hybridization washes, the slides were dried and dipped in diluted Kodak NTB-3 emulsion and exposed for 2-3 weeks. Cellular levels of LHRH mRNA were estimated by measuring optical density of individual labeled cells using an image analysis system. Several cultures taken from the level of the OVLT contained over 100 labeled cells. To date, 1176 labeled cells from 27 cultures have been analyzed. The distribution and total number of labeled cells resembled that seen in cultures stained by immunocytochemistry. We are currently examining whether the addition of the AP, and/or E_2 differentially affected the mRNA level of LHRH cells in the rostral diencephalon, at the level of the OVLT or within the caudal diencephalon.

301.4 MODULATION OF HYPOTHALAMIC LUTEINIZING HORMONE-RELEASING HORMONE SECRETION BY ATRIOPEPTIN IN VITRO. B.T. Miller* and T.J. Collins* (SPON: T.J. Cicero). Dept. of Anatomy & Neurosciences, University of Texas Medical Branch, Galveston, TX 77550.

A recently discovered, major hormonal system - the atriopeptin system - has been shown to be intimately involved in the regulation of water balance, electrolyte balance and systemic blood pressure. New experimental evidence indicates that atriopeptins, which are present in large amounts in cardiac tissue, are also produced in the brain. Immunopositive atriopeptin neurons have been identified in the rat hypothalamus, and fibers containing atriopeptin-like peptides are present in the external zone of the median eminance. Moreover, there is some evidence that hypothalamic atriopeptins might be involved in the control of pituitary hormone secretion, either directly, or by acting as neuromodulators in the hypothalamus. We have found that the presence of micromolar amounts of atriopeptin II (APIII) appears to enhance the secretion of luteinizing hormone-releasing hormone (LHRH) during in vitro incubations of rat or mouse hypothalamic tissue fragments.

Bissected medial basal hypothalami from either Sprague-Dawley-derived adult male rats or C57CL/6 adult male mice were individually incubated in a Kreb's-Ringer's-HEPES medium containing concentrations of APIII ranging from 0 micromolar to 10 micromolar. Matched incubations with neocortical tissue served as controls. The LHRH secreted by the tissues into the media was measured by RIA. Significant increases in the release of LHRH were observed at concentrations as low as 1 micromolar APIII. The APIII-associated increases were completely suppressed in calcium-free media which contained 1 mM EGTA, a calcium chelator. These results suggest that hypothalamic atriopeptin might be involved in the modulation of LHRH secretion in the rat and mouse. Whether hypothalamic atriopeptin exerts only stimulatory effects on LHRH secretion is currently unclear, however since earlier reports suggest that atriopeptins suppress the in vivo secretion of luteinizing hormone from the pituitary.

301.5 A MAPPING OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) NEUROTERMINALS WITH THE PUSH-PULL PERFUSION METHOD IN THE RHESUS MONKEY. M. Gearing* and E. Terasawa. Neuroscience Training Prog. and Wisconsin Reg. Primate Res. Ctr., Univ. of Wisc., Madison, WI 53715.

Previously, we have reported a method for the measurement of *in vivo* LHRH release in conscious monkeys using a push-pull cannula inserted into the stalk-median eminence (S-ME). Our method offers great flexibility and accuracy in positioning the cannula tip, and allows multiple experiments with different cannula locations in the S-ME of a single animal. In the present study, we investigated the relationship between sampling location and LHRH release in 34 experiments performed in 14 ovariectomized female rhesus monkeys. Prior to perfusion, a cranial pedestal was fixed to the skull and a push-pull cannula was inserted into the S-ME through the pedestal. Based on X-ray ventriculography, the ventral edge of the infundibular recess of the third ventricle, at the midline level, was designated as the zero reference point. None of the cannula tip placements were in the third ventricle. Modified Krebs-Ringer phosphate buffer solution was infused through the push cannula and perfusate collected in 10-minute fractions through the pull cannula by identically calibrated pumps at a rate of 20 μl/min for 10-18 hr. LHRH was measured by radioimmunoassay. 1) Within an individual animal in which multiple experiments were conducted with different cannula placements, LHRH pulse frequency was consistent, while LHRH pulse amplitude and mean LHRH release varied considerably with cannula location. 2) Cannula tip placements bilaterally within 1 mm of the midline resulted in higher mean LHRH release (3.7±0.9 pg/ml) than more lateral (1-2 mm) placements (1.7±0.5 pg/ml). 3) When cannula tip placements were within 1 mm of the midline, highest mean LHRH release (10.8±1.5 pg/ml) was obtained at the ventral margin of the median eminence, between the zero reference point and 1.5 mm caudal to it, where the zona externa is located; 4) mean LHRH release was lower (3.1±0.9 pg/ml) when the cannula tip was located in the region between the zona externa and the base of the third ventricle, dorsal to the area described in 3; 5) rostral placements, in the area between the optic chiasm and the zero reference point, resulted in low mean LHRH release (1.3±0.2 pg/ml), as did placements in the area 1.5-2.5 mm caudal to the infundibular recess (1.2±0.9 pg/ml). These physiological results parallel the anatomical distribution of LHRH-immunoreactive fibers (Silverman, et.al., *J. Comp. Neurol., 211*:309, 1982). Therefore, our push-pull perfusion method is reliable and useful for the *in vivo* measurement of LHRH, as well as other neuropeptides and/or neurotransmitters. (Supported by NIH Grants RR00167, HD11355 and HD15433.)

301.6 DEGRADATION OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) BY HYPOTHALAMIC AND ANTERIOR PITUITARY MEMBRANES AND BY INTACT PITUITARY AtT20 CELLS IS INITIATED BY CLEAVAGE OF THE TYR^5-GLY^6 BOND BY ENDOPEPTIDASE-24.15. C.J. Molineaux, C. Michaud* and M. Orlowski*. Dept. of Pharmacology, Mount Sinai Sch. of Med. of the City Univ. of New York, New York, N.Y. 10029

Degradation of LHRH within the hypothalamus and/or the anterior lobe (AL) of the pituitary may play a significant neuroendocrine role in modulating the release of luteinizing hormone (LH) and follicular stimulating hormone (FSH). LHRH (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) was incubated with a particulate fraction of homogenates prepared from rat hypothalamus and from pituitary AL, as well as both membrane preparations and intact cells of the pituitary tumor line AtT20. Products of enzymatic degradation were separated by HPLC and were identified by amino acid analysis. Incubation with hypothalamic fractions yielded two main peptides, pGlu-His-Trp-Ser-Tyr and pGlu-His-Trp, indicating cleavage of the Tyr-Gly and Trp-Ser bonds. Incubation with pituitary fractions under conditions in which 50-80% of the peptide was degraded, yielded only pGlu-His-Trp. No degradation products of the C-terminal part of the peptide were isolated, indicating rapid degradation by the action of aminopeptidases. Incubation of pituitary fractions with LHRH in the presence of captopril, an inhibitor of angiotensin converting enzyme (ACE) led to an accumulation of pGlu-His-Trp-Ser-Tyr and pGlu-His-Trp-Ser-Tyr-Gly, indicating that in the absence of captopril these peptides are rapidly degraded by the action of ACE. Incubation of pituitary fractions in the presence of N-[1(RS)-carboxy-3-phenylpropyl]-Ala-Ala-Phe-pAB (cFP-AAF-pAB), a specific inhibitor of endopeptidase-24.15 synthesized in our laboratory, prevented the formation of any of the above peptides and greatly decreased the degradation of LHRH, indicating that formation of these products is dependent on the presence of endopeptidase-24.15 activity. Incubation of pituitary fractions with LHRH in the presence N-[1(RS)-carboxy-2-phenylethyl]-Phe-pAB, an inhibitor of endopeptidase-24.11 ("enkephalinase"), did not prevent formation of pGlu-His-Trp. Incubation of LHRH with intact AtT20 cells in monolayer culture led to the formation of pGlu-His-Trp. Formation of this peptide and LHRH degradation were completely prevented in the presence of cFP-AAF-pAB. Incubation with captopril alone led to the formation of pGlu-His-Trp-Ser-Tyr. These data are consistent with the conclusion that the primary and quantitatively dominating reaction in degradation of LHRH is cleavage of the Tyr-Gly bond by the action of endopeptidase-24.15, a zinc-metalloendopeptidase previously identified and isolated in this laboratory [Orlowski, M., Michaud, C. and Chu, T. Eur. J. Biochem. 135: 81-88 (1983)]. A minor cleavage in pituitary membrane preparations involves the Gly-Leu bond, apparently catalyzed by endopeptidase-24.11. Supported by grants DK 25377 and DA 04218.

301.7 **SUPPRESSION OF IN-VITRO "BASAL" GONADOTROPIN SECRETION BY GnRH ANTAGONIST: DIFFERENTIAL EFFECTS ON METESTROUS AND PROESTROUS RAT PITUITARIES.** **E.S. Hiatt* and N.B. Schwartz*.** (SPON: L.H. Pinto). Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201.

We have reported a sex-specific change in in-vitro basal gonadotropin secretion rate (BSR; release in the absence of exogenous GnRH) by anterior pituitary fragments 2 and 6 days after gonadectomy (Hiatt and Schwartz, Biol. Reprod. 34:Abst. 212, 1986). In females, BSR of LH and FSH increases in parallel with serum values, while in males BSR is significantly reduced despite elevated serum levels. These findings led us to test the dependence of in-vitro BSR on prior exposure to GnRH in vivo. We chose to compare two estrous cycle stages, proestrus (PRO), when BSR and GnRH receptor levels are high, and metestrus (MET), when levels are low. Rats were given a single s.c. injection (100 ug/.25ml corn oil) of a potent GnRH antagonist (ANTAG; WY 45760; [AcB(2)D-Nal^1-4-F-D-Phe^2-D-Trp^3-D-Arg^6-LHRH] or oil either 12h or 1h prior to sacrifice at 0800h on PRO or MET. Pituitaries were cut into eighths and perifused for 8h using the Endotronics ACUSYST^{TM} perifusion system. For the first 4h, half of the glands from oil-injected rats were given Med 199 containing 1 uM ANTAG; the remainder received Med 199 alone. Starting at 5h all chambers received hourly 15-min pulses of 1 uM GnRH. Perifusates were assayed for LH and FSH by RIA. In PRO rats ANTAG injection lowered LH BSR to 50% and FSH to 70% of oil-treated controls regardless of time of injection. ANTAG administered in vitro suppressed LH BSR to 30% and FSH to 55% of controls. In contrast, in MET rats BSR of LH and FSH are 20% and 70% of control PRO rat values and were unaffected by ANTAG treatment either in vivo or in vitro. In PRO pituitaries LH and FSH responses to the first GnRH pulse were blunted by ANTAG in vivo but subsequent pulses were unaffected; MET responses were not different from controls. In-vitro ANTAG suppressed GnRH-stimulated release to 10-20% of controls at both cycle stages. The lower effectiveness of ANTAG in suppressing FSH has not been shown previously in vitro, but is consistent with in vivo studies in this lab (Grady et al., Neuroendocrinol. 40:246, 1985). Our findings suggest that pituitary fragments, in which cell-cell contact is maintained in culture, retain an appreciable amount of endogenous GnRH that stimulates "basal" gonadotropin secretion. The relative ineffectiveness of the ANTAG in lowering BSR in MET compared to PRO pituitary fragments suggests that there is less GnRH present, as predicted by lower GnRH release and pituitary receptor levels in MET rats. Thus, for questions concerning physiological regulation of gonadotropin secretion, the use of pituitary fragments may be more appropriate than more widely used dispersed cell preparations. Supported by PHS RO1-HD22125 and T32-HD07068.

301.8 INTERMITTENT PULSES OF SEROTONIN ELICIT BIPHASIC EFFECTS ON THE IN VITRO RELEASE OF LHRH IN THE OVARIECTOMIZED-ESTRADIOL-TREATED RAT. D. C. Meyer. Dept. of Physiology, Eastern Virginia School of Medicine, Norfolk, VA 23501.

The neurotransmitter serotonin(5-HT) has well known effects on the release of luteinizing hormone (LH) which may also be estrogen dependent. An in vitro superfusion system was used to assess the potential role of serotonin in modulating the release of luteinizing hormone releasing hormone (LHRH) from the mediobasal-suprachiasmatic-preoptic area (MBH-POA-SCN) in the ovariectomized (OVX) E_2 implanted rat model of LH release. Regularly cycling female Holtzman strain rats, maintained on a photoperiod of 01^{00}-15^{00} H. light were OVX, and silastic capsules containing estradiol-17 beta (150 ug/ml) or sesame oil implanted s.c. Two days later the rats were killed at 10^{00} H and the MBH-POA-SCN isolated. Tissue was superfused in vitro with Krebs Ringer Phosphate buffer (KRP) for 2 hours followed by 3 hours of KRP with intermittent infusion of 5-HT at increasing concentrations: 1 x 10^{-10} M (1st hour); 1 x 10^{-8} M (2nd hour); 1 x 10^{-6} M (3rd hour). Superfusate was collected every 10 min. for the entire 5 hour incubation period and LHRH measured by RIA with the following results(Mean ± S.E.):

Treatment	LHRH (pg/10min)	Period (min)
OVX Control	1.2±0.3	34.8±4.2
OVX 5-HT	0.8±0.2 *	45.6±4.9 *
OVX E_2 Control	1.5±0.3	38.0±3.8
OVX E_2 5-HT	1.9±0.2	30.8±2.7 *

* P = .05 vs. control (paired T test)

These results suggest that the combined effect of all doses of 5-HT have a marked effect on LHRH output and period (frequency), significantly decreasing LHRH output and increasing the period in the OVX model. In contrast in OVX rats given estradiol, 5-HT increased output and significantly decreased the period of LHRH release. This indicates that the effect of 5-HT in modulating LHRH release may be related to the estradiol level in the rat and could serve as one mechanism to coordinate the release of LHRH with photoperiodic and steroid cues. This work was supported by HD 20535 to DCM.

301.9 SEX DIFFERENCE IN THE EFFECT OF MATING ON THE PULSATILE SECRETION OF LUTEINIZING HORMONE IN THE FERRET. R.S. Carroll, M.S. Erskine and M.J. Baum . Dept of Biology, Boston University, Boston, MA 02215.

Unlike males of many other mammalian species, male ferrets retain the capacity to exhibit receptive components of feminine mating behavior in response to treatment with estradiol (E2). The present study was conducted to see whether male ferrets also retain the capacity to exhibit surges in the secretion of luteinizing hormone (LH) after mating which are comparable to the coitus-induced LH surges needed to induce ovulation in estrous females. Female ferrets which were either gonadally intact and in estrus, or gonadectomized and maintained on a 'pulsed' regimen of daily E2 injections (5 ug/kg/2x/day) exhibited a large surge in plasma LH, characterized by an elevation in mean LH levels and an increase in the number of LH pulses, after receipt of an intromission from a stud male. By contrast, no such surge in LH secretion occurred in males which achieved an intromission with a female, regardless of whether they were gonadally intact and in breeding condition, or gonadectomized and given the same 'pulsed' estrogen regimen as that given to the females. In fact, gonadally intact breeding males which achieved an intromission had significantly fewer LH pulses 1-5 h later than unmated males bled serially over the same time period. This decrease in LH pulse frequency was followed by a significant rise in mean plasma levels of androgen 5-12 h later. This sexually dimorphic LH response to intromission probably does not reflect a sex difference in pituitary responsiveness to the endogenous release of gonadotropin releasing hormone (GnRH) because gonadectomized E2-treated male and female ferrets showed equivalent LH responses to the intravenous administration of several doses (2,4,8,16 ug/kg) of GnRH (Carroll et al., Biol. Reprod. 34:112, 1986). Ferrets' sexually dimorphic LH responses to intromission probably reflect a sex difference in the processing of somatosensory inputs from the genitalia or in the neural control of GnRH release into the hypophyseal portal vessels. (This work was supported by HD21094, RCDA MH000392 to MJB and MH09259 to RSC).

301.10 LUTEINIZING HORMONE (LH) SUPPRESSION IN MALE RATS EXPOSED TO A MINIMAL HORMONAL STIMULUS OF TESTOSTERONE, ESTRADIOL OR DIHYDROTESTOSTERONE. M.Y. McGinnis*, M.C. Mirth* and R.M. Dreifuss* (SPON: J.E. Shriver). Dept. of Anatomy, Mt. Sinai Sch. Med., CUNY, New York, NY 10029.

Exogenous testosterone (T) treatment suppresses LH release in castrate male rats. However, estradiol (E_2) and dihydrotestosterone (DHT), two major metabolites of T, have been shown to suppress LH levels to an even greater extent than T. In the present study, Silastic capsules were devised to provide constant serum levels of T, E_2 and DHT in the physiological range for male rats. The ability of these minimal hormonal stimuli to suppress LH, and the time course of LH suppression following capsule insertion were investigated.

Adult, male, Long-Evans rats were castrated 3-4 weeks prior to blood sampling. In the first experiment, castrates received subdermal implants of either two 10mm T-filled silastic capsules, one 5mm 10% E_2 capsule (diluted with cholesterol), one 5 mm DHT capsule, one 10% E_2 capsule plus one 5mm DHT capsule or blank (Bk) capsules. After 14 days of hormone exposure, trunk blood was collected. Results showed that all steroid treatments significantly suppressed LH release. Lowest LH levels were found in rats receiving 10% E_2, DHT or 10% E_2 plus DHT.

In a second experiment, the time course of LH suppression was determined. Open flank pouches were pre-formed the day before blood sampling. Animals were bled at hourly intervals from the tail vein. After the first hourly sample, silastic capsules as described above (T,10% E_2, DHT or Bk) were placed into the flank pouches (without anesthesia), and sampling continued for up to 7 h. Results confirm previous studies showing that LH suppression requires 4-6 h of T exposure. In contrast, LH levels were suppressed within 2-3 h after exposure to 10% E_2 or DHT.

To examine the effects of these hormones on pulsatile LH release, a third experiment was conducted. Castrate males received open flank pouches and indwelling catheters placed in the common carotid artery. The following day blood samples were taken every 6 min. After the first hour of sampling, capsules (T,10% E_2, DHT or Bk) were inserted into the pouches of awake, freely-moving rats and sampling continued for up to 7 h. All three hormone treatments suppressed LH pulses; 10% E_2 was most effective, DHT was intermediate and T was less effective. Blank capsules had no effect on pulsatile LH secretion.

Our results indicate that even when a minimal hormonal stimulus is used, both E_2 and DHT suppress LH more rapidly and to a greater extent than T. It is suggested that major differences in androgenic regulation of copulatory behavior and gonadotropin regulation may exist in male rats.

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301.11 CORRELATION OF RAT PITUITARY PROLACTIN mRNA AND HORMONE CONTENT WITH SERUM LEVELS DURING THE ESTROGEN INDUCED SURGE. A.J. Carrillo, Z.D. Sharp* and L.V. DePaolo. Dept. of Cellular & Structural Biology and Physiology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78284

The model of the serum Prl surge generated in the ovariectomized rat following estradiol benzoate (EB) treatment was used to study the relationship between prolactin (Prl) release and biosynthesis. Adult ovariectomized rats were injected (s.c.) with 7ug of EB or vehicle at noon of day 0. Three days later (day 3) the rats were decapitated every 4 h over a 24 h period (beginning at 0800 h) for determination of serum and pituitary Prl and growth hormone (GH) levels by RIA. In addition, Prl and GH mRNA content was determined using dot blot hybridization with cDNAs.

Administration of EB resulted in a significant rise in serum Prl levels at 1200, 1600 and 2000 h on day 3 when compared to controls. In addition, EB treatment elicited a marked increase in pituitary prolactin levels at all time periods examined except during and after the Prl surge (1600, 2000, and 2400 h) when there was a significant reduction in stored pituitary Prl. Pituitary Prl mRNA content in the EB-treated group was significantly elevated (4 to 6 fold) over control levels throughout the study. Furthermore, Prl mRNA levels in EB-treated rats were significantly higher at 2000 and 2400 h than at other time periods. In contrast to its effects on Prl, EB treatment had a slight inhibitory effect on pituitary content of GH at 2000 and 2400 h when compared to controls; otherwise this steroid had no effect on serum GH levels and pituitary content of GH mRNA. Interestingly, serum GH levels and pituitary GH mRNA content in both treatment and control groups exhibited a circadian periodicity with peak values occurring during the lights-on hours.

These data show that estrogen has a continuous stimulatory effect on pituitary content of Prl and its corresponding mRNA in the rat for at least 3 days after injection. Thus, maintenance of elevated Prl mRNA levels may be necessary for the occurrence of Prl surges. Furthermore, the finding that serum Prl was elevated only at certain times (1200-2000 h) while Prl mRNA content was increased at all times in the EB treated rats suggests a differential regulation between hormone release and biosynthesis.

301.12 EFFECTS OF HYPERPROLACTINEMIA ON LH SECRETION FOLLOWING ELECTRO-CHEMICAL STIMULATION OF THE MEDIAL PREOPTIC AREA. C. Shu* and M. Selmanoff (SPON: D. Ruchkin). Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201.

Chronic hyperprolactinemia is known to decrease LH secretion perhaps by causing decreased secretion of LHRH. We have utilized the 7315a prolactin-secreting pituitary tumor to induce a hyperprolactinemic state sufficient to suppress the postcastration LH rise. We then stimulated LHRH cell bodies in the medial preoptic area (MPOA) to see if the evoked LH secretory profile was different in hyperprolactinemic compared with age-matched controls.

Intact, female Buffalo rats (Harlan Sprague Dawley, Inc.) were innoculated with a suspension of minced tumor tissue. Tissue to initiate our tumor line was kindly provided by Dr. Robert M. MacLeod. Two to three weeks later the tumors had become palpable and rats were ovariectomized (Day 1). On Day 7 rats were implanted sc with estradiol-containing Silastic capsules which maintain levels of circulating estradiol of about 15pg/ml. This concentration of estradiol exerts a negative feedback effect and induces daily LH surges similar to those seen on proestrous afternoon. On Day 8 rats were implanted with an indwelling right atrial catheter. On Day 9 rats were anesthetized with ketamine/acepromazine before the critical period (11am-1pm) such that the afternoon LH surge was blocked. In a stereotaxic apparatus, a bilateral, electrochemical stimulus was applied to the MPOA (100μA, 60sec) and the rats sequentially bled at 0,15,30,45,60,90,120 and 180min thereafter. Cardiac perfusion of the brain was performed with sequential 20ml volumes of 0.9% saline, potassium ferrocyanide and 10% neutral buffered formalin. Cryostat sections of the brain revealed that the blue reaction product extended from the diagonal band of Broca to the caudal extent of the medial preoptic nucleus. At the time of stimulation the tumor volume was about $60cm^3$ (range = $50-80cm^3$).

LH values at time 0 were suppressed in tumor-bearing rats (99 ± 11ng/ml LH-RP-1) compared with controls (343 ± 11ng/ml). Significant suppression was also seen in rats not receiving estradiol (241 ± 35 vs 520 ± 95ng/ml respectively). Electrochemical stimulation resulted in a significant LH increase at 15min with peak levels attained 90min poststimulation. With the exception of the 0 time values, the LH profile of hyperprolactinemic and control rats did not differ.

These results demonstrate that the chronic hyperprolactinemia produced by the 7315a tumor suppresses postcastration LH secretion in the presence and absence of estradiol. Upon stimulation of the LHRH neurons, the LH response is not modified by this hyperprolactinemic state.

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301.13 Effects of Testosterone, Dihydrotestosterone, and Estrogen on Luteinizing Hormone Secretion in Castrated Male Ferrets. Y.P. Tang* and C.L. Sisk (SPON: G.B. Ellis), Neuroscience Program and Department of Psychology, Michigan State University, E. Lansing, MI 48824-1117.

The biological activity of testosterone often depends on the conversion of testosterone within the target cell to an androgenic or estrogenic metabolite. We have previously demonstrated that testosterone exerts negative feedback effects on luteinizing hormone (LH) secretion in male ferrets. Castration results in an increase in LH secretion; testosterone replacement prevents this castration response. The purpose of this study was to compare the relative ability of testosterone (T), dihydrotestosterone (DHT) and estrogen (E) to suppress LH secretion in castrated ferrets.

Adult male ferrets housed under 18 hr of light/day were castrated. Two weeks after castration, the ferrets were divided into 3 groups which over the next five weeks received subcutaneous Silastic capsules containing either T, DHT, or E. Five different doses of the steroid capsules (one dose each week) were administered. T- and DHT-treated ferrets received 1, 2.5, 5, 10, and 15 mm capsule length/100 g body weight; E-treated ferrets received 0.1, 0.25, 0.5, 1, and 1.5 mm capsule length/100 g body weight. Blood samples were collected via heart puncture from lightly anesthetized ferrets immediately before castration, two weeks after castration, and after each week of treatment with the five doses of steroid. Plasma LH concentrations were measured by radioimmunoassay.

Castration resulted in a rise in plasma LH from 0.09 ng/ml before castration to 0.47 ng/ml two weeks after castration. Postcastration LH levels were not different among the groups before steroid treatment began. Plasma LH concentrations were significantly reduced in castrated ferrets by all doses of DHT and E; mean LH levels were nearly undetectable at the four highest doses of these two steroids. In contrast, the lowest dose of T was completely ineffective in suppressing LH, and the next higher dose resulted in only a partial reduction in plasma LH concentrations. These results demonstrate that relatively low doses of DHT (1 mm/100 g body b.w.) and E (0.1 mm/100 g b.w.) inhibit LH secretion, but that T suppresses LH release only at higher doses ($\geq$ 2.5 mm/100 g b.w.). This experiment indicates that E is more effective than DHT, and that DHT is more effective than T, in inhibiting LH secretion in castrated ferrets. This suggests that in intact ferrets, steroid negative feedback on LH secretion may be mediated by the conversion of testosterone to DHT and E within hypothalamic and/or pituitary cells. Supported by HD-21588.

301.14 The Effects of Gonadal Steroids on Vasoactive Intestinal Peptide (VIP) Concentrations in the Hypothalamus of Male and Female Rats. P.N. Riskind, S.M. Gabriel and J.I. Koenig, Neurology Service, Mass General Hospital, and Harvard Medical School, Boston, MA 02114

VIP potently stimulates prolactin (PRL) secretion from *in vitro* cultured pituitary glands. Furthermore, immunoneutralization studies have demonstrated a role for VIP during suckling- and 5-HT- induced PRL secretion. These findings, combined with the high concentrations of VIP in the rat hypothalamus and hypophysial portal blood suggest that VIP may be of importance in regulating the secretion of PRL in this species. Gonadal steroids such as estrogen have profound stimulatory effects upon the concentrations of PRL found in the pituitary and blood of the rat. Therefore, we have sought to determine the involvement of hypothalamic VIP as a mediator of these gonadal steroid effects. Sixty-day-old male and female Sprague-Dawley rats received either bilateral gonadectomies or sham operations under ether anesthesia. Two weeks later, the animals received Silastic capsule implants containing estradiol 17-beta (E2), testosterone (T) or cholesterol (Chol). Animals were sacrificed 12 to 15 days after initiation of the steroid or control treatment. Antisera to VIP were generated in rabbits, using porcine VIP conjugated to key limpet hemocyanin. The assay sensitivity was less than 2 pg VIP/tube. Rat hypothalamic extracts displaced radiolabeled VIP binding to the antibody in a manner parallel to the standard curve. The antibody did not crossreact with porcine PHI or members of the secretin peptide family. Sephadex G-50 chromatography and HPLC of rat hypothalamic extracts revealed single peaks of VIP immunoreactivity which coeluted with VIP standard. Hypothalamic VIP concentrations of male rats were unaffected by castration or gonadal steroid treatments, in agreement with a previous report. However, hypothalamic VIP concentrations were higher in female rats than in male rats (Dl females 237 $\pm$ 8 pg VIP/mg protein, proestrus 213 $\pm$ 22 pg VIP/mg protein vs intact males 157 $\pm$ 13 pg VIP/mg protein). Ovariectomized rats had somewhat lower hypothalamic VIP concentrations (193 $\pm$ 12 pg VIP/mg protein) than either group of intact females, but none of the steroid treatments restored VIP concentrations to ovarian-intact levels. These results indicate that the more dynamic PRL secretory activity of female rats than male rats may be related to higher hypothalamic VIP concentrations in females. Our results also suggest that, in addition to estrogen, other ovarian factors such as progesterone may play a role in maintaining hypothalamic VIP concentrations. Supported by AM 26252.

301.15 APPLICATION OF A CHEMICAL REDOX SYSTEM FOR DELIVERY OF DRUGS TO THE BRAIN: ETHINYL ESTRADIOL. M.E. Brewster,* K. S. Estes and N. Bodor.* Pharmatec, Inc., Alachua, FL 32615 and Ctr. for Drug Design and Delivery, College of Pharmacy, Univ. of Florida, Gainesville, FL 32610.

A redox-based chemical delivery system (CDS), analogous to the $NAD^+ \rightleftarrows NADH$ coenzyme system recently was developed and shown capable of brain-directed delivery of several drugs. Application of the CDS to estradiol (E_2) resulted in sustained LH inhibition in castrate rats and prostate and seminal vessicle weight decrease in male rats which could not be attributed to circulating E_2 levels. This action was far more pronounced than equimolar treatment with estradiol valerate (EV) or E_2 alone (Life Sci. 40:1327-1334, 1987). The CDS was applied to ethinyl estradiol (EE) in an attempt to improve the stability and activity of the estrogen carrier. Distribution studies in rats verified brain directed delivery of EE. Brain concentrations of the oxidized EE carrier complex were sustained with $t_{\frac{1}{2}}$ approximately 2½ days after i.v. administration of CDS-EE while blood levels rapidly fell. Free EE brain levels were similarly sustained after CDS-EE. Levels of EE were not detectable 3 h following treatment with equimolar doses of uncoupled EE. To monitor the duration of central estrogen action, LH was measured in ovariectamized rats. Rats were treated i.v. with 1.0, 0.3, 0.1 or 0.03 mg/kg CDS-EE or vehicle. Twelve days later, serum LH was dampened 67%, 53%, 44% and 14% compared to controls and by day 18 decreased 76%, 46%, 16% and increased 2% in these respective groups. Only the highest dose group had significantly decreased serum LH on day 25. When body weight gain in these rats was monitored, significant and dose-related decreases were observed in rats treated with 0.1 mg/kg or higher doses. Significant decreases were maintained through the 25 day duration of the study. Intact female rats had a similar dose related decrease in body weight gain following a single i.v. dose. Significant decreases were maintained for 27, 12 and 3 days following treatment with 3.0, 1.0 and 0.3 mg/kg, respectively. Uterine weights did not show significant dose-related change. These data showing dose and time relationship of sustained drug action are consistent with the brain directed CDS. The marked effects on body weight gain support a centrally mediated action of E_2 in regulating weight gain in female rats. These pharmacokinetic and pharmacodynamic data indicate a 3- to 5-fold increased potency compared with similar studies of the CDS for estradiol. This increased activity may be attributed to increased brain drug delivery and/or related to the potency of hydrolyzed free EE.

301.16 CHANGES IN PROLACTIN FOLLOWING ACUTE VS CHRONIC ESTROGENIC STIMULI. G.E. Resch* and K. Fair* (SPON: J. Voogt). Div. of Structural and Systems Biol., Univ. Mo. - Kansas City, Kansas City, MO 64110.

Estrogenic action targeted on the brain modulates the function of neuroendocrine systems. Estrogen analogs have been useful tools in understanding estrogenic action. This report describes plasma prolactin (Prl) responses elicited during chronic and acute administration of estradiol (E2) and an estrogenic analog (F2) *in vivo*.

Initial experiments using daily drug injections in oil showed the expected Prl rise to E2. The rise in Prl under F2 stimulation was 50% below that for E2. The marked responsiveness of the CNS to F2 in these data is similar to that of endogenous estrogens.

Subsequently, acute experiments were conducted using intra-arterial bolus injections. These experiments were designed, in part, to study the time course of the Prl response. Serial blood sampling for Prl assay was taken with volume replacement to minimize total blood volume loss. Capillary tube hematocrits were used as an index of sampling stress, previously shown to elevate Prl.

Data show that F2 elicited a rise and fall in Prl levels, which mirrors that for E2. However, the onset of E2-elicited Prl was 30 min. earlier than for F2 and showed a graded increase over time to the peak value. The F2-elicited Prl values were smaller and less consistent in the early phases and reached peak values at the same time as for E2.

In another experiment, pituitary and uterine weights (organ/body weight ratios) showed a preferential increase to E2 vs F2 in pituitary, but not in uterine weight over the time course of the experiments. These data indicate a potent central (pituitary) effect compared to the uterine response.

Comparisons of these data, *in vivo*, with previous *in vitro* pituitary results indicate a slower onset and smaller rise in Prl to intra-arterially administered agents than was seen *in vitro*. Similarly, F2 elicited responses had longer latencies but were comparable to those elicited by E2. The study of estrogenic actions using F2 may facilitate a more detailed understanding of how the brain, as a target of endogenous humoral agents, regulates Prl release.

302.1 ISOLATION OF CDNA CLONES FROM RAT HIPPOCAMPUS RESPONSIVE TO GLUCOCORTICOIDS. J.N. Masters, N.R. Nichols and C.E. Finch. Andrus Gerontology Center, Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191

We have recently characterized the effects of corticosterone (CORT) on steady-state RNA levels in various brain regions of the male rat by 2-D gel electrophoresis of in vitro translation products (Nichols et al., 1986, Soc. Neuroscience Abstract 188.14, p691). In all regions examined, three translation products were increased (35, 33 and 20 kdalton polypeptides) and one product decreased (50 kdalton) in response to administered CORT. Furthermore, the 35, 33 and 20 kdalton products were increased in response to stress (Nichols et al., this vol.).

In order to further characterize these products and possibly increase our sensitivity in identifying other RNAs that change in response to CORT, we screened a rat hippocampal cDNA library in lambda gt10 by differential hybridization using cDNA derived from adrenalectomized (ADX) and ADX+CORT treated (3d) animals. From approximately 20,000 recombinants screened, 25 showed a differential response with the primary screen. Following the secondary screen, 7 clones were further characterized by binding purified phage DNA to nylon membranes in a slot blot apparatus and hybridizing duplicate blots with the ADX or ADX+CORT cDNA probes. Four of the seven clones were confirmed to respond to CORT; clones 1-1, 5-5 and 11-3 were increased 2-3 fold and clone 2-2 increased about 10 fold.

Total RNAs isolated from the hippocampus from three separate animal experiments(3 day treatment) were fractionated on a formaldehyde-agarose gel, transferred to a nylon membrane and probed with the insert of clone 2-2. An RNA of about 3.7kb was found to hybridize and showed a 10-fold response between ADX and ADX+CORT RNA. Another clone which did not change in response to CORT was used as a control in a duplicate blot.

Since the 35, 33 and 20 kdalton in vitro translation products show large increases to administered CORT, we speculate that clone 2-2 may represent one of these peptides. Hybrid arrested or selected translation will be used to confirm this hypothesis. Additionally, clones 1-1, 5-5 and 11-3 may represent RNAs which we did not detect in the original in vitro translation experiments which will need to be confirmed with other experiments.

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302.2 VIBRATORY STRESS ELLICITS RAPID GLUCOCORTICOID RESPONSES IN RAT HIPPOCAMPAL RNA. N.R. Nichols, A.S. Khan* L.K. Maedo* J.N. Masters and C.E. Finch. Andrus Gerontology Center, Dept. of Biological Sciences, Univ. of Southern Calif., Los Angeles, CA 90089-0191.

Rapid effects of glucocorticoids on rat hippocampus include stimulation of cellular metabolism, behavioral changes, and neuroendocrine regulation. Previously, we described select increases in three poly(A)-containing RNAs, coding for 35, 33, and 20 kdalton polypeptides, in response to administered corticosterone (CORT) in rat hippocampus, and also cortex, striatum, cerebellum and hypothalamus (Nichols et al., 1986, Soc. Neurosci. Abstract 188.14, p.691). We postulated that similar responses of large magnitude (5- to 50-fold) in different brain regions might represent a new class of stress responses in rodent brain. We now show that rapid increases within 2 hr of CORT treatment are seen for these RNAs. Moreover, similar increases are induced by the physiological stress of shaking for 2 hr in a cage mounted on a Dubnoff metabolic shaker. Hippocampal RNA responses in three groups of male Fisher 344 rats (intact, intact + stress, and adrenalectomized + stress) were compared by in vitro translation of isolated total RNA and subsequent 2-dimensional gel electrophoresis and fluorography. Translation products were quantitated on 10 d film exposures by computerized videodensitometry and densities were normalized between fluorographs with reference to unchanging spots (0.8- to 1.2-fold). Responses are expressed as fold-change with respect to intact and adrenalectomized (ADX) control values:

POLYPEPTIDE M_r	INTACT + STRESS / INTACT	INTACT + STRESS / ADX + STRESS	2 HR CORT / ADX
35 kdalton	5-fold	6-fold	12-fold
33 kdalton	1	14	20
20 kdalton	3	4	6

All three responses exhibited glucocorticoid specificity (RU 28362 $\geq$ CORT >> aldosterone > dihydrotestosterone = ADX control; e.g. 35 kdalton polypeptide fold-changes with respect to ADX control were 21, 13, 2, 1 and 1, respectively). These responses represent specific gene products which are being differentially regulated by elevated glucocorticoid levels and vibratory stress, and may be important in neuronal/glial functions governing behavioral and neuroendocrine events in the physiological adaptation to stress. Their relation to the well-known heat-shock/stress responses remains to be established.

Supported by John D. and Catherine T. MacArthur Foundation Research Program on Successful Aging, Office of Naval Research Grant #N00014-85-K-0770 and NIH Training Grant #T32-AG00037-09.

302.3 REGULATION OF GLUCOCORTICOID RECEPTOR MESSENGER RNA CONCENTRATIONS IN RAT BRAIN REGIONS.
N. Barden & A. Peiffer. Ontogénèse et Génétique Moléculaire, Le Centre Hospitalier de l'Université Laval, Quebec G1V 4G2, Canada.

We have studied the regulation of glucocorticoid receptor (GR) mRNA concentrations in two brain regions: the hippocampus, which has a particularly high GR content, and the hypothalamus, an important neuroendocrine integration site for the control of stress hormone secretion. Past studies have shown that both adrenal and gonadal steroids differentially influence glucocorticoid binding site concentrations depending upon the brain region. To determine if these changes are also exerted on GR mRNA concentrations, we have used a Northern blot assay to quantify GR mRNA. Total RNA was extracted from hypothalamus or hippocampus by a guanidium isothiocyante method and purified by centrifugation on CsCl density gradients followed by phenol/chloroform extraction. Northern blots were hybridized with [^{32}P]-labelled Riboprobe corresponding to a 2.2 Kb fragment of GR mRNA and quantified by densitometry. GR mRNA levels were expressed in relation to the ß-actin mRNA concentration of the same sample. Autoradiographic results indicated that the GR mRNA found in both hippocampus and hypothalamus migrated in the same 6.5-7Kb region as did GR mRNA isolated from liver or pituitary gland. The concentration of GR mRNA in hippocampus was approximately 70% greater than it was in the hypothalamus. We recently reported that ovariectomy increases and that estrogens suppress GR mRNA concentrations in rat pituitary gland. GR mRNA concentrations were also increased in the brain of bilaterally ovariectomized female rats 12 days after surgery. However, this effect was significantly greater in the hypothalamus than it was in the hippocampus. In contrast, the GR mRNA concentration of hippocampal tissue showed a greater decrease in response to exogenous corticosterone than did hypothalamic GR mRNA levels. The hypothalamic GR mRNA concentration of ovariectomized-adrenalectomized animals was greater than that of either ovariectomized only or adrenalectomized only animals. It remains to be determined by *in situ* hybridization whether or not these actions of adrenal and gonadal steroids are exerted on the same hypothalamic cell populations.

302.4 GLUCOCORTICOID RECEPTOR GENE EXPRESSION IN RAT PITUITARY GLAND INTERMEDIATE LOBE.
A. Peiffer & N. Barden. Ontogénèse et Génétique Moléculaire, Le Centre Hospitalier de l'Université Laval, Québec, Qc, G1V 4G2, Canada.

We have recently reported that estrogens influence glucocorticoid receptor (GR) mRNA concentrations in rat pituitary anterior lobe. The presence of GR mRNA and its regulation by estrogens was subsequently investigated in the rat pituitary intermediate lobe using Northern blot and *in situ* hybridization techniques. Female rats (250 g) which had been bilaterally ovariectomized under light ether anaesthesia were (at different times after surgery) perfused with 4% paraformaldehyde, pH 7.4 (for the *in situ* hybridization studies) or decapitated. Total RNA was isolated from intermediate lobe tissue by the guanidium isothiocyanate method and purified by centrifugation on CsCl gradients followed by phenol/chloroform extraction. Autoradiographic results of Northern blots hybridized with [^{32}P]-labelled Riboprobe corresponding to to a 2.2 Kb fragment of GR mRNA showed that GR mRNA concentrations are barely detectable or undetectable in normal animals. These findings concord with the lack of immunodetectable GR in the pituitary gland intermediate lobe of intact animals. In ovariectomized rats, however, a strong hybridization signal was given by the presence of a 6.5-7Kb GR mRNA. This GR mRNA species was identical, on Northern blots, to that found in liver and anterior pituitary gland. The effect of ovariectomy on intermediate lobe GR mRNA, which was detected at 12 days and was further increased at 4 weeks post-operatively, was reversed by administration of near-physiological doses of 17-ß-estradiol. *In situ* hybridization experiments on 10 µm frozen pituitary sections affixed to poly-L-lysine-coated slides were done in order to confirm the findings of the Northern blot studies. Slides were incubated with a [^{3}H]-labelled cRNA probe diluted in hybridization buffer (50% formamide, 10% dextran sulfate, 3x SSC,1x Denhardt's, 100 µg denatured salmon sperm DNA, 100 µg yeast tRNA) at a concentration of 3.0 x 10^6 cpm/ml,then washed, dried and coated with film emulsion. The results of these studies confirm our observations made in the Northern blot studies on the effect of ovariectomy on GR mRNA concentrations in rat pituitary intermediate lobe. It remains to be determined whether or not the GR mRNA induced by ovariectomy is translated into a functional receptor protein.

302.5 GLUCOCORTICOID REGULATION OF PREPROENKEPHALIN mRNA LEVEL IN RAT STRIATUM. H.M.Chao*, M.Blum[1], J.L.Roberts[1] and B.S.McEwen. Lab. of Neuroendocrinology, Rockefeller University, N.Y., N.Y. 10021 and [1]Dept. of Neurobiology, Mt. Sinai School of Medicine, N.Y., N.Y. 10029

In order to investigate the regulation of preproenkephalin mRNA in rat brain, we have applied a sensitive assay for determining mRNA levels. This assay employs a radioactively labeled antisense riboprobe derived from the rat brain preproenkephalin cDNA of S.Sabol. This riboprobe is hybridized in solution to total RNA from brain tissue and subsequently treated with RNAse T2, in a modification of the SP6 quantitative nuclease protection assay (Zinn, K., et al, Cell 34:865, 1983). Using this assay we determined that the relative abundance of preproenkephalin mRNA in total RNA from five specific brain regions was as follows: striatum >> hypothalamus, pons-medulla > cerebellum > hippocampus. In Northern analyses, this riboprobe hybridizes to a single mRNA species of approximately 1.5 kb, in these five brain regions.

Previous studies have shown that preproenkephalin gene expression can be regulated by hormones. Administration of dexamethasone results in an increase in immunoreactive enkephalin levels in rat striatum (Rossier, J., et al, Life Sci.25:2105, 1979). Moreover, in vitro experiments have indicated that glucocorticoid treatment can result in an increase in preproenkephalin mRNA levels (Yoshikawa, K. and S.L.Sabol, BBRC 139:1, 1986). Using the RNAse T2 protection assay , we compared the levels of striatal preproenkephalin mRNA in adrenalectomized (ADX) rats with those of ADX rats injected s.c. with corticosterone (40 mg/kg per day for 5 days). The animals treated with corticosterone showed an approximately 2-fold higher level of preproenkephalin mRNA than the ADX animals injected with vehicle. Parallel measurements of actin mRNA showed no such increase in actin gene expression with corticosterone treatment. Preproenkephalin mRNA levels in the other brain regions investigated (where the basal levels of expression are significantly lower than in striatum) seemed largely unaffected by the corticosterone treatment. Studies are currently in progress to determine whether stress will mimic the effects of glucocorticoids on the level of preproenkephalin mRNA in the striatum. (Supported by NS07080.)

302.6 ROLE OF THE PITUITARY-ADRENOCORTICAL AXIS IN THE CONTROL OF TYROSINE HYDROXYLASE (TH), PHENYLETHANOLAMINE-N-METHYL TRANSFERASE (PNMT) AND PROENKEPHALIN (pEK) mRNA AND ENKEPHALIN LEVELS IN THE SYMPATHOADRENAL SYSTEM. O.H. Viveros, P. Lee, R.J. Rigual*, J.-S. Hong, M.K. Stachowiak*. Laboratory of Behavioral and Neurological Toxicology, NIEHS, NIH , P.O. Box 12233, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

pEK A derived peptides are colocalized with catecholamines (CA) in adrenal medulla and sympathetic ganglia. During stress enkephalins are released together with CA and produce unique physiological effects. Recently we began to investigate the mechanisms which control hormonal output from the sympathoadrenal system. We have shown that the adaptation of adrenomedullary cells to enhanced transmitter release involves increased mRNA levels, synthesis and postranslational processing of CA enzymes and pEK. The purpose of this study was to examine roles of the pituitary-adrenocortical axis in the control of these processes in the rat adrenal medulla (AM) and superior cervical ganglia (SCG). Male Fisher rats were hypophysectomized (HPX) or sham-operated (SO) at the age of 8 weeks and were supplemented with 5% sucrose and saline. Two weeks later animals received injections of saline or dexamethasone (DEX, 1 mg/kg, i.p.) for 5 days, and were decapitated 12 hours after last injection. Total RNA and protein content decreased by 25-50% after hypophysectomy and were not restored by DEX. The relative abundance of specific mRNAs (units/mg total RNA) was estimated by dot blot hybridization and northern analysis using cloned TH, PNMT, pEK cDNA probes. The abundance of adrenomedullary PNMT mRNA decreased in HPX by 50%. DEX injections reversed these changes completely, but did not affect PNMT mRNA levels in SO. TH mRNA abundance in AM and SCG was not altered in HPX, but it was increased after DEX treatment (30-50% increase in both SO and HPX animals). pEK mRNA levels in SCG and AM were decreased after hypophysectomy by 16 and 27%, and responded to DEX treatment only in HPX (60-80% increase). To assess posible changes in enkephalin precursor processing both cryptic (CM - after trypsin and carboxypeptidase B digestions) and native (NM - without enzyme digestions)[Met-5]-enkephalin-like immunoreactivity was measured. In AM specific content of CM and NM (pmoles/mg protein) increased by 25% and 100% after hypophysectomy suggesting an enhanced processing of precursor peptides. This increase in NM/CM ratio was not affected by DEX. In SCG, hypophsectomy produced 35% decrease in NM/CM ratio, which was reversed by DEX. In conclusion, the relative abundances of TH, PNMT, and pEK mRNAs are differentially controlled by the pituitary-adrenocortical axis. This control is mediated by glucocorticoid receptors which also stimulate synthesis (AM) and processing (SCG) of enkephalins. In addition, the pituitary-adrenocortical axis exerts an inhibitory influence on the processing of adrenomedullary enkephalins by glucocorticoid independent mechanisms.

302.7 ROLE OF THE SPLANCHNIC NERVE ACTIVITY IN THE CONTROL OF TYROSINE HYDROXYLASE (TH), PHENYLETHANOLAMINE-N-METHYL TRANSFERASE (PNMT) AND PROENKEPHALIN (pEK) mRNA LEVELS IN THE RAT ADRENAL MEDULLA. J.-S. Hong, L. Thai*, R.J. Rigual*, O.H., Viveros, M.K. Stachowiak*, Laboratory of Behavioral and Neurological Toxicology, NIEHS, NIH, P.O. Box 12233, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

The effects of splanchnic nerve (SPN) stimulation on the levels of TH, PNMT, and pEK mRNA were investigated in the rat adrenal medulla. Male Fisher rats were anesthetized with chloral hydrate (320 mg/kg i.p.). Both adrenal glands were decentralized by transecting branches of SPN just below ganglia. Distal portion of the left transected nerve was stimulated at either 15 or 5 HZ, with 1 msec, 40 Volt pulses for 1 hour. Subsequently rats were sutured and allowed to recover from anesthesia and killed at different time intervals. TH, PNMT, and pEK mRNA levels were estimated by RNA-dot blot hybridization and northern analysis. Two to four hours after nerve transection, TH and pEK mRNA levels were increased by 30-60% in the decentralized side when compared to the nonoperated controls. Stimulation of SPN at 15 Hz resulted in additional increases. Increase in TH mRNA was found already 1 hour after the end of stimulation and the changes in pEK mRNA occurred 1 hour later: both increases achieved maximal levels (50-80%) 3 hours following the end of stimulation. In contrast, relative abundances of PNMT mRNA in denervated and stimulated glands were not changed. In another experiment, SPN branches were stimulated at 5 Hz. Two hours later TH, PNMT, and pEK mRNA levels in the stimulated gland were 21-35% lower than those of the nonstimulated side. To study possible mechanisms underlying the above-mentioned changes, rats were injected with atropine alone (1 mg/kg i.p.) or in combination with chlorisondamine (10 mg/kg i.p.) 10 min before anesthesia and at the end of 1 hour SPN stimulation. Atropine alone did not alter the effect of stimulation on mRNA levels. Combined treatment with both antagonists did not prevent the increases in mRNA levels in denervated adrenal medulla. However, such treatment completely reversed 15 Hz induced increases in mRNA levels of TH and pEK. In fact, decreases of mRNA levels (30-40%) compared to non-stimulated side were found. In contrast, 5 Hz stimulation-induced decreases in mRNA levels of TH, PNMT and pEK were not affected by atropine in combination with chlorisondamine. In conclusion, depending on stimulation frequency, SPN exerts both stimulatory and inhibitory effects on the expression of TH and pEK mRNA, but only an inhibitory effect on the expression of PNMT mRNA was observed. Stimulatory effects are mediated through nicotinic mechanisms, however, mechanisms underlying inhibitory effects remain unclear.

302.8 **DIFFERENTIAL REGULATION OF THE mRNAs FOR THE CATECHOLAMINE ENZYMES TYROSINE HYDROXYLASE AND PHENYLETHANOLAMINE N-METHYLTRANSFERASE IN BOVINE CHROMAFFIN CELLS.** *M.J. Evinger, J.M. Carroll, S.E. Hyman, H.M. Goodman*, and T.H. Joh.* Lab. of Molec. Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021 and Dept. Mol. Biol., Mass. Gen. Hosp., Boston, MA 02114

Tyrosine hydroxylase (TH) and phenylethanolamine N-methyltransferase (PNMT) are the enzymes responsible for synthesis of the catecholamine (CA) neurotransmitters dopamine and epinephrine, respectively. Because adrenergic cells of the brainstem and adrenal medulla express TH and PNMT, both coordinate and differential control of the genes for these two CA biosynthetic enzymes may be analyzed concurrently in these tissues. This study compares the transcriptional regulation of TH and PNMT mRNAs in response to major regulatory effectors of CA production in the adrenal gland. Specifically, hormonal and neural modulation of the steady state levels of TH and PNMT mRNAs have been analyzed in response to treatment with glucocorticoid hormones, to increased levels of cAMP and to depolarization with potassium.

Bovine adrenal medullae were dissociated by treatment with collagenase, fractionated by centrifugation on Percoll density gradients, and plated at a density of 3×10^5 cells/ cm^2 in DMEM:F12 (1:1) medium containing 10% fetal calf serum (FCS). Following treatment with a specific effector, cells were lysed with NP-40 (0.5%) and SDS (0.2%) and total RNAs, isolated by extraction with phenol and chloroform, were fractionated on agarose-formaldehyde gels. Northern blot analyses permitted the detection and quantitation of specific CA mRNAs by hybridization with either bovine TH cDNA (700 b.p. Kpn-Pst I fragment) or PNMT cDNA (1100 b.p.).

Glucocorticoids are required for maintenance of PNMT activity *in vivo* in the rat adrenal gland. We have previously demonstrated that both the rate of transcription and the steady-state levels of adrenal PNMT mRNA increase following treatment with dexamethasone in hypophysectomized rats. In the present studies, bovine adrenal chromaffin cells were established *in vitro* for 2 days in the presence of 10% FCS before replacement with serum-free or glucocorticoid-depleted media. Cultures were maintained for 3-5 days prior to treatment with 10 μM dexamethasone. In serum-free medium, PNMT mRNA increased significantly within 24 hr of treatment with dexamethasone. Moreover, an increase in PNMT mRNA was detectable within 5 hr of addition of exogenous hormone to cells in glucocorticoid-depleted medium. These results indicate that glucocorticoids exert a direct influence on production of bovine adrenal PNMT mRNA and that the effect observed *in vitro* is analogous to that observed in rat adrenal cells *in vivo*.

Synthesis of PNMT mRNA, however, responds differently to certain effectors which stimulate production of other CA enzyme mRNAs. Specifically, TH and PNMT mRNAs respond differently to forskolin, an activator of adenylate cyclase. In the presence of the phosphodiesterase inhibitor isobutylmethylxanthine, 1 and 10 μM forskolin produces a rapid and marked increase in TH mRNA, while the level of PNMT mRNA remains the same as that seen in control cultures. Therefore unlike TH mRNA, accumulation of PNMT mRNA is not stimulated by increased concentrations of the second messenger cAMP. Furthermore, depolarization of chromaffin cells by 50 mM K^+ in the presence of 5 mM Ca^{2+} results in an increase of TH mRNA although comparable changes were not observed for PNMT mRNA.

These studies suggest that modulation of CA mRNA levels constitutes one major means for exerting coordinate and differential regulation on the genes in the CA biosynthetic pathway. Moreover, these results indicate that bovine adrenal chromaffin cells *in vitro* provide an excellent system for investigating molecular mechanisms of CA gene transcriptional regulation.

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302.9 THE REGULATION OF TYROSINE HYDROXYLASE EXPRESSION IN THE PERIPHERAL NERVOUS SYSTEM OF THE RAT. D.R. Studelska*, R. Soriano*, M.I. Johnson and K.L. O'Malley (SPON: S. Brimijoin). Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Tyrosine hydroxylase (TH) is the initial and rate-limiting enzyme necessary for catecholamine biosynthesis in adrenergic neurons. The intracellular and transsynaptic regulation of its activity has been studied for many years. Now that the rat TH gene has been cloned, the study of the genetic regulation of TH production at the level of mRNA production is feasible. The present studies employed the technique of in situ hybridization.

Neurons were dissociated from embryonic superior cervical ganglia (SCG) and explanted on collagen-coated coverslips in serum-supplemented media containing fluorodeoxyuridine to destroy nonneuronal cells. The cultured neurons were then transferred to N2, a defined medium. The cells were then subjected to various treatments such as exposure to drugs thought to perturb TH expression, i.e., dexamethasone. Following treatment, cells were rinsed, fixed and stored in ETOH.

Anti-sense RNA probes were made from portions of a full length rat TH cDNA inserted in the Bluescript plasmid vector. Cells were rinsed and prehybridized at 42°C. The radiolabeled probe was then applied at a concentration of 1.2×10^6 DPM/ml in the same buffer for an overnight incubation at 42°c. The next day the cells were treated with RNase A and then washed at 42°C to remove unhybridized probe. The cells were then airdried, coated with Kodak NTB2 nuclear track emulsion, and kept in a light tight box for an exposure of one week before the grains were developed.

Dexamethasone increased TH mRNA production, however, the expression of TH message in the explanted cells was quite variable. This variability may reflect the heterogeneity of the SCG neurons, which innervate diverse tissues, and which are known to be quite different in their neuropeptide content. Since similar experiments in sectioned adult ganglia did not demonstrate as much variability, it is possible the variable expression in the cultured cells was due to maturational differences. TH message is also expressed in the cholinergic neurons of nodose ganglia, which are of placodal rather than neural crest origin. The comparison of the regulation of the TH gene in these anatomically contiguous, but embryologically distant neurons may provide insights into the regulatory mechanisms of genes that are integral to the function of neuronal transmission.

302.10 FLUPHENAZINE-N-MUSTARD (FNM)-INDUCED CHANGES IN LEVELS OF mRNAs FOR PREPROTACHYKININ (PPT) AND TYROSINE HYDROXYLASE (TH) IN THE STRIATUM AND SUBSTANTIA NIGRA (SN) OF THE MOUSE AS DETECTED BY IN SITU HYBRIDIZATION CYTOCHEMISTRY (ISHC). L.T. Weiss (1), N. Mahy (2)*, and M.-F. Chesselet (1), Depts. of Pharmacology, The Medical College of Pennsylvania (1) and University of Pennsylvania (2), Philadelphia, PA.

Chronic alteration of dopaminergic neurotransmission in the striatum and SN occurs in Parkinson's disease and with chronic neuroleptic treatment. In this study, we sought to determine whether blockade of dopaminergic receptors modifies the expression of specific genes in striatal and nigral neurons. Male Swiss Webster mice were injected 2 times, 8 hours apart, for 2 consecutive days with a dose of FNM (4 um/kg), which irreversibly blocks D_2 but not D_1 dopaminergic receptors. The mice were sacrificed the following day. The right striata were dissected out and used for HPLC analysis. The left side of the brains were frozen, cut on a cryostat and processed for ISHC using ^{35}S-labelled RNA probes with emulsion autoradiography (1). Levels of hybridized mRNAs were compared by visual and computer-assisted grain analysis. Probes complementary to mRNA for PPT (H.U. Affolter) coding for substance P and K, for TH (D. Chikaraishi) and glutamic acid decarboxylase (GAD; A.J. Tobin) were used. In FNM-treated mice, a decrease in labelling for PPT mRNA was observed in the striatum and for TH mRNA in the SN pars compacta. The specificity of these changes was assessed by the lack of modification of GAD mRNA levels in pallidal neurons of the same animals. The decrease in PPT mRNA in striatal neurons of FNM-treated mice confirms that interruption of dopaminergic transmission in the striatum decreases tachykinin gene expression and suggests an involvement of D_2 dopaminergic receptors in this effect. The decrease in TH mRNA in nigral neurons of FNM treated mice contrasted with a two-fold increase in the striatal turnover of dopamine (DA), measured as the ratio of the levels of the DA metabolite DOPAC to DA. This suggests that a compensatory decrease in TH gene expression occurs when dopaminergic nigrostriatal neurons are activated by sustained blockade of D_2 dopaminergic receptors. These results provide evidence that changes in specific gene expression measured in single cells by ISHC of specific mRNA's can be induced by short term blockade of striatal and nigral dopaminergic receptors and suggest that such changes can be involved in the adaptation of striatal and nigral neurons to changes in dopaminergic transmission.

1. Chesselet et al. J. Comp. Neurol., 1987, in press.
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302.11 INCREASED STRIATAL PREPROENKEPHALIN mRNA INDUCED BY HALOPERIDOL DECANOATE. M.R. Emmett*, P.L. Wood and B. Petrack, Neuroscience Research, Research Department, Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, New Jersey, 07901.

Haloperidol, injected daily into rats for 2-3 weeks, increased preproenkephalin mRNA in striatum but not in other brain regions (Tang et al, Proc. Natl. Acad. Sci., 80: 3841, 1983). The authors suggest that the antipsychotic action of haloperidol might result from its ability to increase striatal enkephalin levels. Recently, haloperidol decanoate, a pro-drug, has been used as a long-acting neuroleptic in schizophrenic patients. In the present study, we tested the possibility that two injections of haloperidol decanoate during a 3-week period might be as effective as daily injections of the parent drug in elevating preproenkephalin mRNA levels in rat striatum.

Four groups of Sprague-Dawley rats were injected according to the following schedule.

1. Haloperidol, 1 mg/kg, subcutaneously (s.c.), daily for 21 days.
2. s.c.-Control, saline, 0.2 ml, s.c., daily for 21 days.
3. Haloperidol decanoate, 20 mg/kg, intramuscularly (i.m.), Day 1 and Day 11.
4. i.m.-Control, saline, Day 1 and Day 11.

On Day 21, the rats were killed by decapitation. Brain regions were dissected, immediately frozen in liquid nitrogen and stored at -70°C. Liver was taken as a negative control.

Total RNA was extracted from the tissues and quantitated via both northern blot and slot blot analyses. The preproenkephalin mRNA was hybridized with a 911 base-pair fragment from pRPE-2/SP64, a plasmid containing the cDNA for rat preproenkephalin. The probe was labeled with ^{32}P-CTP, using a random priming procedure. Preproenkephalin cRNA, synthesized via a riboprobe system, was used as a positive control.

The probe identified an mRNA in striatum and hypothalamus (from all groups) of approximately 1400 bases, whereas no hybridization was observed with liver RNA. The amount of preproenkephalin mRNA in striatum from haloperidol and haloperidol decanoate treated rats was approximately triple that in striatum from the two groups of saline controls. Haloperidol decanoate also mimicked the parent drug in increasing preproenkephalin mRNA in slot blot analyses. In contrast, neither drug affected preproenkephalin mRNA levels in hypothalamus.

These studies demonstrate that haloperidol decanoate functions as a long-acting haloperidol in selectively elevating striatal preproenkephalin mRNA, consistent with its action as a pro-drug antipsychotic agent.

302.12 POST-MORTEM STABILITY OF PREPROENKEPHALIN mRNA IN RAT BRAIN. M.M. GARCIA* AND R.E. HARLAN. (Spon: C.H. Norris) Departments of Pharmacology and Anatomy, Tulane University School of Medicine, New Orleans, LA 70112.

The measurement of mRNA levels in brain tissue is becoming an increasingly important technique for the neuroscientist. As the use of such methods becomes more common, the influence of exogenous factors such as anesthesia on RNA levels and the stability of RNA in unfixed post-mortem tissue become matters of interest. The latter problem is especially of interest to those working with human tissue.

In order to study these questions, we killed 6 groups of rats (3-4 rats per group) by decapitation. The brains were removed from the skulls and frozen on dry ice at 0 min, 15 min, 1 hr, 4 hr or 8 hr after decapitation. One groups of animals at 0 min was anesthetized with pentobarbital prior to decapitation. Except for the zero-time group, in which brains were removed immediately, the brains were left in situ in the skulls at room temperature until removal for freezing. The brains were stored at -80°C until use. Total RNA was extracted from each brain using the guanidinium isothiocyanate-cesium chloride method, and the RNA content determined spectrophotometrically. One and 0.5 µg of RNA from each sample was dotted in duplicate onto nitrocellulose with vacuum filtration. The filter was air-dried and baked at 80°C under vacuum for 2 hr. The RNA on the filter was hybridized to a ^{32}P-labeled cDNA complementary to rat preproenkephalin (PPE) mRNA (gift of R. Howells). Hybrids were detected autoradiographically and quantitated with a scanning densitometer.

Analysis of these results showed no significant difference in PPE mRNA levels between the untreated rats and those treated with pentobarbital. There were also no significant differences between the 0 min values and those in the 15 min, 1 hr, 4 hr, or 8 hr groups. In addition, RNA from each sample was electrophoresed on a 1% agarose gel and stained with ethidium bromide. There was no detectable degradation of the 28S and 18S ribosomal RNA bands even 8 hours post-mortem.

These results suggest that PPE mRNA is stable in post-mortem, unfixed brain tissue for at least 8 hours. This should be of considerable interest and importance to those working with human models, while the additional finding that pentobarbital anethesia does not effect PPE mRNA levels should be of interest to those using animal models for the study of gene expression in the brain. Supported by NS24148

302.13 COMBINED IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY FOR CCK AND TYROSINE HYDROXYLASE IN RAT VENTRAL MIDBRAIN. K. Seroogy[1], M. Schalling[1], S. Brene*[1], A. Dagerlind*[1], S.Y. Chai*[1], H. Persson*[2], J. Dixon[3], D. Filer*[4], M. Goldstein[4], J. Walsh*[5] and T. Hökfelt*[1]. [1]Department of Histology, Karolinska Institutet, Stockholm, Sweden, [2]Department of Medical Genetics, Uppsala University, Uppsala, Sweden, [3]Department of Biochemistry, Purdue University, West Lafayette, IN, USA, [4]New York University, Medical Center, New York, NY, USA, [5]CURE, VA Medical Center-Wadsworth, Los Angeles, CA, USA.

As further analysis of dopamine/cholecystokinin (CCK) coexistence within neurons of the ventral midbrain at both the mRNA and post-translational product levels, we have combined the techniques of in situ hybridization and immunohistochemistry on the same tissue section. Normal and colchicine-treated rat brains (either unfixed or paraformaldehyde-fixed) were cut in a cryostat at 14 µm and sections through the ventral mesencephalon were thaw-mounted onto glass slides. Sections were initially processed for hybridization histochemistry using either cDNA probes for CCK and tyrosine hydroxylase (TH) (the biosynthetic enzyme for dopamine) labelled with $\alpha(^{35}S)$-dNTP's by nick-translation or, alternatively, synthetic oligonucleotide probes for both CCK (48 bp) and TH (48 bp) labelled on the 3' end with $\alpha(^{35}S)$-dATP using terminal deoxynucleotidyl transferase. Hybridization was conducted at 42°C for 16-18 h using $1\text{-}2\times10^6$ cpm per slide. Following X-ray film autoradiography, the sections were then processed for the simultaneous immunohistochemical detection of CCK and TH using a double-label immunofluorescence method and photographed. Finally, the slides were dipped in nuclear track emulsion, exposed and processed autoradiographically for cellular localization of CCK and TH mRNA.

Neurons throughout the substantia nigra (A9), ventral tegmental area (A10) and several midline nuclei were found to contain CCK or TH mRNA. The results obtained with either type of probe were the same. Both CCK and TH immunoreactivity were detected within TH or CCK mRNA-labelled perikarya, respectively. We are currently analyzing the effects of several neuroleptics on CCK and TH mRNA levels in ventral midbrain neurons. In additon, the distributions of CCK and TH mRNA-containing somata are being examined in the ventral mesencephalon of other species, including cat.

302.14 IN SITU HYBRIDIZATION HISTOCHEMISTRY OF TYROSINE HYDROXYLASE mRNA IN RAT BRAIN. Frank Baldino, Jr.*, Ariel Deutch, Robert H. Roth, Rudolph G. Krause, II*, and Michael E. Lewis. Medical Products Department, E. I. du Pont de Nemours, Wilmington DE and Department of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, CT.

Tyrosine hydroxylase is the rate-limiting enzyme in catecholamine biosynthesis in the CNS. Recently we have developed a synthetic oligonucleotide probe to resolve transcripts coding for this enzyme within individual neurons. Here we report the distribution and relative abundance of TH messenger RNA throughout the midbrain and brainstem regions of the rat CNS.

In situ hybridization and the appropriate controls were performed according to our previously published methods. A 30 base oligodeoxynucleotide probe, complementary to the 3' exon coding region of rat tyrosine hydroxylase mRNA, was used to detect transcripts coding for the rat TH precursor. Probes were radiolabeled on the 3' end using [^{35}S]dATP with terminal transferase according to our previously published procedures. Alternate 15 m sections were processed for immunohistochemistry using the avidin-biotin method with antibodies directed against the C-terminus of tyrosine hydroxylase. Hybridization signal was detected with emulsion autoradiography.

Localization of mRNA was characterized by an extremely dense accumulation of silver grains in the neuronal cytoplasm with little or no label over the nucleus. There was a marked concordance in the distribution of hybridization signal and TH-immunoreactive neurons. Labeled neurons were observed in the substantia nigra pars compacta, ventral tegmental area, locus coereleus, ventral lateral medulla, nucleus tractus solitarius, subcoereleus and arcuate nucleus. Destruction of the A8 neurons with 6-OHDA resulted in a loss of hybridization signal associated with these neurons. Thus, single cell resolution of this relatively rare transcript, coupled with immunodetection of TH, should provide the basis for further studes on the regulation of TH expression in the CNS.

302.15 COMPUTER-AIDED ANALYSIS OF CELLULAR RESOLUTION IN SITU HYBRIDIZATION HISTOCHEMISTRY IN BRAIN. W. T. Rogers*, J. S. Schwaber and M. E. Lewis, Engineering and Medical Products Departments, E. I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898 (SPON: M.D. Dibner)

We describe here a method for relative quantitation of mRNA levels with single neuron resolution. Following processing for in situ hybridization histochemistry, tissue sections are emulsion-dipped, exposed, and developed. The method relies first on locating autoradiographically-labeled cells by dark-field microscopic visualization of silver grains over counterstained neuronal profiles by a computer-aided method of "flying-field mapping" (Rogers et al., Applied Optics, '87). Each location is then stored in a digital database with an accuracy of 1 micron. Once a schematic map of the tissue section and of labeled neurons has been created, the stage is automatically driven to the location of each mapped neuron. Computer-based image analysis of sequentially located, high resolution, small fields containing individual cell images is then performed. Each cell image is digitized and subjected to a dynamically determined video threshold. We establish that the number of pixels above threshold is proportional to the number of silver grains by calibration against manually counted grains in a representative sampling of labeled cells. A sampling of background fields is also digitized and the average grain count subtracted from each cell's grain count. Maps of cell distributions are then constructed, with cell marks color coded for relative mRNA levels. These maps yield information heretofor unavailable on regional distribution of quantitative cellular expression of specific mRNA in brain. This method should greatly facilitate the analysis of changes in gene expression during development and following various physiological and pharmacological manipulations.

302.16 *IN SITU* HYBRIDIZATION FOR TYROSINE HYDROXYLASE: HYPOTHALAMIC DISTRIBUTION AND SEXUAL DIMORPHISM WITHIN THE ANTEROVENTRAL PERIVENTRICULAR NUCLEUS. R.B. Simerly and L.W. Swanson. Howard Hughes Medical Institute and The Salk Institute, La Jolla, CA 92037.

It is generally accepted that the hypothalamus contains 5 major dopaminergic cell groups which extend from rostral parts of the A14 and A15 groups, to the caudal A13, A12, and A11 cell groups. We have examined the distribution of cells within the hypothalamus that express mRNA encoding tyrosine hydroxylase (TH), the rate limiting enzyme for catecholamine biosynthesis, by applying *in situ* hybridization histochemistry in the rat brain with ^{35}S labeled cRNA probes (~10^7 cpm/ml). SP6 polymerase was used to transcribe antisense riboprobes from the 280 b.p. insert that was subcloned into the SP65 vector system by Drs. E. Lewis and D. Chikaraishi (see Lewis et al., J. Biol. Chem. 258:14632, '83) who generously provided this template for the present study. TH-mRNA containing cells were found in the region of each hypothalamic dopaminergic cell group with distributions that were virtually identical to those of TH-immunoreactive cells. Hypothalamic nuclei that contained heavily labeled cells include the anteroventral periventricular nucleus, suprachiasmatic preoptic nucleus, the preoptic and anterior parts of the periventricular nucleus, the paraventricular and arcuate nuclei, as well as the zona incerta, posterior hypothalamic area, and perifornical region of the lateral hypothalamus.

Of particular interest is the population of dopaminergic cells within the anteroventral periventricular nucleus (AVPv). As compared to males, the AVPv in female rats contains over 3 times as many dopaminergic cells, and this sexual dimorphism appears to be dependant on the perinatal levels of gonadal steroids (see Simerly et al., Neuroendocrinol., 40:501, '85). In order to evaluate the possibility of a similar sexual dimorphism in the number of cells that express TH mRNA we prepared 2 adjacent 1-in-3 series of 20µm thick sections through the AVPv of male and female Sprague-Dawley rats and processed one of the series for *in situ* hybridization histochemistry. The adjacent series of sections was processed for immunohistochemistry by using an antiserum against TH that was provided by Dr. T. Joh. Consistent with our earlier findings, over 3 times as many TH-immunoreactive cells were found within the AVPv of female rats as were found in males. In addition, a comparable sexual dimorphism was found for cells that express TH mRNA. No sexual differences were detected for either the number of TH-immunoreactive, or TH mRNA containing cells located within the ventrally adjacent suprachiasmatic preoptic nucleus.

These findings suggest that perinatal gonadal steroids specify the number of cells within the AVPv that express TH in detectable amounts by determining the number of cells that are capable of expressing sufficient quantities of TH message, as opposed to a sex-specific alteration in the post-translational processing of the enzyme.

302.17 TRANSNEURONAL REGULATION OF TYROSINE HYDROXYLASE mRNA EXPRESSION IN THE RODENT MAIN OLFACTORY BULB. M. E. Ehrlich, T.H. Joh, F.L. Margolis, and H. Baker. Dept. of Neurology, Cornell Univ. Med. Coll., New York, NY 10021 and Roche Inst. Mol. Biol., Nutley, NJ 07110.

Peripheral deafferentation of the main olfactory bulb produced by destruction of olfactory sensory neurons results in profound alterations in a population of juxtaglomerular dopamine neurons. In surgically deafferented rats and three weeks following chemical deafferentation (intranasal irrigation with 0.17M $ZnSO_4$) in mice, activity and immunoreactivity of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, were reduced to 10-20% of control levels. Several lines of evidence indicated that these dopamine neurons do not die. For example, in rat, the activity and immunoreactivity of aromatic amino acid decarboxylase, the second enzyme in dopamine biosynthesis, are only minimally decreased. In this study, we investigated the molecular mechanisms underlying the alteration in expression of the dopamine phenotype. Poly A^+ mRNA was isolated from control and chemically deafferented CD-1 mice, and the relative amounts of TH-like mRNA was determined by Northern blot analysis. The probe was a 400 bp nick-translated Eco RI - Kpn fragment of a rat TH cDNA. Hybridization to equal amounts of control mouse adrenal and olfactory bulb A^+ mRNA yielded a single band, of approximately the same intensity, at 1.9 Kb, the same size as that reported for rat adrenal and brain TH mRNA. Densitometric analysis of hybridization intensity to equal amounts of A^+ mRNA from control and chemically deafferented mouse olfactory bulb revealed an 85% decrease in hybridization. Therefore, a change in the stable amount of TH mRNA easily accounts for the loss in detectable TH protein. Further experiments are required to determine whether the alteration in TH mRNA levels may be attributed to a lower rate of TH gene transcription, or increased mRNA degradation. In addition, we demonstrated that olfactory marker protein mRNA, as previously reported (PNAS 84: 1704-1708, 1987), is present in olfactory bulb and interestingly, also decreased markedly following deafferentation. We conclude that transneuronal regulation of TH gene expression significantly affects TH activity and immunoreactivity in the rodent main olfactory bulb.

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302.18 **TYROSINE HYDROXYLASE IN THE YOUNG AND AGED MOUSE BRAIN - HYBRIDIZATION HISTOCHEMISTRY AND IMMUNOCYTOCHEMISTRY.** B.K. Gupta*[1], M. Gupta[1], M.H. Stoler*[2], L.H. Fossom[3], and A.W. Tank[3] (SPON: S. Demeter). Departments of Neurobiology and Anatomy[1], Pathology[2], and Pharmacology[3], U of R Sch. Med., Rochester, NY 14642.

Tyrosine hydroxylase (TH) is the rate-limiting enzyme in the biosynthesis of catecholamines. Immunohistochemical studies have demonstrated the presence of TH-positive dopaminergic neurons in substantia nigra (SN) and the ventral tegmental area (VTA). It has been reported previously that the activity of TH in the striatum decreases in aged rats compared with their younger counterparts. The present study was undertaken to investigate if this decrease is due to: (a) altered TH levels in the neurons of SN, (b) altered expression for TH mRNA in the dopaminergic neurons of SN, or (c) a decrease in the number of TH-positive neurons, and if such a decrease also may be present in the VTA of aged mice compared with the young adults. Male C57BL/6 young adult (2-3 months old) and aged (24 months old) mice were divided into four groups. Fresh pieces of substantia nigra and the ventral tegmental area were dissected out from one group of animals, frozen, and processed to detect TH levels. A second group was perfused intracardially with 4% paraformaldehyde. The brains were removed, 10μm thick sections were cut through the SN and VTA, and processed for in situ hybridization using ^{3}H-RNA probe complementary to TH mRNA. ^{3}H-labeled sense probe was used as negative control. Alternate sections were stained immunocytochemically for TH. Brains from similarly perfused third group were removed, 40μm thick serial sections were cut through SN and VTA and processed for TH immunocytochemistry. Fresh tissue samples were taken from a fourth group and TH mRNA levels were measured from SN and VTA using dot-blot and Northern blot analysis. In both SN and the VTA, silver grains were observed over dopaminergic cells that were positive immunocytochemically for TH in the alternate sections. Although the number of cells expressing TH mRNA using in situ hybridization in SN and the VTA were similar in both the young and aged mice, we are further investigating if the expression of TH per cell in the two age groups varies. Results of these studies will be presented.

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302PO MOLECULAR AND GENETIC ANALYSIS OF A GENE SELECTIVELY TRANSCRIBED IN PRIMARY NEURAL CULTURES OF *DROSOPHILA MELANOGASTER*. E.K. Neumann*, A. Furst*, and A.P. Mahowald*. (SPON: J. Kriegler) Dept. of Developmental Genetics and Anatomy, Case Western Reserve Univ. Sch. of Med., Cleveland, Ohio 44106.

We have previously described the isolation of a genomic clone containing a gene whose transcript is selectively expressed in embryonic neural cultures of *Drosophila*. The gene maps to the 23A region of the second chromosome near the Decapentaplegic complex. A 2.3 kb transcript appears at low levels in early embryos and increases at 14 hrs of embryogenesis. This is approximately the time we observe a five-fold increase of the transcript in the neural cultures. In addition, a 1.3 kb transcript, whose spatial and temporal pattern of expression is distinct from the 2.3 kb transcript, is transcribed nearby and in the opposite direction.

Initial results of strand-specific *in situ* hybridization experiments with cultured cells and embryonic sections reveal a localization of the transcript to neural cells and the salivary gland. Cultured cells show labelling only in a subset of neurons in some differentiated neural clusters and not to any other cell types. Hybridization with the opposite strand results in labelling of non-neural cell types and not neural clusters.

Three genetic deficiencies uncovering this region have allowed us to screen a set of 27 lethal mutations obtained from W. Gelbart, which are potential candidates for this gene. Nine of these mutants belong to one complementation group. Four out of the nine show lethal neural defects in embryos as visualized by anti-HRP. Northern, Southern, and *in situ* hybridization analyses of the mutants are now underway to determine a correlation between the mutants and the gene.

303.1 SACCADIC REACTIONS TO DOUBLE-STEP STIMULI: THE INFLUENCE OF MOVEMENT AMENDMENT DIRECTION. G.K. Kerr* and R.J. Lockwood*. (SPON: S. Dunlop). Neuromuscular Lab., Dept. of Human Movement Studies, Univ. of Western Australia, Nedlands 6009, Australia.

The response of the saccadic control system to continuing (C) or reversing (R) a previously initiated saccade was assessed by means of double-step stimuli. Six subjects practised an initial 15 degree horizontal saccade to a central 0.12 degree target on day 1. On two subsequent days a 10 degree C or R amendment of the initial saccade was required. Initial saccades were triggered by a pulse step of the start target and for movement amendments by a second target step at one of eight different inter-stimulus intervals on 57% of trials. Eye movements were recorded by EOG with head movements being restricted by a rigid head and chin support.

Plots of initial movement amplitudes and inter-response intervals as a function of the amount of shared processing time (TO) of the two stimuli revealed two phases of movement amendment. When TO was less than 105ms (SD=18.85) the initial saccade was lengthened (C) or shortened (R) prior to producing a second saccade. This appeared to be accomplished by modulation of the peak velocity. As TO decreased from 105ms the time taken to produce a second saccade increased to reach a peak at almost zero overlap (TO= -1.29ms, SD=8.77). This rate of increase was greater for R than C amendments ($t(5)=3.759$, $p=.0132$).

The results support a continuous parallel processing model of visual afferent information (Becker and Jurgens, Vis. Res. 19:967-983, 1979). An initial refractory period, during which a second saccade cannot be generated, results from a prior modification of the initial saccade. This period is analagous to a duty cycle of action, reported for ballistically initiated arm movements, which must be completed before a second response can occur (Terzuolo et al., Brain Res. 58:217-222, 1973). Subsequent delays are dependent on the direction of the amendment and indicate that the initial patterns of neural and muscular discharge may be the limiting factors.

303.2 SACCADES TO TARGETS IN VISUAL FIELDS CONTRALATERAL TO OCCIPITAL LOBECTOMIES A.D.Epstein*, R.J.Tusa, S.E.Gordon*, and N.R.Miller* (SPON: R.S.Fisher). Departments of Neurology and Ophthalmology, Johns Hopkins University, Baltimore, Maryland 21205-2191.

Previous studies suggest that patients with occipital lobe strokes have the ability to make accurate saccades to targets in the opposite, "blind" visual field. Several objections have been raised to these studies: 1)possibility of residual striate cortex, 2)scatter of target light into the intact field, 3)failure to stabilize the head, and 4)lack of eye movement recordings.

We tested three patients with complete homonymous hemianopias produced by 6, 7.5, and 10 cm occipital lobectomies performed 14, 8, and 10 years before entry. Each target was presented unpredictably 10-60° away from the previous target in the horizontal meridian, and was generated by either 3 fL light-emitting diodes (LED) or 1000 fL He-Ne laser spots. The patient had to fixate the target light before being presented with a new one. Each patient performed 700-900 trials. The head was stabilized with a bite bar, and eye movements were recorded by electro-oculography.

With LED targets there was a weak correlation of the amplitude of the first saccade and target step size into the blind field. The mean value of all first saccade amplitudes to 20, 40, and 60° target steps from all positions is shown in line A of the table. This trend disappeared when the amplitudes of the first saccade initiated from only one orbital position were analyzed (line B). This suggests that these were target-searching saccades whose amplitudes were affected by the orbital position from which the saccade started. With laser targets the amplitude of the first saccade in one patient correlated with target step size even for saccades initiated from the same orbital position (line C). This patient had the smallest lesion and was age 9 at operation, whereas the others were 14 and 25.

These findings suggest that some occipital lobectomy patients have the ability to make reasonably accurate saccades to targets presented in their blind fields. This ability may depend on high stimulus intensity, minimal lesion size, and minimal age at operation.

Target step size ->		20°	40°	60°
Amplitude of first saccade	A	12.8	16.5	17.1
	B	17.3	21.9	17.1
	C	17.5	23.9	44.3

(Support: NIH 5T32-EY07047-09 and the Heed Foundation)

303.3 **SACCADIC EYE MOVEMENTS WITHOUT VISUAL FEEDBACK: MEMORY-LINKED SENSORIMOTOR SPATIAL TRANSFORMATION.** J.W.Gnadt, R.M.Bracewell*, R.A.Andersen. The Salk Institute, La Jolla, CA 92037

Three rhesus and 5 human subjects were trained to make saccade-like movements to remembered target locations defined by visual targets. Experiments were performed in a dark room. Because no visual feedback was available during the delay between target presentation and eye movement, nor during the eye movement itself, the task afforded a behavioral measure of memory-linked spatial sensorimotor transformation. Eye movements were measured using the scleral search coil technique. Compared to control saccades made to visible targets, saccades to remembered target locations were grossly spatially distorted (upwards saccades hypermetric, downward saccades hypometric), often had markedly curved trajectories, had reduced velocities, and were more variable. The precise form of the distortion was idiosyncratic. Subjects received different degrees of feedback of targeting accuracy: For monkey M02, the target came back on after the eye movement. Monkeys M13 and M88 were rewarded for successfully completing the movements with a spatial accuracy of ± 10 deg. or ± 20 deg., respectively; no visual feedback was presented. The distortion was smallest for monkey M02 (mean vertical error = +2.0 deg., $p<.01$; mean horizontal error = +0.7 deg., NS), intermediate for monkey M13 (vertical = +5.8 deg., $p<.01$; horizontal = +1.7 deg., $p<.01$), and largest for monkey M88 (vertical = +8.7 deg., $p<.01$; horizontal = +3.5 deg., $p<.01$). Humans received no feedback during trials. For humans, the mean errors were +2.2 deg. vertical ($p<.01$), and +2.0 horizontal ($p<.01$). There were significant nonlinear interactions between direction and length of eye movements for the 2 monkeys and 2 humans tested. Although most movements had an upward component of error, each direction of movement had a unique direction and magnitude of error. Main sequence analysis revealed that movements to remembered targets had slower velocities than corresponding movements to visible targets. The error accumulated rapidly for the first 500 ms of response delay with little additional accumulation. The addition of visual cues improved performance significantly in the one monkey and one human tested: The spatial accuracy and movement dynamics improved slightly in a dimly lit room and substantially when a random dot pattern was superimposed on the viewing screen. Analysis of equivalent eye movements originating from different orbital positions demonstrated similar, though statistically different, patterns of distortion in each of 2 monkeys and 2 humans tested. The similarities demonstrated that the distortions were oculocentric (i.e. retinotopic or related to movement vector) and not to a tendency to look towards a specific orbital or spatial position. The differences, however, suggested that the trajectories of movement were not the only factors involved in the distortions.

303.4 EYE MOVEMENTS EVOKED FROM SITES BETWEEN THE FRONTAL AND SUPPLEMENTARY EYE FIELDS. A. R. Mitz and M. Godschalk. Laboratory of Neurophysiology, National Institute of Mental Health, Bethesda, MD 20892.

Two eye fields have been identified in the monkey frontal lobe with the aid of low current (under 50 μA) microstimulation: the frontal eye field (FEF) in the rostral bank of the arcuate sulcus, within area 8 (Bruce et al., J. Neurophysiol. 54:714, 1985) and the supplementary eye field (SEF), on the medial aspect of area 6, slightly rostral to the FEF (Schlag and Schlag-Rey, J. Neurophysiol. 57:179, 1987). By using a modified microstimulation technique (Mitz and Wise, J. Neurosci., 7:1010, 1987) we have found a continuous representation of eye movements from the FEF to the SEF.

A recording chamber was implanted over areas 6 and 8 in each of 2 rhesus monkeys. Current pulses of up to 65 μA were delivered through glass-coated platinum-irridium electrodes every 200 μm in depth along 150 tracks in each monkey. The stimulation electrodes had tip exposures of 1000 μm^2, 5 to 10 times greater than typically used for microstimulation. Constant current, biphasic (negative-positive, 0.2 ms each phase) pulses were delivered at 330 pulses/s in trains of 31 pulses. Eye movements were watched by two observers in both monkeys and, in the second monkey, recorded by infrared oculometry.

Due to the effectiveness of the unique electrode and long stimulus trains, the boundaries of the FEF and of the SEF were not distinct. Including the FEF and SEF, conjugate eye movements were evoked from a 3-6 mm wide cortical strip, 21 mm in mediolateral extent. Movement thresholds were usually below 40 μA, but only occasionally below 15 μA outside area 8. Stimulation at sites in medial area 8 and adjacent area 6 evoked either conjugate eye movements, orofacial movements, or both. Eye movements evoked from this general region were typical for FEF stimulation sites, i.e. repeated stimuli delivered at a given site evoked conjugate eye movements of a fixed direction and angular displacement. More medially, evoked movements were more like some described for stimulation in the SEF, i.e. conjugate saccades towards a single final (contralateral) eye position. The direction, displacement and reliability of each saccade depended upon the initial eye position. In addition, saccades seemed to be less reliably evoked if the animal was visually fixating or tracking an object in space. Saccades were more reliably evoked if the initial eye position was in the ipsilateral hemifield.

From these results we infer that there is a continuum of eye movement representation from the FEF to the SEF, and possibly a gradual functional transition between these two areas.

303.5 FUNCTIONAL AND ANATOMICAL RELATIONS OF CENTRAL THALAMUS AND CORTICAL OCULOMOTOR AREAS. M. Schlag-Rey, I.M. Jeffers, S. Sampogna, and J. Schlag. Dept. of Anatomy and BRI, UCLA, Los Angeles, CA 90024-1763.

The central thalamus (intralaminar and paralaminar complex = IMLc) is known to carry oculomotor-related signals. Connections of the IMLc with the major cortical areas controlling the gaze - frontal eye field (FEF), supplementary eye field (SEF), inferior parietal lobule (IPL) - have been demonstrated but which signals are transmitted through which projections is unknown. The purpose of this study was to clarify our understanding of the cortico-thalamic network concerned with eye movements by using a combined physiological and anatomical approach.

In 4 trained Macaca nemestrina implanted with eye coils the IMLc was probed with microstimulation (20 to 40 cathodal pulses, 0.2 ms duration, 250 Hz, 10-80 μA). A consistent topographical organization was observed: goal-directed saccades were evoked caudally and laterally, fixed-vector saccades rostrally and medially. Following the systematic mapping of the IMLc by microstimulation in one hemisphere, a small injection of tracer (WGA-HRP or Bisbenzimide) was placed in SEF or FEF, in the same or opposite hemisphere. Control injections were placed in SMA or area 4 (2 additional monkeys).

WGA-HRP injected at a focal SEF site yielding goal-directed saccades labeled the caudal IMLc near or at the site where similar goal-directed saccades had been evoked. This result parallels our previous finding that units encoding eye position are more frequently encountered in the caudal part of the IMLc than in the rostral one. Label from SEF sites yielding small, fixed-vector saccades was found in more anterior and medial parts of IMLc but not at its most rostral level.

A small injection in the FEF region known to produce small saccades (confirmed by the labeling of the anterior superior colliculus) produced discrete labels in the mediodorsal and intralaminar nuclei, partially overlapping the region labeled by SEF. Some thalamic sites (particularly ventral ones) yielding small saccades (<5°) at low threshold (20 μA) were not labeled by either FEF or SEF injections.

In contrast with SEF and FEF results, injections placed in area 4 and SMA failed to label the oculomotor IMLc. Compared at the same antero-posterior levels, small injections of FEF, SEF, SMA and area 4 labeled distinct thalamic zones organized from the most medial (FEF) to the most lateral (area 4). (Supported by USPHS grants EY02305, EY05879 and NS07898).

303.6 *VISUAL NEURONS IN THE SUPERIOR TEMPORAL SULCUS (STS) OF BEHAVING MACAQUE MONKEYS CAN DISCRIMINATE PASSIVE AND SELF-INDUCED RETINAL SLIP.* *R.G. Erickson*, P. Thier* and W. Koehler*. (Spon: M.Slaughter). Department of Neurology, University of Tübingen, 7400 Tübingen W. Germany.*

The ability to maintain a constant spatial reference requires discrimination between externally and self-induced retinal slip.

*To test the hypothesis that this discrimination is reflected in the response profiles of directionally selective cortical visual neurons, the activity of single units in the motion sensitive visual areas of the STS was recorded and their responses to passive and self-induced retinal slip were compared. The cells recorded so far appear to fall into two broad categories: 1) Large field (LF) directional cells (eg 40*40deg or larger) on the anterior bank that respond to passive movement of any size stimulus (up to 90*90deg) in the preferred direction, and at least some of which respond even to rotation of a fullfield optokinetic drum. Despite the lack of evidence for inhibitory surrounds, none of the LF cells tested responded to the same visual movement occurring as a consequence of active eye movements. 2) Small field (SF) directional neurons (eg 5*5deg or smaller, less than 10deg eccentricity) on the posterior bank, a portion of which responded only to passive visual stimulation and not to stimulus movement generated by active eye movements. Some of these cells did not respond to large patterns and may have silent inhibitory surrounds. Other SF neurons responded identically to both active and passive stimulation.*

Our results clearly show that many neurons in the STS can discriminate self-induced and passively generated retinal slip. There are two mechanisms that could account for this ability. One is the reception of an efference copy signal (Holst, von and Mittelstaedt, Naturwissenschaften 37,1950). The recent finding of motion sensitive cells with antagonistic silent inhibitory surrounds, however, has led to the suggestion that purely visual mechanisms could also cancel the effect of self-induced retinal slip (Allman et al., Ann. Rev. Neurosci. 8, 1985), thereby mimicing the effect of an efference copy. Although the responses of SF neurons could be accounted for by visual mechanisms, the response properties of LF neurons leave open the possibility that they receive a true efference copy signal.

303.7 INTERACTION OF VISUALLY GUIDED SACCADES WITH SACCADES INDUCED BY ELECTRICAL STIMULATION OF POSTERIOR PARIETAL CORTEX. D.D.Kurylo* (SPON: A.A.Skavenski). Psychology Department, Northeastern University, Boston, MA 02115.

This was an extension of my prior work which demonstrated that saccades can be elicited by micro-electrical stimulation of two discrete zones of posterior parietal cortex (PPC). The extension involved exploring the way in which these electrically induced saccades interact with normal visually guided saccades. Electrical stimulation was made to sites within PPC of two alert nemestrina monkeys while they performed saccadic eye movements to follow a target which was stepped to an eccentric position. Onset time of electrical stimulation was varied to occur before, during, or after the onset of eccentric visual targets. Stimuli were arranged to yield saccades in orthogonal directions. There was a competition between the two types of saccades which prevented their simultaneous production. When electrical stimulation started anytime during the latency period of the visual saccade, that saccade was aborted and only the electrical saccade appeared. Vector summation of the two saccades was never seen. With sufficient temporal separation of the two stimuli, both saccades occurred separated by a latency of no less than 170 msec. Visually guided saccades remained accurate even when the visual target went off before either saccade started. These interactions of electrical stimulation and visually guided saccades suggest that the execusion of these two types of saccades are mutually exclusive, probably due to the utilization of common elements during their programming.

303.8 DOES MICROSTIMULATION GENERATE THE TRAJECTORY OR SPECIFY THE GOAL OF FIXED-VECTOR SACCADES? J. Schlag and M. Schlag-Rey. Dept. of Anatomy and BRI, UCLA, Los Angeles, CA 90024-1763.

Electrical stimulation (250 Hz) was performed at several sites of the monkey's forebrain which produced fixed vector saccades when the eyes were steady at stimulation onset. Twelve sites were studied in the central thalamus, 4 in the arcuate frontal eye field, and 11 in the supplementary eye field. Latencies ranged from 60 to 120 ms, threshold current from 10 to 100 μA. When the same stimulus trains were applied during or immediately after a spontaneous eye movement, the saccade trajectory was considerably modified in all cases: the eyes were driven from wherever they happened to be after the spontaneous movement to the destination where the fixed-vector saccade would have brought them, had no spontaneous movement occurred. Thus, the transformed trajectory of the evoked saccade compensated for the preceding spontaneous deviation of the eyes. This consistently occurred if the stimulation started within 10 to 150 ms from the onset of the spontaneous saccade. At 150 to ~ 200 ms intervals, the saccade course became less predictable. After ~ 200 ms, evoked saccades recovered their fixed-vector characteristics: they moved the eyes for a fixed distance in a fixed direction from the position after the spontaneous saccade.

This finding has several implications: 1) It suggests that the "fixed-vector" saccades studied were directed toward a goal defined with respect to an eye position sampled before the spontaneous movement. Our hypothesis is that the electrical stimulation evoked a retinotopic goal (such as the retinotopic representation of a target) whose spatial coordinates were then computed at a further stage to calculate the appropriate saccadic vector. 2) The signal of eye position which serves as reference to locate the goal must be internally delayed to compensate for the long time taken for transmission and processing of visual input signals.

Evoked goal-directed saccades (converging toward a point in space) were not modified in their course when coming on the trail of a spontaneous eye movement. Theoretically, fixed-vector saccades due to the elicitation of a motor error signal should not be altered either. If this is confirmed, the present paradigm may be valuable for testing whether a given brain site processes a visual error or a motor error signal. (Supported by USPHS grants EY02305 and EY05879).

303.9 LATERAL HEAD AND BODY MOVEMENTS DUE TO ELECTRICAL STIMULATION OF FIVE SEPARATE AXON BUNDLES. E.J. Tehovnik, J.S. Yeomans and K. Buckenham*, Dept. of Psychology, Univ. Toronto, Toronto, Canada M5S 1A1.

Lateral eye, head, and circling movements are evoked by electrical stimulation in many brain regions from the frontal cortex to the pons. These movements are due to activation of at least five separate axon bundles in rats. Videotapes showing circling movements in each of these bundles have been analyzed and are available. Within each bundle, similar behaviors and refractory period distributions are observed. Collision-like effects are observed within each bundle when stimulating two points concurrently, which defines the trajectory of these axons, and allows estimation of the distribution of conduction velocities (e.g., Yeomans & Linney, 1985). Collision-like effects have never been seen when stimulating between different bundles.

The most reliable lateral movements occur in the uncrossed tegmentobulbar bundle near the midline anywhere from rostral midbrain tegmentum to caudal pons. No habituation to maintained stimulation occurs. The conduction velocities range from 2 to 20 m/sec. Less reliable circling occurs in superior colliculus, because two bundles producing opposite movements originate in the tectum. The crossed tectoreticulospinal bundle produces contraversive circling and dominates in most sites (Sahibzada et al., 1986; Ellard & Goodale, 1986). The uncrossed tectopontine bundle produces ipsiversive turning which is less smooth and more "cringing". Ipsiversive circling is observed when the crossed axons are cut. The conduction velocities of these tectal axons are slightly slower than the tegmentobulbar axons.

Over 90% collision has been obtained between substantia nigra and internal capsule (Tehovnik & Yeomans, 1986). Habituation to maintained stimulation occurs in 30-90 sec. The conduction velocities (0.9 to 4.4 m/sec) and trajectories are similar to striatonigral axons, although too fast to be nigrostriatal dopamine axons. The only lateral movement sites producing collision with anteromedial cortex are in the rostral striatum, suggesting that the corticostriate path also produces circling. The responses are very unreliable, require high currents, and often seizures are seen. Habituation occurs within 10 sec.

We propose that lateral eye, head and body movements are due to activation of corticostriate, striatonigral, tectopontine, tectoreticulospinal or tegmentobulbuar bundles.

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303.10 MULTIMODAL INTERACTIONS IN SUPERIOR COLLICULUS: INDEPENDENT EFFECTS ON ON-, OFF-, AND PREMOTOR RESPONSES OF INDIVIDUAL CELLS. Carol K. Peck and Franklin S. Wartman III. School of Optometry, University of Missouri-St. Louis, St. Louis, MO 63121.

Most neurons in the intermediate and deep layers of the superior colliculus respond to both the onset and offset of visual targets through their receptive field. Moreover, visual, auditory and somatosensory inputs converge on single cells in these laminae, and their simultaneous presentation can markedly enhance or depress the activity of a given neuron. The present study shows that individual cells often show selective enhancement of their on-, off- and premotor responses.

Multimodal (visual/auditory) cells were identified in extracellular recordings from 3 chronically-prepared, alert, trained cats. The head was free to move in the horizontal plane (up to ± 45 deg.). Horizontal and vertical components of gaze (eye position in space) were measured with a scleral search coil technique. Horizontal head position was measured with a low-torque linear potentiometer, and horizontal eye position (in the head) was calculated from these signals. Cells were tested while the cat performed a delayed saccade task. Gaze saccades (with head free) and eye saccades (with head fixed) were made toward, or close to, the center of the cell's movement field. Response latency, duration, mean impulse and number of impulses per trial were measured. Single-modality and combined-modality trials were interleaved.

Forty-five cells were studied, and complete data sets (on-, off- and movement-related) were collected from 25 of these. In a significant number of cells (11/25) for which complete data sets were collected, presentation of multimodal (visual/auditory) stimuli had differential effects upon the on-, off-, and movement-related responses of individual collicular neurons. Multimodal depression was generally greatest in the on-responses of cells showing long-latency tonic responses to the onset of a target in either modality. Such cells typically discharged phasically in response to the offset of a target in either modality and exhibited little, if any, multimodal enhancement (or depression) of the off-response. Movement-related discharges were often more vigorous with visual-auditory targets than with either modality alone.

These data suggest that, in some collicular neurons, neither the presence nor the sign (enhancement or depression) of multimodal sensory interactions permit simple predictions of the effects of multimodal targets on that cell's premotor responses during controlled orienting behavior. Supported by USPHS grant NS-21238 and by the Weldon Spring fund of the University of Missouri.

303.11 **THE EFFECT OF FRONTAL EYE FIELD AND SUPERIOR COLLICULUS LESIONS ON THE SACCADIC AND PURSUIT EYE MOVEMENT INITIATION.** P.H. Schiller, N.K. Logothetis. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA

In humans and monkeys smooth-pursuit eye movements (EM) can be generated with considerably shorter latencies than can saccadic EM. This fact is one of several observations suggesting that the pursuit and saccadic systems are controlled by different neural mechanisms. Ablation studies have shown that the latency of saccadic EM in monkeys is increased following superior colliculus (SC) but not frontal eye field (FEF) lesions; FEF lesions on the other hand produce deficits in predictive pursuit EM, suggesting that this area may be involved both in saccadic and pursuit EM generation. In order to further examine this question the effect of FEF and SC lesions were examined on the latency of pursuit and on saccadic EM initiation.

Monkeys were trained to fixate a target. After a variable delay the target was turned off and reappeared several degrees to the left or to the right while performing a ramp movement at various velocities. The animal's task was to saccade to the moving target. Accomplishment of the task was rewarded with drops of apple juice. Because of the shorter latency of pursuit initiation, pursuit movement began prior to the saccade to the moving target (usually within 80 to 100 ms in our situation) and then continued after target acquisition suggesting that the pursuit system can use pre-saccade image velocity information from peripheral retina to set the pre and post-saccadic pursuit velocity whenever a ramp movement is initiated in the periphery. The effects of FEF and SC lesions were determined on the performance of this task.

Our results showed increased saccadic latencies following SC lesions and changes in the metrics of saccades following FEF lesion. The pursuit performance of the animal was unaffected by either lesion. Both computation of the pre-saccadic velocity and saccade size through this velocity information were unaffected.

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303.12 SACCADE-RELATED BURST CELLS IN THE SUPERIOR COLLICULUS: RELATIONSHIP OF ACTIVITY WITH SACCADIC VELOCITY. W.H. Rohrer*, J.M. White*, and D.L. Sparks. Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, AL 35294.

Reversible inactivation of the superior colliculus (SC) produces dramatic alterations in the direction, amplitude and velocity of visually-guided saccades (Hikosaka & Wurtz, 1986). However, cells related to saccadic velocity have not been reported in the primate SC. The saccade-related burst cells of the SC generate a high frequency discharge beginning 18-20 msec before saccade onset. This saccade-related burst encodes the direction and amplitude of a saccade by the location of the active neuronal population, not by the frequency or other characteristics of the burst (Sparks & Mays, 1980). Sparks and Mays failed to find a relationship between saccadic velocity and the parameters of the burst (Sparks & Mays, unpublished observations) but they studied only visually-guided movements which have a narrow range of velocities for equal amplitude movements. The range of saccadic velocities can be extended by requiring saccades to the remembered location of a target as well as saccades to a continuously present target. We used these tasks to re-examine the burst characteristics of saccade-related cells in the SC preceding saccades covering a wide range of velocities.

Rhesus monkeys, with implanted scleral search coils, were trained to perform two behavioral tasks: one requiring a saccade to a continuously present visual target (visually-guided saccade), the other requiring a saccade to the location of a briefly flashed target (remembered saccade). Extracellular recordings were made from single units in the deep layers of the superior colliculus during visually guided and remembered saccades.

We selected remembered and visually-guided saccades matched for direction and amplitude (within 1 °). The velocity of remembered saccades was lower and their duration lengthened relative to visually-guided saccades. The mean spike rate and peak frequency (for the interval beginning 30 msec before saccade onset and ending 20 msec after saccade onset) of most saccade-related cells were positively correlated with the peak velocity of the saccade. Some cells which burst for visually-guided saccades did not burst at all during remembered saccades. Only a few cells fired as vigorously before remembered as before visually-guided saccades.

These results (and those of Berthoz et al., 1986 for the cat) suggest that in addition to encoding the amplitude and direction of saccades by location of cell activity within the topographic map, the superior colliculus can influence saccadic velocity. Variations in saccadic velocity may reflect changes in both the size and level of activity of the active population of collicular burst neurons. (Supported by NIH EY05486 and EY01189).

303.13 **SACCADE ENDPOINT SCATTER AND PROPERTIES OF THE COLLICULAR MOTOR MAP.** J.A.M. Van Gisbergen* and A.J. Van Opstal*, (SPON: C. Gielen). Dept. Med. Phys. Biophys., Univ. of Nijmegen, 6525 EZ Nijmegen, The Netherlands

According to Deubel (1987), human visually-elicited saccades have relatively more scatter in amplitude (R) than in direction (Φ). We have found that this holds also for saccades obtained by collicular electrical stimulation in the rhesus monkey. To explore how collicular mechanisms may cause saccade precision to be different along the R and Φ dimensions, we have made simulations with a slightly extended version of a recent model for the role of collicular ensemble coding in the saccadic system (Van Gisbergen et al., Neuroscience, 1987). This model has an anisotropic logarithmic mapping from the retina onto the motor colliculus whereby the target creates a dome-shaped motor activity profile. The four parameters specifying the mapping and the spatial extent of the motor activity were estimated earlier by Ottes et al., (Vision Res., 26: 857-873, 1986) from electrophysiological data. The efferent section of the model specifies how neural activity in the motor map is translated into a normometric movement. To account for saccade endpoint scatter, the location of maximum motor activity has a small trial-to-trial variability around the mean position. This noisy variation in the afferent mapping of the model is characterized by a Gaussian distribution (standard deviation: s mm) which is translation invariant when target position is changed.

Simulations show how saccade endpoint scatter depends upon the properties of the model: 1) The nonhomogeneity of the logarithmic afferent mapping causes endpoint scatter to increase with saccade amplitude; 2) The amount of R vs Φ scatter depends upon the degree of anisotropy in the afferent mapping.

We have measured R- and Φ-scatter of visually-elicited saccades (2<R<50 deg) in three human subjects with the scleral coil technique. The results confirm Deubel's finding and can only be matched by the model if the collicular magnification factor for Φ exceeds magnification for the R dimension. The amount of R-scatter in the data was roughly proportional to R but, unlike in the model, showed a tendency to saturate at very large amplitudes (R = 50 deg). When expressed in collicular dimensions the saccade endpoint scatter appears to be quite small (s = 50 μm) compared with the width of the dome shaped motor activity profile and the size of the collicular map which are both in the order of millimeters. Consequently, if the collicular motor map is indeed anisotropically organized, a small amount of scatter in the location of recruited motor activity would already be sufficient to explain our saccade-scatter data.

303.14 HOW TO COMPUTE EYE POSITION QUATERNIONS. D. Tweed* and T. Vilis. Depts. of Physiology and Ophthalmology. University of Western Ontario, London, Ontario, Canada N6A 5C1.

We have argued elsewhere[1] that quaternions, which express rotations in terms of axes and amplitudes, are the most convenient and meaningful representation of eye position in three dimensions. One advantage is that quaternions are more symmetric than other representations. For example, the Fick system defines horizontal position with respect to a head-fixed axis and torsion with respect to an eye-fixed axis. If experiments show that these two components behave differently, it may not be clear how much of the difference is due to their dissimilar definitions. With quaternions all components are defined using a single rotation axis.

In this poster we describe how to compute eye position quaternions and angular velocity vectors from the outputs of two search coils on one eye in two magnetic fields; the coils need not be orthogonal, nor need their locations on the eye be known. We describe three data processing programs. The first computes eye position quaternions and velocities. The second finds primary position using the quaternions for several gaze directions. The third transforms the eye position quaternions so they are expressed relative to primary position (i.e. so that primary position is represented by the quaternion 1) and moves the quaternions and velocity vectors into a coordinate system where Listing's plane is the yz plane. The method's accuracy is chiefly limited by non-uniformity of the magnetic fields; within 45° of centre, we can compute rotation amplitudes to within about 1° and axes to within 2°. In some cases the data processing can be simplified without an excessive loss of accuracy: if the two search coils are orthogonal and the eye is within 20° of centre, then three coil signals, appropriately scaled but with no other processing, give the vector of the eye position quaternion with an error of less than 11%; and differentiating the signals yields a reasonable estimate of eye velocity. Other coil arrangements or larger eccentricities would increase the error.

Velocity plots computed by the three programs show that the axes of human and monkey saccades tilt out of Listing's plane as predicted by a feedback model[1] in which motor error is the quotient of desired and actual eye position. The axes show that the saccadic eye velocity signal has three degrees of freedom, and hence that the two pools of nonhorizontal short lead burst neurons function independently for saccades.

This work was supported by the Medical Research Council of Canada.

1. Tweed, D. and Vilis, T. Implications of rotational kinematics for the oculomotor system in three dimensions. J. Neurophysiol. (In press)

303.15 VOLUNTARY, VESTIBULAR-CONTINGENT SACCADES TO JUST VIEWED EARTH-FIXED TARGETS FOLLOWING HEAD MOVEMENT IN DARKNESS. J. Bloomberg*, G. Melvill Jones and B. Segal. Aerospace Med. Res. Unit, Dept. of Physiol., McGill Univ., Montréal, Québec, Canada H3G 1Y6

To foveate a target with head fixed, the superior colliculus appears to utilize a retinal error (retinotopic) signal together with an internal signal representing eye position re head (craniotopic) to specify ocular saccade direction and amplitude (e.g. Mays & Sparks, J. Neurophysiol. 43:207, 1980). However, when the head is free to move, saccade generation requires the addition of a head movement signal to operate in a spatiotopic frame of reference. The present study investigates whether a vestibular response to head rotation could suffice to generate volitional saccades to a just-viewed earth-fixed target.

Human subjects sat on a servo-controlled rotating chair, with head fixed to it by a dental bite. First, a central earth-fixed target was visually fixated. Fixation was then transferred to a low intensity (LED) head-fixed target just under the first. The subject was then passively rotated in darkness to the right or left through one of five randomly chosen amplitudes (5°-30°), while suppressing the vestibulo-ocular reflex by fixation on the head-fixed target. After cessation of head rotation and while still in the dark, a voluntary saccade was made to the remembered earth-fixed target. Finally, in the light, the actual earth-fixed target was re-foveated to record the oculomotor error. For 9 subjects, angular amplitude was controlled by varying the duration of turn (0.1-0.6s) at constant velocity (40°/s). For 4 subjects, velocity was varied (20°/s-80°/s) with constant duration of turn (0.35s). Accuracy of saccades to the just viewed target was quantified by saccadic gain calculated as the amplitude of the saccade in the dark divided by the ideal amplitude to have attained the target (i.e. unity gain = perfect target acquisition). All subjects consistently made appropriately-sized saccades towards the earth-fixed target, in the dark, independent of whether the controlled variable was duration or velocity. Mean saccadic gain in variable duration tests was 1.01 ± 0.016 (SE), n = 533, and in variable velocity tests was 1.00 ± 0.014, n = 218.

Since the saccade was generated in darkness and eye movements were suppressed during head rotation, it is inferred that the relevant input to the saccadic system most probably derived from a memory of the preceding vestibular response to head rotation. Hence we conclude that a remembered vestibular-contingent input to the saccadic system can suffice for the generation of appropriate volitional saccades within a spatiotopic frame of reference. More generally the vestibular-contingent signal could provide the necessary input for spatiotopic saccadic gaze control during head movement (Guitton et al. J. Neurophysiol. 52: 1030, 1984).

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303.16 **SPATIAL PROPERTIES OF SIGNALS CARRIED BY SECOND ORDER VESTIBULOOCULAR RELAY NEURONS IN THE CAT.** B. W. Peterson, W. Graf and J. F. Baker, Dept. of Physiology, Northwestern Univ. Med. School, CHI, IL 60611 and Rockefeller Univ., NY, NY, 10021.

We have continued experiments to determine at what levels in the 3-neuron vestibulooocular reflex arc the sensorimotor transformation from vestibular to extraocular muscle coordinates is implemented. Activity of second-order vestibular neurons, identified by their monosynaptic response to electrical stimulation of the labyrinth, was recorded intra-axonally using micropipettes filled with horseradish peroxidase (HRP) dissolved in KCl-Tris buffer solution. Responses to natural vestibular stimulation were recorded during 0.5 Hz rotations in the horizontal plane and in 4-20 vertical planes. Selected neurons were stained intracellularly with HRP for single cell morphology which established their projections to oculomotor nuclei.

To date we have recorded from 50 second order vestibulooocular neurons (2VONs) recorded in the medial longitudinal fasciculus between abducens and trochlear nuclei. Neurons were classified according to whether they were activated from the labyrinth ipsi- or contralateral to the recording site and according to the canal from which they received their strongest input. Sixteen 2VONs received their primary input from the ipsi- or contralateral anterior canal (AC). Eight non-convergent AC neurons responded maximally to rotation in a plane within 10° of the AC plane while 4 AC neurons received sufficient convergent input from the orthogonal vertical canals to shift their plane of maximum response more than 10° from the AC plane. These shifts were symmetrical about the AC plane so that the population average was within 5° of that plane. Of 23 ipsi- and contralateral posterior canal (PC) neurons, 7 were non-convergent while 11 received sufficient convergent input from the orthogonal vertical canals to shift their plane of maximum response more than 10° from the PC plane. The shift was almost always towards the roll plane so that the population average was 3.5° from the activation plane of the oblique eye muscles. The rotational responses of 4 AC and 10 PC neurons indicated that they received convergent input from the horizontal canals. However, when responses were averaged, these horizontal responses tended to cancel leaving little net population response to horizontal rotation. We also encountered 11 ascending axons of second order neurons activated primarily from the horizontal canals. Six of these received significant input from the vertical canals but the mean population response was within 4° of the horizontal canal plane.

While some of the neuronal populations are still small, the data show that multiple canal convergence upon 2VONs can shift the response of vertical canal neurons into the plane of the oblique eye muscles. We conclude that this convergence is one mechanism for generating the appropriate spatial response of these muscles. Interestingly, we have never found an AC or PC neuron whose response was shifted far enough to align with the plane of vertical rectus muscles. Motoneurons of these muscles often receive convergent input from second order neurons of all 4 vertical canals. Thus their spatial properties may depend upon the bilateral divergent projections of second-order AC and PC neurons.

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303.17 RESPONSE PROPERTIES OF HEAD-AND-EYE-VELOCITY CELLS IN THE NUCLEUS PREPOSITUS HYPOGLOSSI AND THE MEDIAL VESTIBULAR NUCLEUS OF THE BEHAVING MONKEY. J.L. McFarland and A.F. Fuchs. Regional Primate Research Center and Department of Physiology & Biophysics, University of Washington, Seattle, WA 98115.

The vestibulo-ocular reflex (VOR) stabilizes images of stationary objects on the retina as the head moves. When the head rotates, the VOR generates compensatory eye movements which rotate the eyes in the opposite direction. In order to stabilize the image of an object that is moving with the head this reflex must be suppressed.

The VOR is served by a three neuron arc that includes the eighth nerve, vestibular nuclei interneurons and the extraocular motor neurons. At least one interneuron, the TVP cell, continues to be deeply modulated with head velocity even when the monkey suppresses the VOR to hold the eye fixed in the head. It has been suggested that the unwanted head velocity signal from TVP cells is cancelled at the motoneuron. We have recorded from cells in the medulla which might serve such a role.

Some single units in the medial vestibular nucleus (MVN) and the nucleus prepositus hypoglossi (NPH) discharge during horizontal head rotation and smooth pursuit eye movements in the same direction. They discharge approximately in phase with head velocity during suppression of the VOR and in phase with eye velocity during smooth pursuit. The sensitivities to head and pursuit velocity are nearly equal. Two thirds of the cells increased rates for movements toward the recording site (i.e. Type 1 for head rotation), while the remainder were Type 2. During the VOR in the light these cells manifest little, if any, modulation. They resemble, therefore, the "gaze velocity" cells of the flocculus and Y-group. In addition, to head and eye velocity sensitivity, many of these cells (60%) also increased their steady firing rate for eye position (static position coefficients averaged 1.9 spikes/second/degree). About half of the cells exhibited a burst of spikes during saccades in their "on" direction. These horizontal head-and-eye-velocity cells usually operated at slower rates than most other oculomotor-related neurons.

Medullary head-and-eye-velocity cells were recorded in areas that project to the ipsilateral and contralateral abducens nucleus. They also carry head and eye velocity signals that are appropriate to cancel the VOR and to participate in the control of voluntary eye movements. Therefore, we suggest that these head- and-eye-velocity cells project to the abducens nucleus where they provide signals that help modulate the VOR.

This work was supported by NIH grants EY00745 and RR00166.

303.18 VESTIBULAR NUCLEUS SIGNALS AFTER INACTIVATION OF "EYE VELOCITY STORAGE" BY BILATERAL VESTIBULAR NEURECTOMY IN PRIMATES. W.Waespe*, U.Schwarz. Dept of Neurology, University Hospital Zurich,CH-8091 Zurich,Switzerland (WW); NIH, Bethesda (US)

The existence of an "eye velocity storage" element has been postulated in the Vestibulo-ocular reflex (VOR) to account for the dynamically "slow" changes in the eye velocity of OKN, and the occurrence of OKAN. Activity changes of some vestibular nuclei neurons have similar dynamics during OKN and OKAN as activity of the "storage" element. Bilateral vestibular neurectomy (BVN) permanently abolishes VOR, OKAN and the "slow" component of OKN. As primary vestibular neurons do not mediate visual or eye velocity signals the effects of BVN on OKN and OKAN are puzzling. It was suggested that BVN inactivates not only primary but also secondary vestibular neurons.

To test this hypothesis we recorded several months after BVN the activity of all neurons encountered in the vestibular nuclei during spontaneous eye movements, OKN, SP (smooth pursuit) and suppression of OKN in trained monkeys.

The results are in agreement with the idea that BVN interrupts the process of "eye velocity storage" at the level of the vestibular nuclei. The recordings, however, failed to identify which neurons are inactivated by BVN. The number and classes of recorded neurons seem to be similar to those in normal monkey. Many neurons were found which behaved -save the absent labyrinthine response- during spontaneous eye movements and SP exactly as "tonic (position) -vestibular-pause ("TVP" or "PVP") cells and vestibular-plus-position (vI/eII) cells in normal monkey. During OKN, these cells were also modulated in relation to eye velocity but sensitivity was substantially smaller than in normal monkey. All other neurons, some of which probably corresponded to "pure-vestibular" or "vestibular-plus-saccade" neurons,known to be activated during OKN in normal monkey, failed to do so after BVN.

We conclude that probably only a few vestibular nuclei neurons are functionally inactivated by BVN which disrupts the process of "eye velocity storage". Their number is obviously too small to be detectable by the method of cell sampling. We can exclude, however, that a particular class of functionally defined vestibular nuclei neurons are inactivated by BVN.

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303.19 A BRAIN STEM SITE OF PLASTICITY IN THE VESTIBULO-OCULAR REFLEX IN MONKEYS. S.G. Lisberger and T.A. Pavelko, Department of Physiology and Division of Neurobiology, University of California, San Francisco, CA, 94143.

The vestibulo-ocular reflex (VOR) generates smooth eye movements that are equal to and opposite head movement, even in total darkness. As a result, the retinal images from the stationary surroundings remain stable during head turns in the light. If retinal image stability is disrupted persistently during head turns, the VOR undergoes long-term adaptive plasticity. In the lab, we induce large increases or decreases in the gain of the VOR (eye velocity divided by head velocity) by fitting monkeys with magnifying or miniaturizing spectacles.

We now report that the site of synaptic changes underlying VOR plasticity is in vestibular inputs to flocculus target neurons (FTNs). FTNs are inhibited at latencies of 1.0 to 1.9 msec by single shock stimulation of the flocculus. They are located in the medial vestibular nucleus. The majority discharge in relation to contralaterally directed eye movement during steady fixation, smooth pursuit and the VOR. When VOR gain is normal, the relationship between FTN firing rate and eye velocity is the same during smooth pursuit with the head fixed as during the VOR in the dark. Thus, the firing of each FTN during pursuit is an absolute against which the firing rate during the VOR can be compared in monkeys with altered VOR gains. After adaptive changes in the VOR, FTNs show dramatic changes in firing. 1) During the VOR in the dark, they express changes that exceed the magnitude of the change in the VOR. When VOR gain is low, for example, many FTNs show increased firing for an *ipsilaterally* directed VOR. In normal monkeys, FTNs show increased firing for a contralaterally directed VOR. This neural exaggeration of the behavioral change is expected in the modified pathways to overcome the effects of other, parallel VOR pathways that are not modified. 2) During tracking of a target that moves exactly with a sinusoidal head rotation, FTNs also show changes in firing. After decreases in VOR gain, for example, the firing rate of FTNs becomes modulated out-of-phase with their excitatory input from the ipsilateral vestibular nerve. Normally, FTNs show little or no modulation of firing rate in this stimulus condition.

Previous work showed that the change in FTN firing probably is not inherited from changes in the firing of afferents to FTNs. Second-order vestibular neurons, which presumably project to FTNs, do not show changes in firing in adapted monkeys. Purkinje cells in the flocculus *do* show changes in firing in adapted monkeys, but the direction of the changes is opposite that which would either account for the behavior of FTNs in adapted monkeys or cause the observed changes in the VOR. This suggests that the site of plasticity is across a synapse onto FTNs from either 1) floccular Purkinje cells or 2) brain stem vestibular neurons. We have excluded the possibility of changes across the inhibitory synapse from Purkinje cells onto FTNs, since stimulation of the flocculus evokes an eye movement that is not modified in relation to changes in the gain of the VOR.

We conclude that plasticity of the VOR is mediated by changes in the synaptic efficacy of brain stem vestibular inputs to flocculus target neurons. Previous work argues that there may also be synaptic changes in the flocculus, but these cannot be the primary modification that causes plasticity in VOR gain. Rather, the changes in the flocculus attempt to miminize the modulation of Purkinje cell simple spike firing during the adapted VOR. (Supported by NIH grant EY03878).

303.20 MULTIDIMENSIONAL GEOMETRIES INTRINSIC TO COGNITIVE CNS HYPERSPACES, AND THEIR METAORGANIZATION BY A SENSORIMOTOR APPARATUS. ***A.J. Pellionisz,*** Department of Physiology & Biophysics, New York University Medical Center, New York, NY 10016

Tensor Network Theory of the CNS is built on the axiom that the CNS expresses physical invariants of the external world in coordinates intrinsic to the organism. In such *general* frames dual geometrical representations are possible by assigning to invariants both sensory (covariant) and motor (contravariant) vectors. Neither, however, fully characterizes the invariant; one passively measures it without yielding a knowledge how to generate it, the other can produce it but not necessarily with appropriate metrical properties. Understanding the link between the two representations is all the more important, since in a sensorimotor system they are connected via a neuronal network, whose role will then be revealed.

Indeed, the general question how the sensory is related to the motor realm is a central problem in *experimental neuroscience* (which yields anatomical and physiological knowledge of the transfer), in *brain theory* (which mathematizes of its understanding) and in the *neurocomputer & neurobotics* fields (which utilize this understanding by physically implemented means). In neuroscience it is known that for a representation of the external world (for its *cognitive model)* such as obtained by *vision,* employing solely a sensory apparatus is not sufficient (the retina goes blind if our eye cannot move around). *An interaction of sensory and motor expressions is necessary for a geometrical model of invariants in the CNS.*

Neuronal networks that are required for moving the eye according to the retinal image (either by saccadic, or smooth pursuit manner) are known at least partially. Also, it is evident that the retina, the sup.colliculus, pontine saccadic bursters (etc; even the visual cortex) and finally the eye muscles all utilize various vectorial coordinate systems intrinsic to these structurofunctional units. In terms of tensor network theory, the connection between the sensory and motor domain is the metric tensor that transforms covariant descriptive vectorial representations into contravariant constructive vectorial expressions. Mathematically, if this link between dual representations is established, *all geometrical features of a multi-dimensional CNS hyperspace are comprised in such metric tensor.* This is why both experimental research (of how the structure and function of arrays of neurons in existing networks implement metric properties of body geometries) and theoretical neurophysics (revealing mathematical features of CNS hyperspaces) are expected to focus on physical and functional brain geometries.

For the visuo-oculomotor system, both the physical geometrical properties of the muscles, and the matching functional geometry that is necessary to coordinate the action of the overcomplete muscles are well explored. Tensorial models of how sensory and motor metrics can evolve in an interaction with the physical geometry of a respective sensory or motor apparatus have been elaborated for the vestibulo-collic, retino-collic, retino-ocular and vestibulo-ocular neuronal arc. It was also postulated by the Metaorganization-principle, however, that once a dual sensorimotor representation emerges, a hierarchical representation of the function of the total circuit can be obtained, which comprises an internal cognitive geometrical model of the interrelation of sensory and motor expressions; called (in this case) *vision.* A model of how neuronal networks of the tectum and visual cortex may implement such a cognitive space, invoked by the primary retino-ocular arc, will be presented. — Support: NS22999 & NS13742

304.1 GABA INHIBITION AT THE PERIARCUATE CORTEX OF THE RHESUS MONKEY AND THE VISUAL DISCRIMINATION REVERSAL TASK WITH GO/NO-GO PERFORMANCES. K. Kubota, A. Mikami and T. Oishi*. Dept. of Neurophysiology, Primate Res. Inst., Kyoto Univ., Inuyama, Aichi, Japan, 484.

To examine roles of GABAergic inhibitory interneurons of the monkey periarcuate cortex in the control of behavior, a GABA antagonist,Bicuculline,was injected locally into the cortical depths of left periarcuate cortex of one rhesus monkey, while the monkey was performing a visual discrimination reversal task with GO/NO-GO performances with right hand (symmetrically reinforced, correction method). Yellow circle presented on the display was erased by monkey's lever press. After a fixed WAITing period,CUE(red or green spot) appeared for 1 s, and was followed again by yellow circle. The monkey continued to press the lever for more than 1.2 s, if the CUE was green(NO-GO RESPONSE). He released the lever within 1.2 s, if the CUE was red(GO RESPONSE). Correct responses were rewarded. Whenever the monkey responded correctly for succesive 11 trials, the task was reversed, that is, associations of two colors and two responses were changed.

After a single injection of Bicuculline methiodide (SIGMA) dissolved in physiological saline (5-15 ug, 5 ug/1uL;10 uL syringe, ITO. Type MS-10;injection speed, 1 uL/min), the monkey made more errors in NO-GO RESPONSE period with decrease or no changes of number of errors in GO RESPONSE period. The monkey tended to release the lever in WAITing and CUE periods. Occurrences of task reversals decreased. Correct performance rate decreased by 5-10% from preceding control level of 70-90%. The effect started about 20 min after the injection and continued for 40-60 min. Performance rate returned to the control, stayed at the same level,or gradually went down until the monkey discontinued the task. In the extreme case, 10ug injection induced muscle contractions in fore-and upper-limbs for 1 hr and performance rate went down to 15%.

Thus, Bicuculline made the monkey difficult to continue to press the lever during the task. It is speculated that neurons of the contralateral periarcuate cortex, specifically active in NO-GO RESPONSE period, are of GABA inhibitory and give inhibitory influences to neurons with facilitatory influence which leads to the lever relsease movement.

304.2 SPATIAL DISCRIMINATION PROPERTIES OF PREFRONTAL CORTICAL NEURONS OF MONKEYS PERFORMING A DELAYED ALTERNATION TASK. S. Carlson*, H. Tanila*, K. Jokelainen*, H. Hämäläinen and A. Pertovaara.

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Spatially discriminative delay related neurons have been studied earlier in the prefrontal cortex of monkeys (Niki, H. Brain Res 68: 197, 1974). The function of some of such neurons has been shown to be dependent on the spatial relations of the choice keys. In the present work dorsolateral prefrontal cortical neurons were studied in monkeys performing a delayed alternation task in which the choice pads in the panel were to the right or left or up and down from the central pad. Two monkeys were trained to hold the central pad for 1-5s. This delay period was indicated by a visual cue above the pad. At the end of the delay period the hold cue went off and a pair of visual cues above a pair of choice pads was illuminated to indicate that the monkey could release the central pad and touch either of the choice pads. Alternating touching of the choice pads after the hold period resulted in a juice reward. Errenous touching was not rewarded. The monkey was first instructed to perform the task to the right for 15-20 trials then to the left and finally in the upward direction. The monkey had also been trained to perform the task according to auditory cues. After a neuron had been recorded during the first three modes of the task (right/left/up) the visual cues were turned off and the auditory cues on and the neuron was again recorded in the different modes of the task. Hand movements were monitored with EMG and eye movements with EOG.
Preliminary analysis of the task related neurons showed response specific and reward related neurons. Both spatially discriminative and nondiscriminative delay related neurons were recorded. The responses of some spatially discriminative neurons were diminished by the occurrence of mistakes in the performance of the monkeys. Most of the delay related spatially discriminative neurons were not dependent on the relative position of the choise pads to each other.The preliminary analysis indicated , however, that the delay related spatially discriminative neurons included units which code the relative position of the choice pads (left/right/up and down). The activity of these neurons was neither dependent on the direction of the impending hand movement nor on the modality of the cue (auditory/visual).

304.3 INVESTIGATION OF THE ROLES OF DORSOMEDIAL AND VENTROLATERAL PREMOTOR REGIONS AND THE FRONTAL EYE FIELDS IN VISUALLY GUIDED MOVEMENTS. J.D. Schall, S.E. Mann and P.H. Schiller Dept. Brain and Cognitive Science, M.I.T., Cambridge, MA 02139

Frontal cortex contains a number of regions which are involved in the generation of visually guided movements. The purpose of this investigation was to determine if the dorsomedial frontal cortex (DMFC), the post-arcuate premotor region (PM) and the frontal eye fields (FEF) could be distinguished on the basis of neuronal activity recorded during performance of visually guided movements. We also investigated whether the DMFC accesses the oculomotor nuclei through the superior colliculus or FEF.

Four rhesus monkeys were trained to perform visually guided eye and arm movements. In each region we found examples of neurons which responded in relation to all elements of the task. Many cells discharged following the appearance of the visual target. Some of these neurons responded transiently; others discharged until the cue for movement, and others fired until the saccade. Such visually responsive cells were most common in the FEF and DMFC and less common in PM. Cells which discharged before saccades were common in the FEF and DMFC, and cells which fired before arm movements were found in DMFC and PM. These movement related neurons were most active before goal-directed movements and less active before spontaneous movements. We found many other neurons in each of these regions which exhibited clearly modulated activity during the task but were less closely linked to the individual elements of the task.

It has been demonstrated in this laboratory that the FEF and superior colliculus have independent access to the oculomotor nuclei. To ascertain whether the DMFC influences the oculomotor nuclei through the FEF or the superior colliculus, unilateral lesions of both of these structures were performed in one monkey. Microstimulation of DMFC elicited saccades following ablation of either the FEF or the superior colliculus. Moreover, stimulation of the DMFC ipsilateral to the ablated FEF at sites which initially evoked contralateral saccades now elicited ipsilateral saccades.

These results indicate that each of these regions contain neuronal activity which may provide a substrate for the generation of visually guided movements and that DMFC accesses the oculomotor nuclei independent of the FEF or superior colliculus. (supported by NRSA EY05959 to JDS and NIH EY00676 and NSF BNS 8310399 to PHS)

304.4 MOVEMENT FIELDS OF NEURONS IN THE PREMOTOR CORTEX OF THE PRIMATE. S.-K. Park, J.-J. Wang*, J.H. Kim and T.J. Ebner. Departments of Neurosurgery and Physiology, Univ. of MN, Mpls., MN 55455.

This study evaluated the representation of horizontal, two dimensional movements in the premotor cortex of the primate, emphasizing the characteristics of the movement fields of these cells. Starting from a central start zone two Rhesus monkeys were required to move to one of 30 randomly presented target zones displayed on a computer controlled, horizontally positioned video screen using a draftsman's arm style manipulandum. A cursor was projected on the video screen and provided real time visual update of the animal's position. The task required placing the cursor within the start and target boxes. Target zones were arranged in five sets of equally spaced targets (60°) around a central start zone, each set at a different distance. Chronic unit recording techniques were used to record the activity of 198 premotor cortical neurons before and during this task. In addition to recording the position of the hand (manipulandum position) and features of trajectory kinematics in the horizontal plane, chronic EMG electrodes were used to monitor shoulder and forearm EMG activity. Horizontal and vertical eye movements were monitored using an infrared oculometer. Three dimensional plots of the cells firing rate above background for the discharge prior to (premovement component) and after the onset of movement (movement component) were constructed. Premotor cortical cells which increased their discharge prior to or during the task showed a preferential modulation for a specific region (direction and amplitude) of horizontal extrapersonal space in which the movement was made. Although the extent of the movement field could be large, the discharge was clearly restricted to a region of the horizontal work space. Also the movement fields exhibited a distinct center where the discharge was maximal. In most cells both the premovement and movement components increased over the same region of horizontal space. In a few cells the importance of the starting position was evaluated. In this group of cells the modulation was dependent on the movement origin. These preliminary observations on the characteristics of the movement fields of premotor cortical neurons raise the intriguing possibility that aspects of extrapersonal space are represented in these cells discharge. Supported by NSF grant NSF/BNS-8318885.

304.5 FRONTAL CORTEX: NEURONAL ACTIVITY PRECEDING DIRECTIONAL AND NON-DIRECTIONAL CUES. E. Vaadia* and S.P. Wise, Lab. Neurophysiology, NIMH, Bethesda MD 20892

The activity of 429 frontal cortex neurons of a rhesus monkey was recorded before and during forelimb reaching in two tasks, presented in blocks of 20 trials each. In both tasks, we studied firing-rate modulation during the period between trial initiation (when the monkey touched the central of three keys) and the onset of a visuospatial timing cue. The monkey was required to begin its forelimb movement to either the left or right target key within 2 s of cue onset, but not before 1 s had elapsed. In one task, the cue (a "directional cue") provided information about the timing and direction of limb movement that would be rewarded on that trial. In the other task, the cue (a "nondirectional cue") provided only timing information; the direction of limb movement that would be rewarded was inversely related to the number of prior consecutive movements in a given direction.

Of 252 task-related neurons, 75 showed pre-cue activity. Pre-cue activity could begin from soon after trial initiation to >3 s later. From trial to trial, the change in pre-cue activity began at a highly variable time before the cue and the firing rate was less consistent than other neural activity patterns observed in this area. However, the offset time of the pre-cue activity was, in most cases, highly consistent and time-locked to the cue-onset.

Leftward vs. rightward movements. No units showed significant differences in pre-cue activity dependent on the direction of limb movement on the forthcoming or previous trial. Thus, the data do not support the hypothesis that pre-cue activity reflects the preparation for a specific limb movement, i.e., motor set.

Directional vs. nondirectional cues. Most (64/75) units with pre-cue activity showed no clear activity differences between the two tasks. Thus, these data do not support the notion that pre-cue activity reflects aspects that distinguished the two tasks: (a) the possibility of using movement, reward or stimulus information from the previous trials to make a choice on the next trial, (b) the predictability of reward, or (c) attention to the instructional content of the cue. We therefore hypothesize that most pre-cue activity reflects or contributes to the main aspect common to the two tasks: the animal's attention to the timing of the cue.

For those few (11/75) cells that differed in pre-cue activity between the two tasks, on the basis of additional testing and analysis we can argue against the above points a (pre-cue activity was greater during directional-cue trials) and b (changing the reward probability in directional-cue trials did not affect pre-cue activity). It therefore remains possible that this pre-cue activity reflects or contributes to attention toward the instructional information transmitted by the cue, as well as to its timing.

304.6 NEURONAL ACTIVITY BEFORE HINDLIMB AND FORELIMB MOVEMENT IN PREMOTOR CORTEX OF RHESUS MONKEYS. K. Kurata and S.P. Wise, Laboratory of Neurophysiology, NIMH, Bethesda, MD 20892

We recorded single-unit activity in the premotor cortex (PM) of two rhesus monkeys in order to study movement- or set-related activity associated with hindlimb or forelimb movement. Each monkey was trained to either press a key by flexion of the right hand or to lift a pedal by dorsiflexion of the right foot. The monkeys' leg and arm were loosely fixed to restrict large movements to the distal limbs. In the task, after a 1 s intertrial interval, either a yellow or a green light emitting diode (LED) was illuminated as an instruction stimulus. When the yellow LED was illuminated, a foot movement was required, and when the green LED was illuminated, a hand movement was required. The monkeys then had to withhold movement for a randomized delay period of 1.5-3.6 s, after which a red LED was illuminated as a trigger stimulus (TS). After the TS, the monkeys had to make the appropriate movement within 800 ms to receive a juice reward.

EMG analysis showed that while the monkeys were performing the task there was no consistent change in muscle activity during the delay period and that changes in muscle activity were limited to the appropriate limb on each trial. Out of 572 task-related neurons recorded in the left PM of the two monkeys, 149 neurons showed set-related activity, defined as significant increases or decreases in discharge rate throughout most of the delay period, and 299 neurons showed movement-related activity, defined as significant changes in discharge rate starting ≤300 ms before the onset of movement.

	Number of PM Neurons		
Activity	Hand only	Foot only	Mixed
Set-related	57	62	30
Movement-related	112	114	73

In both monkeys, foot set- and movement-related neurons were concentrated near the superior precentral sulcus, and hand set- and movement-related neurons were located lateral to the "foot" neurons. The distribution of mixed (foot and hand) set- and movement-related neurons overlapped with the location of "hand" and "foot" neurons.

The results show that most PM neurons contribute to the preparation for and execution of specific limb movements rather than movement per se. Further, the differential distribution of neurons with activity related to hindlimb vs. forelimb movement support previous indications* that PM is topographically organized.

*K. F. Muakkassa, P. L. Strick (1979) Brain Res. 177: 176-182
K. Kurata, K. Okano, J. Tanji (1985) Exp. Brain Res. 60: 188-191

304.7 THE EFFECTS OF COOLING MIDLINE CEREBRAL CORTEX, INCLUDING THE SUPPLEMENTARY MOTOR AREA, ON MOVEMENT-RELATED NEURONAL RESPONSES IN AREA 4 OF CONSCIOUS MONKEYS. E. M. Schmidt, R. Porter and J. S. McIntosh. NIH, NINCDS, LNLC, Bethesda, MD 20892 (EMS and JSM) and The John Curtin School of Med. Res. Canberra, Australia 2601 (RP).

The supplementary motor area (SMA) has been shown to play a role in the performance of movements by a variety of techniques including neurophysiological recordings, intracortical microstimulation, and cerebral blood flow studies. The hypothesis that SMA may influence the responsiveness of area 4 neurons to kinesthetic stimuli (Wiesendanger, et al. '73) has received support from the observation that conditioning stimulation in SMA modifies the latency and/or magnitude of responses of area 4 cells that were produced by passive arm displacements (Hummelsheim, et al. '86). To further test this hypothesis, the movement and perturbation-related responses of area 4 cells were examined before, during and after SMA cooling.

A Rhesus monkey was trained to flex and extend the wrist in response to movement of a visual target on a video monitor. The monkey's hand was held in a molded form coupled to a torque motor which produced a simulated spring load. The monkey was required to match a wrist-movement coupled cursor to a target for a period of at least one second to receive a reward. Halfway through the random duration hold period, a 50 ms torque pulse was applied to perturb the wrist in either the flexion or extension direction.

After training, the following items were implanted under pentobarbital anesthesia and aseptic conditions: 1) six bipolar EMG leads in the right forearm muscles; 2) a recording chamber over the arm area of the contralateral precentral motor cortex; 3) a cooling chamber placed within the sagittal fissure overlying the territory of SMA; and 4) a head restraint device.

The only effect observed on wrist movements when the chamber was cooled to 10°C, and cortex on both sides of the midline was cooled below 25°C, was a possible reduction in their spontaneity. The animal could perform the task with the same speed as before cooling, and the accuracy of positioning and holding the target was unimpaired. No tremor or drift away from the intended displacement was observed. Thus far, the activities of 39 task-related area 4 neurons have been analysed for periods before, during and after bilateral SMA cooling. Eleven of these neurons exhibited a torque pulse response but showed no qualitative difference in the response during cooling.

Under the test conditions employed, we found no evidence that SMA modulates sensory responses of area 4 neurons. This absence of an effect of short term, reversible disturbance of function of SMA, contrasts with the apparent long term modulation of long-latency stretch reflexes which follows unilateral SMA lesions in man (Dick et al. '87).

304.8 ROSTROCAUDAL TOPOGRAPHY IN THE CORTICO-CORTICAL CONNECTIONS OF MEDIAL AGRANULAR CORTEX IN RATS. Gregory S. Goodwin*, Roger L. Reep and James V. Corwin* SPON: (Richard D. Johnson). Departments of Physiological Sciences and Neuroscience, University of Florida, Gainesville, FL 32610; and Department of Psychology, University of New Orleans-Lakefront, New Orleans, LA 70148.

Medial agranular cortex (AGm) is a component of rodent sensorimotor cortex which appears to be a frontal eyefield and may also function as a supplementary motor area. In addition, unilateral lesions of AGm result in neglect to visual, auditory and somatic sensory stimuli presented in the contralateral hemispace. Previous studies have indicated rostrocaudal differences in AGm with respect to various aspects of these functions. In order to delineate the cortico-cortical circuitry which may participate in these functions, we have investigated the topography of cortical afferents to AGm using 3% aqueous Fluorogold (FG) as a retrograde tracer, delivered in focal injections (0.02-0.10ul) through a 33g Hamilton syringe or glass micropipettes with tip diameters of 20-30um. When FG injections were made in AGm at levels rostral to the genu of the corpus callosum, large numbers of labeled cells were seen bilaterally in ventrolateral orbital cortex. In the caudal part of ipsilateral somatic sensory cortex, extensive labeling is present as fairly continuous sheets of cells in layers III and V, extending from the lateral margin of AGm down to the rhinal fissure. Visual cortex contains moderate to heavy labeling arranged as intermittent clusters of labeled cells in layers III and V. FG-positive cells were also found in the retrosplenial, perirhinal and entorhinal areas, primarily ipsilaterally.

As injections are centered in progressively more caudal portions of AGm, the orbital cortex labeling shifts medially such that only the medial orbital area contains FG-positive cells in cases with injections in far caudal AGm. In somatic sensory cortex there is a steady decline in the number of labeled cells as injections are located more caudally in AGm. This is expressed as a breakup of the continuous pattern of FG-positive cells into clusters of cells which become increasingly more separated from each other. Eventually, with injections in very caudal AGm, only two clusters remain: one just lateral to AGm and another just dorsal to the rhinal fissure. In visual cortex the already intermittent pattern is progressively extended such that fewer clusters are seen with more caudal AGm injections. Labeling in the perirhinal and entorhinal areas becomes gradually sparser with more caudally placed injections, without showing any clustering effects. One region which shows increased labeling with more caudal AGm injections is the dorsal taenia tecta immediately rostral to the genu of the corpus callosum.

These findings illustrate a rostrocaudal topography in the cortical connections of AGm, and indicate possible anatomical substrates by which certain functional differences throughout AGm may be expressed.

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304.9 CIRCULAR ORGANIZATION OF CONNECTIONS LINKING MIDBRAIN AREAS, SECTORS OF THE MEDIODORSAL THALAMUS, AND PREFRONTAL AREAS IN THE RAT. T. W. Deacon, A. Sokoloff, and D. Wecht. Biological Anthropology, Harvard Univ., Cambridge, MA 02138.

Numerous studies have noted that the dorsal medial (areas Cg1, Cg3, and F2 of Zilles) and the rhinal sulcal divisions (areas AIv and AId) of the prefrontal cortex in the rat brain are distinguished by non-overlapping projections from different subsectors of the mediodorsal nucleus (MD) of the thalamus. In order to determine if this topographic distinction is maintained with respect to mesencephalic connections as well, we have investigated connections from prefrontal areas to midbrain and tectal areas and from these mesencephalic areas to MD using both autoradiographic (tritiated proline and leucine) and enzymatic (WGA-HRP) tracers. The present report focuses on the interconnections between these structures.

Tracers injected into the dorsomedial prefrontal area (DMPFC) label cells and/or axon terminals (depending on the tracer employed) in the dorsal and lateral sector of MD, and label axon terminals in the intermediate and deep layers of the superior colliculus, the dorsolateral sector of the central gray area, and the deep mesencephalic nucleus. Tracers injected into the sulcal prefrontal area (RSPFC) label cells and/or axon terminals in the medial sector of MD, and label axon terminals in the deep mesencephalic nucleus and the adjacent ventrolateral sector of the central gray. Tracer injections into the far rostral pole of the cortex that include medial, sulcal, and orbital sectors of the prefrontal area label cells and/or axon terminals in the ventral sector of MD, and label axon terminals in the deep gray layer of the superior colliculus and the adjacent tegmental area medial to the brachium of the inferior colliculus. Tracer injections centered in the intermediate and deep layers of the superior colliculus label axon terminals in the dorsal and lateral sector of MD and cells in layer V of DMPFC. Tracer injections centered deep to the superior colliculus within the dorsal tegmental area label axon terminals in the ventral sector of MD and cell bodies in layer V of both DMPFC and RSPFC near the frontal pole. Tracer injections centered within the deep mesencephalic nucleus label axon terminals in the posterior medial sector of MD and cells in layer V of RSPFC. These results suggest that the connections linking areas of the midbrain, MD thalamus, and prefrontal cortex are organized into at least three spatially segregated circuits that each exhibit a circular topology.

304.10 CORTICAL PROJECTIONS OF THE ROSTRAL INTRALAMINAR NUCLEI. G.J. Royce, S. Bromley*, C. Gracco* and R.M. Beckstead. Dept. Anatomy, Univ. of Wisconsin, Madison, WI 53706 and Dept. Anatomy & Cell Biology, Med. Univ. of South Carolina, Charleston, SC 29425.

The organization of the projections from the rostral intralaminar thalamic nuclei to the cerebral cortex was examined in the cat by autoradiography after deposits of ^{3}H-amino acids into the central lateral (CL), paracentral (Pc), central medial (CeM), and para-stria medullaris (paraSM) nuclei.

Following injections into the CL nucleus, label was found on the lateral side within the presylvian (PrS) sulcus, throughout most of the suprasylvian (Ss) gyrus, including the adjacent lateral and suprasylvian sulci, and in the posterior corner of the ectosylvian gyrus. Also on the medial side, label was present in the orbitofrontal (Of), precentral agranular (Prag), anterior limbic (La), retrosplenial (Rs), and postsubicular (Ps) areas. Label was also present throughout the cingulate (Cg) gyrus and on both banks of the splenial (Sp) sulcus, as well as on both banks of the cruciate (Cru) sulcus (areas 4 and 6).

Projections from the Pc nucleus were similar to the CL projections, in that on the lateral side label was found within the PrS sulcus, Ss gyrus and adjacent lateral and Ss sulci, and posterior ectosylvian gyrus. Also on the medial side, label was present in the Of, Prag, La, Rs, and Ps areas, within the cruciate and splenial sulci, and in portions of the lateral gyrus that were clearly within area 17.

Injections of the CeM nucleus which also included the rhomboid nucleus were followed by labeling in the PrS, but in contrast to the CL and Pc projections, the Ss gyrus was labeled only in its posterior part. However, the CeM clearly, if sparsely, projects to the posterior lateral gyrus, part of area 17, both laterally and medially. In addition, medially, CeM projects to the rostral cortex (Of, Prag, La, Cru sulcus, and rostral cingulate gyrus), but largely spares the more posterior limbic areas.

The paraSM nucleus, which had been included previously with the lateral posterior (LP) nucleus, was shown to differ from the rest of LP by projecting to the striatum (Beckstead, '84). The paraSM nucleus projects only to the presylvian sulcus and orbitofrontal cortex laterally, but on the medial side has an extensive input which is very similar to the CL and Pc projections, i.e., label is present in the Of, Prag, La, Rs, and Ps areas, in the cruciate and splenial sulci and in the posterior lateral gyrus (area 17).

The laminar distribution of label was as follows: the CL, Pc and paraSM nuclei projected primarily to layers I and III, whereas the CeM input was found in layers I and VI. Also, the CL projection had a patchy appearance in the posterior limbic field.

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304.11 TOPOGRAPHICAL DISTRIBUTION OF CELLS OF ORIGIN OF THALAMIC EFFERENTS TO POSTERIOR PARIETAL CORTEX IN RHESUS MONKEY. Jeremy D. Schmahmann* and Deepak N. Pandya. Departments of Anatomy and Neurology, Boston University School of Medicine, Boston, MA 02118, and E.N.R.M. Veterans Hospital, Bedford, MA 01730.

A previous WGA-HRP study demonstrated that certain thalamic nuclei project to the superior parietal lobule (SPL) and inferior parietal lobule (IPL) in rhesus monkey (Schmahmann and Pandya 1986). The patterns of thalamic afferents were different for each architectonic area of the SPL and IPL, however some thalamic nuclei were seen to project to more than one area. In order to define the precise topography within individual thalamic nuclei of the cells projecting to different subregions within the posterior parietal cortex, the present study was undertaken using fluorescent retrograde tracers. After sectioning the corpus callosum, fast blue, rhodamine latex microspheres and diamidino yellow were placed in three rostral to caudal sites in the SPL (areas PE and PEc) and IPL (areas PFG, PG, and Opt of Pandya and Seltzer 1982) in the left and right hemispheres respectively.

The SPL receives projections predominantly from the LP and PO nuclei, whereas the IPL afferents are mainly from PM, with a lesser input from LP. Unique to caudal IPL, area Opt, is strong input from LD and the anterior nuclei, AV and AM. Pulvinar oralis sends projections only to the most rostral region of IPL. Both SPL and IPL receive light but consistent projections from VL, VPL, the lateral part of MD, and the intralaminar nuclei (Pcn, CL, CM).

Within nuclei projecting to both SPL and IPL, and most notably in LP, more lateral portions project to SPL, more medial portions to IPL. In the regions of LP and PM that have connections with the posterior parietal cortex, thalamic neurons projecting to the rostral parietal lobe are located in ventral positions, whereas those projecting to the caudal parietal lobe are situated more dorsally. Thalamic neurons projecting to the mid-parietal lobe occupy an intermediate position. Within individual nuclei there is only minimal overlap of neurons projecting to the different subregions of the SPL and IPL. No double-labelled neurons were observed projecting to the IPL, and only occasional double-labelled neurons were found in LP following injections of rostral and middle regions of SPL.

Thus there is topographic specificity in the medio-lateral and dorso-ventral dimensions within individual thalamic nuclei of neurons projecting to the posterior parietal cortex.

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304.12 SPATIAL RELATIONSHIPS OF CALLOSAL AND ASSOCIATION NEURONS IN FRONTAL AND PARIETAL CORTICES OF MONKEYS. P.B. Johnson*, A. Angelucci*, R. M. Ziparo*, M. Bentivoglio, D. Minciacchi* and R. Caminiti. Institute of Physiology, Univ. of Rome and Institute of Neurology, Catholic Univ., Rome, Italy.

The spatial relationships of callosal and association neurons of frontal and parietal cortical areas were studied with a double-labelling strategy. Fast blue and nuclear yellow were injected at appropriate time intervals in the left frontal (areas 4 and 6) and right posterior parietal (area 5) cortices, respectively, of two Macaca fascicularis. This allowed the study of the following retrogradely labelled cells: in the frontal lobe, callosal cells projecting to the contralateral frontal lobe and association neurons projecting to ipsilateral area 5; in the parietal lobe, callosal cells projecting to contralateral area 5 and association cells projecting to the frontal lobe. In all cortices studied, the distribution of callosal and association neurons was uneven. They were found in layers II, III, V and VI, although the former predominated in the lower part of layer III while the latter were more numerous in the upper part of the same layer. Spectral and coherency analyses were used to study the distribution of the labelled cells in the tangential cortical domain. The spectral analysis describes the periodic variations in cell number contained in these distributions, here examined along the anteroposterior cortical axis. The coherency analysis expresses, for a given range of periodicities, the amount of linear relationship between two distributions. The spectral analysis showed that the predominant feature of the tangential arrangement of callosal and association cells in both frontal and parietal areas consisted of periodicities in their number with peak-to-peak distances of more than 4 mm. In reconstructed 2-dimensional flattened images of the cortex these periodicities were observed in the form of bands with various shapes and orientations. Further, the spectrum contained additional periodicities with peak-to-peak distances of 1-3 mm, which would correspond to a more minute and local arrangement of labelled neurons in groups of 500-1500 µm width. These latter periodicities were not the main component of the spectrum. In our case the coherency study provided a quantitative description of the degree of similarity between callosal and association distributions in the cortical space, together with their phase relationships. This analysis showed a high degree of similarity between supra and infragranular distributions of the same cell population. However, the coherency showed varying degrees of similarity and phase between callosal and association neurons of frontal versus parietal cortex. These results indicate that the cells of origin of callosal and association connections in both frontal and parietal areas are independently distributed in the cortical space. However, when the periodic content of their distributions is considered, a common principle underlies their spatial organization.

304.13 DOPAMINERGIC PROJECTIONS TO PREFRONTAL AND PARIETAL CORTICAL REGIONS IN MONKEY (MACACA FASCICULARIS). D.A. Lewis, S.L. Foote, M. Goldstein, J.H. Morrison. Res. Inst. of Scripps Clin., La Jolla, CA 92037 and NYU Med. Cen., New York, NY.

Using an antiserum directed against tyrosine hydroxylase (TH), which appears to selectively label dopaminergic (DA) fibers in monkey neocortex (J. Neurosci. 7:279, 1987), we characterized the distribution of labeled fibers in the prefrontal and parietal regions of Old World cynomolgus monkeys (Macaca fascicularis). The density of TH-IR fibers exhibited substantial heterogeneity, both across and within cortical cytoarchitectonic regions. In prefrontal cortex, fiber density was greatest in area 9; within this region, the medial surface had a greater density of labeled processes than the dorsal surface. Numerous TH-IR fibers were also present in area 24 (anterior cingulate), where fiber density was greater in the supracallosal than in the pregenual or infracallosal portions. Adjacent area 25 on the medial surface had a lower and more homogeneous density of labeled fibers. An intermediate density of TH-IR fibers was present on the orbital surface. The density of labeled fibers was lowest in areas 46 and 10. Within area 46, fiber density was greatest on the dorsal surface, decreased in both banks of the principal sulcus and was lowest in the fundus of this sulcus. In parietal cortex, the density of TH-IR fibers was markedly diminished in the posterior bank of the central sulcus compared to the adjacent motor regions. Fiber density increased substantially in both lateral and caudal directions such that area 7 had a greater density of labeled fibers than area 5. Some posterior cingulate areas also contained a high density of TH-IR fibers. The laminar pattern of fiber distribution in a given area of cortex was systematically related to the overall fiber density of that area. For example, in the lightly innervated area 46, labeled fibers were present primarily in layer I and in layers V-VI, whereas in the densely innervated area 9, TH-IR fibers were present in all layers.

The presence of DA projections to parietal cortex was confirmed by the injection of fast blue, a retrogradely transported dye, into area 7 in 2 monkeys. In both animals, fast blue-positive, TH-IR neurons were identified in the ipsilateral substantia nigra-ventral tegmental area (SN-VTA). Fast blue-positive neurons without TH-IR were also found in the SN-VTA.

Thus, in addition to possessing a number of other common anatomical features, monkey prefrontal and parietal regions also both receive DA projections which exhibit substantial inter- and intraregional differences in fiber density and/or laminar distribution. These findings suggest that the influence of DA on the function of association areas of monkey neocortex may be both widespread and regionally specific. Supported by RSDA MH00519, AG15131 and the MacArthur Foundation.

304.14 **INTERDIGITATION OF CALLOSAL AND METABOLIC COLUMNS IN PRIMATE PREFRONTAL CORTEX: A DOUBLE LABEL HRP AND 2DG STUDY.** A.S. Clark, H.R. Friedman, M.L. Schwartz, and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Major classes of cortical input to the prefrontal association cortex display a modular organization; following injection of different anterograde tracers into the cortex of the principal sulcus (PS) of one hemisphere and the posterior parietal cortex of the opposite hemisphere, terminals form an alternating pattern of ipsilateral and contralateral columns (Goldman-Rakic and Schwartz, Science 216:755, 1982). Columnar properties of organization also hold true for other aspects of cortical information processing. Functional activity, as revealed in 2DG metabolic labeling, is not uniformly distributed throughout the prefrontal cortex, but forms columns of high metabolic activity alternating with equivalent areas of low metabolic activity (Goldman-Rakic, Trends Neurosci. 7(11): 419, 1984). This raises the possibility that the columnar pattern of metabolic activity may be related to the patterns of specific afferent connections in association cortex. Combining the ^{14}C-2DG metabolic labeling method with HRP histochemistry, we report the relationship between two aspects of columnar organization of the cerebral cortex in the monkey; anatomical columns of callosal origin and functional metabolic columns.

WGA-HRP was injected into the posterior third of the PS which projects contralaterally via callosal fibers to the prefrontal cortex (PFC) of the opposite hemisphere. Two days after the HRP injection, the monkey received a single i.v. injection of ^{14}C-2DG (100 μCi/kg), followed by a 45 min. period during which he sat quietly in a primate chair. An overdose of sodium pentobarbital was administered, the animal was perfused with mixed aldehydes and the brain was dissected into tissue blocks to be quick frozen in isopentane cooled in dry ice.

Tissue blocks from the PFC contralateral to the injected PS were cryostat sectioned in the coronal plane at a thickness of 20-30μ. Adjacent sections were (1) histochemically reacted with TMB to reveal the distribution of anterograde and retrograde HRP labeling or, (2) apposed to x-ray film for 6-7 days to visualize the pattern of 2DG metabolic labeling. Additional sections were stained with cresyl violet. Camera lucida drawings of HRP terminal label were superimposed onto photographic prints of 2DG images taken from adjacent tissue sections to examine the relationship of the different labeled territories.

HRP callosal columns with widths ranging from 0.5-1.0mm and 2DG columns of high metabolic activity having similar widths were present in the dorsal and ventral banks of the PS as well as the areas bordering them, and were particularly demarcated in the dorsal bank. In this region, callosal columns were interdigitated with columns of high metabolic activity. The apposition of some adjacent columns was striking; the borders of HRP columns abutted columns of high metabolic activity with no overlap between them. Other regions of the PS appeared to have more complex labeling relationships: in some regions, areas of high metabolic activity and callosal label were in precise register and in other regions the relationship was less clear. We are currently conducting studies to clarify the role of technical, anatomical and behavioral variables to the generation of these relationships.
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304.15 CONNECTIONS OF THE PULVINAR AND ADJACENT THALAMIC NUCLEI WITH THE PRIMARY MOTOR AND PREMOTOR CORTEX IN MACAQUE MONKEYS. G.P. Standage and G.S. Doetsch. Depts. of Anatomy, Surgery (Neurosurgery), and Physiology and Endocrinology, Medical College of Georgia, Augusta, GA 30912.

Possible interconnections of the pulvinar and adjoining thalamic nuclei with motor areas of the cerebral cortex were examined using the HRP method. Single injections (.01 ul) of wheat germ conjugated HRP were made into the anterior portion of the pulvinar, involving both the medial (pul m.) and oral (pul o.) pulvinar subdivisions. Single or multiple adjacent injections of HRP (.025 ul each) were made into the primary motor and premotor cortex, two in cortical area 4 and two in cortical area 6.

Following injections into the pulvinar, HRP labeled cells were found in areas 4 and 6, primarily confined to layer V. Additional labeling was found in areas 8, 45, and 46 of the frontal lobe and areas 5, 7, and the medial surface of the parietal lobe. Labeled cells were typically found in clusters of three to six neurons separated by regions devoid of labeled cells.

A cortical injection into the hand region of area 4 resulted in labeled cells and axon terminals located primarily within the ventral and anterior parts of pul m. and in the ventral part of pul o. Labeled cells and axon terminals formed a single band oriented from dorsomedial to ventrolateral within each of the two subdivisions of the pulvinar. In addition, labeling was present in the border region between the caudal ventroposterior lateral nucleus (VPLc) and the lateral posterior nucleus (LP).

Cortical injections into area 6 produced labeling within pul m. and pul o., and less consistently within the lateral subdivision of the pulvinar (pul l.). An injection near the midline, anterior to the foot region of area 4, produced labeling of cells located dorsally within all three subdivisions of the pulvinar. Conversely, an injection placed laterally in area 6, anterior to the hand region of area 4, resulted in labeled cells located ventrally in pul m. and pul o., but produced no labeling in pul l. Labeled cells and terminals formed two bands oriented from dorsomedial to ventrolateral within the two subdivisions. Additional labeling was found within VPLc and LP.

These preliminary data suggest that the pulvinar complex is reciprocally connected with the primary motor and premotor cortex. The interconnections appear to be differentially distributed, at least those of pul m. and pul o. The connections of the pulvinar with areas 4 and 6--together with those of areas 5 and 7--may play an important role in the control of intentional movements of the upper limbs and eyes (see Acuna et al. Exp. Br. Research:52,411-422,'83).

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304.16 THE PRIMATE MEDIAL PULVINAR (MP) AND ITS CONNECTIONS WITH THE FRONTAL LOBES AND OTHER CORTICAL AREAS. M. Giguere and P.S. Goldman-Rakic. Sec. Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Recently it has become clear that the prefrontal cortex (PFC) in primates receives a major thalamic innervation from the medial pulvinar nucleus (MP) in addition to its well known projection from the mediodorsal nucleus. To elucidate the regional and laminar distribution of MP connections with the cerebral cortex, WGA-HRP or tritiated aminoacids were injected in various portions of MP in six rhesus monkeys. Sections were examined for HRP-positive cells and terminals under bright and dark field illumination. Every other section was charted and the results reconstructed on standard diagrams in each case.

Following injections restricted to the lateral part of MP, labeling was observed most densely in the principal sulcus, the lateral orbital cortex and inferior convexity, while injections placed more medially in MP labeled medial areas of the frontal lobe more intensely. This topography corresponds precisely to that obtained following HRP injections in PFC (Goldman-Rakic and Porrino,J.C.N.242:535,1985). As described for the mediodorsal connection with the PFC (Giguere and Goldman-Rakic, Neurosc. Abst.11:671, 1985), the cortical cells of origin of MP afferents are located in layer VI, with occasional cells labeled in superficial layer V, whereas the MP terminals are located in the middle layers III and IV and form a disjunctive pattern of bands throughout the labeled areas of the PFC. No labeled terminals are found in layer I. The density of labeled terminals often surpassed that of labeled cells in some parts of the PFC.

In addition to its prefrontal connections, MP projects to an extensive network of ipsilateral association areas. In agreement with other studies, the superior temporal sulcus, the granular and dysgranular parts of the insular cortex, the lateral and medial posterior parietal cortex, the posterior cingulate, the retrosplenial area as well as the parahippocampal gyrus are all extensively and reciprocally connected with the medial pulvinar. The distribution of labeled cells and terminals in these areas corresponds to that observed in prefrontal cortex, though no disparity in density of labeled cells and terminals was noticed. Recent studies in this laboratory have demonstrated that the principal sulcus is reciprocally connected with all of the areas that receive MP afferents (Selemon and Goldman-Rakic, Neurosc. Abst. 11: 323). It therefore appears that the medial pulvinar, which is particularly prominent in primates, is in position to recruit or activate an entire distributed neural system dedicated to a common function. Supported by MS38546 and a fellowship from the FRSQ to MG.

304.17 MULTIMODAL EFFERENT PROJECTIONS FROM THE POSTERIOR CINGULATE CORTEX IN THE RHESUS MONKEY. C.L. Barnes and D.N. Pandya. Bedford Veterans Hospital, Bedford, MA 01730 and Depts. of Anatomy and Neurology, Boston Univ. Sch. of Med., Boston, MA 02118.

In recent years, several multimodal areas have been identified in the frontal lobe, parietotemporal area and the parahippocampal gyrus. These multimodal areas have been proposed from the anatomical and physiological demonstration as recipient areas of converging efferents from parasensory association areas of the somatosensory, auditory and visual modalities. In the present study, we have observed that specific regions of the caudal cingulate gyrus (area 23 and retrosplenial cortex, area 29) seem to project to specific parts of the parasensory association areas in the parietal lobe, superior temporal gyrus and inferotemporal region.

In eleven rhesus monkeys, Fluorescent Retrograde Dyes (FRT-Rhodamine labeled latex microspheres, Diamidino Yellow, Fast Blue) were injected in different subdivisions of the parietal lobe (somatosensory), superior temporal gyrus (auditory) and inferotemporal region (visual) in a single animal. The resulting differentially labeled neurons were traced in the hemispheres. We observed an overlapping pattern of retrogradely labeled cell bodies in areas 23 and 29 following FRT injections confined to areas Opt and caudal PG of the inferior parietal lobule, areas paAlt and Ts3 of the superior temporal gyrus and areas TF and TL (Rosene and Pandya, Soc. Neurosci. Abstr., 1983) of the posterior parahippocampal gyrus. In contrast, FRT injections restricted to the surrounding areas POa and rostral PG of the inferior parietal lobule, areas Ts1 and Ts2 of the superior temporal gyrus, and areas TE2 and TE3 of the lateral inferotemporal region did not produce labeled neurons in these parts of cingulate cortex. The labeled neurons in the cingulate region were found in layers III and V-VI. Despite the overlapping pattern of retrogradely labeled neurons, only few double labeled neurons were identified in the deep layers of these regions, especially following injections of areas Opt and TF. Thus, these posterior cingulate areas send projections to those areas known to subserve the peripheral vision, audition, and highly integrated somatosensation.

Multimodal areas have been identified in the superior temporal sulcus and the frontal lobe as well as the paralimbic areas of parahippocampal gyrus based on the convergence of afferent connections. However, the multimodal nature of the posterior cingulate and retrosplenial cortices is based on their efferent connections. These findings indicate that specific parasensory association areas are influenced by a common limbic source. (Supported in part by ENRM VA Hospital, Bedford, MA and Grant 16841 to DNP)

304.18 PROJECTIONS TO A TONOTOPICALLY-ORGANIZED VOCAL MOTOR REGION OF ANTERIOR CINGULATE CORTEX IN THE MUSTACHED BAT. W.E. O'Neill, D.M. Gooler and R.D. Frisina, Dept. of Physiology, Univ. of Rochester Sch. of Med. and Dent., Rochester, NY 14642.

The mustached bat emits multiharmonic biosonar signals consisting of a long constant frequency (CF) component followed by a terminal frequency modulation. Relative motion between the bat and its surroundings induces a Doppler-shift in the echo frequency, which the bat compensates by adjusting the frequency of its emitted sonar pulses. Doppler-shift compensation stabilizes the echo frequency at the frequency to which the bat's inner ear is sharply tuned. This permits the resolution of small frequency and amplitude modulations in the echo caused by the wing movements of insect targets.

We have previously discovered that microstimulation in the anterior cingulate cortex (ACg) elicits biosonar cries rostrally, and audible social vocalizations caudally. Moreover, the region from which sonar cries can be elicited is tonotopically organized, so that the dominant 2nd harmonic of the elicited pulses increases systematically from 58 to about 61 kHz along a rostrocaudal axis. Thus, unlike the other vocal control regions previously studied in bats, this area contains a neural substrate for the control of vocal frequencies emitted during Doppler-shift compensation. In an effort to determine the pathways by which echo frequency information reaches the ACg, we have studied the connections to this region after focal injections of HRP. Ipsilaterally we found retrogradely labeled cell bodies in layers II and III of the dorso-rostral auditory cortex, in a location likely to contain neurons sensitive to harmonic combinations (CF/CF neurons) discovered in previous studies (Suga et al., Science 203:270-274, 1979). Additionally, a very small number of labeled cells are consistently found in the dorsal division of the medial geniculate body of the thalamus. Other non-auditory regions of thalamus showed many more retrogradely labeled cells. These included cell groups in the medial and lateral parts of the medial lateral nucleus, and cells scattered throughout the ventral anterior nucleus, both of which are components of thalamic motor systems. Contralaterally, labeled cells were found in layers II/III of both the ACg and prefrontal/frontal cortex dorsal and lateral to ACg. These are directly adjacent and rostral to the injected site in ACg. These cortical projections may provide auditory information indirectly to ACg, since previous studies have shown connections to frontal cortex from auditory cortex (Kobler et al, Soc. Neurosci. Abstr. 9:956, 1983).

In conclusion, direct and indirect pathways exist which may provide the auditory feedback necessary in ACg for control of vocal frequency for Doppler-shift compensation. Supported by PHS-NINCDS grant no. 1 RO1 NS21268 to WEO.

304.19 POSTNATAL MATURATION OF HUMAN FRONTAL CORTEX:NEW CYTOARCHITECTONIC CRITERIA. I.Kostović, M.Judaš+ and N.Bogdanović+, Sect. of Neuroanatomy Dept.of Anatomy, Medical Faculty, Univ.of Zagreb, Yugoslavia.

Classical cytoarchitectonic studies have described postnatal development of human cerebral cortex as a sequence of progressive maturational events.The aim of this study was to develop new approach using criteria for evaluation of regressive histogenetic events,modular cell arrangements and establishment of cortical projections.The following criteria were applied:(1) for regressive events:a) disappearance of transient fetal layers (sobplate zone) and cells,b) resolution of granular layer (IV) and disappearance of six-layered pattern,(2) for modular and tangential rearrangements: cell clustering and radial columnation,(3) for progressive differentiation: standard criteria (laminar and cytological maturation, decrease in cell-packing density).This parameters were tested on selected frontal areas (agranular-premotor,dysgranular-intermediate and granular-prepolar) by analyzing serial Nissl-stained celloidin sections prepared from postmortem frontal lobe of 11 children ranging between neonatal period to 14 years (y.).In the newborn all frontal non-central areas appear very immature when compared on the basis of regressive (1) and modular (2) criteria.In addition, boundaries between areas are poorly defined.Thus,prominent granular layer (IV),fetal subplate layer and clusters of layer V pyramidal (3-6) neurons are present throughout frontal cortex.This indicates rather uniform development across the entire frontal lobe.However, based on the progressive criteria (3) premotor area appear to be more mature then prepolar cortex or Broca area.Period between 3-9 months is characterized by gradual emergence of pyramidal cell clusters in layer III,rearrangements in vertical alignement and disappearance of fetal layers.At age of 3 y. frontal agranular (premotor) cortex shows relative maturity due to the disappearance of granularity (criterion-c-1),dispersion of small cell clusters (c-2) and cytoarchitectonic differentiation (c-3).In contrast, frontal granular (prepolar) cortex and Broca area show signs of protracted development:abundance of fetal cells in deep cortex, presence of layer IV (c-1) and immature appearance of layer III (c-3).Between 11-14 y. additional change was observed:Appearance of deep staining among the large layer III pyramidal cells which tended to be distributed in a spatially periodic pattern which is different from small cluster system.This study demonstrated validity of regressive and modular cytoarchitectonic criteria as complements to the "standard" criteria.The following conclusions are drown based on this approach:During infancy there exists a rather synchronous development of all frontal areas.Pyramidal cell cluster formation reflects growth of cortical efferents.The cytoarchitectonic organization of the frontal granular cortex continues to develop during childhood and puberty.
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305.1 NEURONAL ACTIVITIES OF THALAMO-CORTICAL AND HIPPOCAMPAL SYSTEMS DURING AUDITORY WORKING MEMORY PROCESSING IN THE RAT. Y. Sakurai. Dept. of Psychology, Toyama Med. & Pharmaceu. Univ., Sugitani, Toyama 930-01, JAPAN.

Certain aspects of memory involve thalamo-cortical function. Unit activity in the prefrontal cortex (PFC) and dorsomedial thalamus (DMT) was related to working memory for response (Sakurai & Sugimoto, Behav. Brain Res., 17:213, 1985; 20:295, 1986). Many previous studies have shown relationships between hippocampus (HPC) and several types of memory. The present study was designed to examine the unit activity of the thalamo-cortical (PFC and DMT) and the hippocampal (CA1, CA3, DG) systems during a task for auditory working memory, about which little has been known as compared to visual and spatial memory. The task was an auditory version of continuous nonmatching-to-sample (CNM) task, originally introduced by Pontecorvo (Animal Learn. & Behav., 11:356, 1983). At the start of each trial, a tone (8 kHz or 1 kHz) was presented and continued for 10 sec. One second after the onset of the tone, the guillotine door opened and the response-panel was available for 3 sec. During a nonmatch trial (NM), the stimulus was different than that during the preceding trial and a panelpress (Go) provided food reward. During a match (M) trial, the stimulus was the same as that during the preceding trial and a Go response turned on a buzzer. With this training schedule, the optimal strategy for the rat was a Go response on NM trials and a No-Go response (not pressing the panel) on M trials. The intertrial interval was 3 sec which functions as a delay period. Following completion of the training, the rat was anesthetized and nichrome wires (25μ) for single unit recording were chronically implanted stereotaxically into the PFC, DMT, CA1, CA3, and DG. The wires were connected to a 60-wire plastic plug, and the whole assembly was fixed with dental cement to the skull. Five days after surgery, the rat was retrained in the CNM task and each unit was recorded during performance of the task. Unit activity was analyzed in three temporal periods of the task: the last 3 sec of the tone in one trial, the first 1 sec of the tone during the next trial, and the 3 sec of delay period between them. During these three periods, six types of frequency histograms classified the activity by stimulus (high tone or low tone), responses (Go or No-Go) and outcomes of the responses (correct or error). By this analysis, each unit could be judged to be related to the auditory perception, the motor control, or the working memory processing. The ratios of each type of units in the PFC, DMT, CA1, CA3, and DG are currently under investigation. Comparison among those structures in relation to the auditory working memory processing will be discussed.

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305.2 MEMORY DEFICIT IN PASSIVE-AVOIDANCE LEARNING IN BULBECTOMIZED LONG-EVANS HOODED RATS. B.M. Thorne and J. Rowles*. Miss. State Univ., Miss. State, MS 39762.

Complete olfactory bulb removal in rats typically results in increased muricide, increased activity, hyperirritability to handling, and a deficit in passive-avoidance (P-A) learning. Because bulbectomized rats are usually hyperactive, it is possible that the P-A deficit stems from an inability to withhold a response rather than from a lack of memory. To compare the memory loss hypothesis with the hyperactivity hypothesis of the P-A learning deficit, we employed a modified P-A apparatus which permitted an alternative to behavioral inhibition.

The modified P-A apparatus consisted of an illuminated common platform connected to two discriminably different chambers. Rats were given 6 acquisition trials in which entry into each of the dark chambers was alternated. On the last trial, each subject received a 3-sec, 1 ma footshock. Twenty-four hours later, all animals were tested on retention of the footshock by being placed on the platform with access permitted to both chambers. A correct response on the postshock trial was defined as either failing to leave the platform within 5 min or choosing the chamber in which shock had not been presented during acquisition.

In agreement with most previous studies, bulbectomy resulted in significant weight loss in the males, increased irritability in both sexes relative to the controls, elevated muricidal behavior in the males, and increased open-field activity in both sexes. On the P-A task, bulbectomy decreased response latencies on 4 of the 6 acquisition trials. In addition, control animals showed clear evidence for habituation to the platform, i.e., increasing response latencies across trials. Bulbectomized rats showed no evidence for habituation. On the postshock trial, 25 of 26 operated control rats stayed on the platform, while this was true for only 6 of 30 bulbectomized animals. According to the definition, all of the control animals made the correct response, while 9 of 30 OB rats made an incorrect response. The difference was significant indicating a memory defect in bulbectomized rats on the P-A task.

305.3 AN ATTENTIONAL-ASSOCIATIVE NETWORK THAT SIMULATES THE TOPOGRAPHY OF THE NICTITATING MEMBRANE RESPONSE, HIPPOCAMPAL AND SEPTAL NEURONAL FIRING, AND THE EFFECTS OF HIPPOCAMPAL LESIONS AND STIMULATION, DURING CLASSICAL CONDITIONING. Nestor A. Schmajuk. Center for Adaptive Systems. Boston University. Boston, MA 02215.

The present study introduces a real-time version of the Pearce-Hall (1980) model that is capable of associations among conditioned stimuli (CS), and incorporates performance rules that convert learning variables into topography of the rabbit nictitating membrane (NM) response and associated neural firing.

By assuming that hippocampal lesions (HL) and hippocampal stimulation (HS) affect computations of attentional variables, the model is able to simulate HL and HS effects on many classical conditioning paradigms. In the normal case, associability of CS_i when presented with event k is given by: $\alpha_i^k = |\lambda^k - B^k|$, where B^k is the aggregate prediction of event k made upon all CSs present at a given moment, and λ^k represents the intensity of event k. In the HL case, associability is given by $\alpha_i^{US} = |\lambda^{US} - V_i^{US}|$, where V_i^k is the net associative value between CS_i and event k. Computer simulations for the HL case were carried out for the following protocols: acquisition under different interstimulus intervals (ISI), conditioned inhibition, extinction under different ISIs, latent inhibition, blocking, overshadowing, discrimination reversal, and sensory preconditioning. Regarding HS, the present study assumes that α^{US} increases when HS is applied.

Neural activity in the hippocampus was simulated by assuming that hippocampal neurons code the instantaneous magnitude of B^{US}. Neural activity in the medial septum was simulated by assuming that septal neurons code the instantaneous magnitude of $\Sigma_j \alpha_j$, i.e., by the sum of CS and US associabilities. Simulations of CA1 hippocampal unit activity during acquisition and extinction, and medial septum unit activity during acquisition of conditioning, are presented.

Although some discrepancies between simulation results and relevant literature were noted, the network proved capable of simulating a large portion of the experimental data.

305.4 LOCAL AND GLOBAL FEATURE EXTRACTION USING COHESIVE AND DISCONNECTED TEMPLATES WITH MACHINE VISION CROSS-CORRELATION PROGRAMS. R. B. Glassman. Dept. of Psychology, Lake Forest College, Lake Forest, IL 60045.

Vision may be a result not only of a hierarchical building of local perceptual "bricks" at low levels of the visual system into larger perceptual "houses" at higher levels of the brain; rather parallel processing of local and global information may take place beginning at the lowest levels of connections from retina to cortex (Hughes, H. C. & Sprague, J. M., Exp. Brain Res., 61:332-354, 1986). While primarily serial-processing computers can simulate such processes only in a slow, roundabout way, they can help in evaluating the logic of hypotheses about image processing.

Two programs, written using the convolution function of the IRI P256 vision machine with array processor, were used to run cross-correlations, normalized for local brightness, between a template comprising pixels representing a small piece of an object-image, and every translational position in a scene in which the same object appeared. Two-dimensional correlogram plots represented the degree of match as brightness, at each pixel position. In one program all pixels were contiguous within a rectangular border ("cohesive template") while the other program permitted an arbitrary spatial distribution of small patches of pixels ("disconnected template"). With the latter program, it was possible more precisely to iconically define a feature in terms of the critical information about form and gray level, while excluding irrelevant information.

A higher degree of selectivity was achieved with disconnected templates. In the special case of a simple, high-contrast object, good recognition occurred over a distance factor of two ("perceptual size constancy"), but more conceptual and empirical work is needed to more generally determine the conditions under which the disconnected template technique helps or hinders feature extraction.

Some of the correlograms have a visually intriguing three-dimensional quality or an embroidery-like quality; these seem to be the result of redundancies within template and scene. Beauty may or may not be truth, but perhaps these effects can be used to facilitate the search for a match in a computer pattern recognition process. This would be so if the effects can function as filters or amplifications of relevant information, or as guides in a second stage of the search process. If evolution has made such a discovery, perhaps these effects are analogous to some aspects of organismic visual processing.

(Work carried out at Oak Ridge National Laboratory, Center for Engineering Systems Advanced Research.)

305.5 **FACILITATION OF LEARNING, ENHANCEMENT OF MEMORY, AND PROTECTION AGAINST AMNESIA BY A BENZODIAZEPINE ANTAGONIST IN MICE.** H. Lal, P. Bhakthavatsalam*, and M.J. Forster. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107-2690.

Benzodiazepines are known to impair learning and memory performance. The present investigation provides evidence that a benzodiazepine antagonist, Ro 15-1788, facilitates learning and memory in mice. In one experiment, 3-month-old HSD:(ICR)BR mice were injected i.p. with either saline or Ro 15-1788 (40 mg/kg i.p.), 10 min prior to acquisition of a discriminated escape habit. Acquisition consisted of training trials on which the mice were required to enter the correct goal arm of a T-maze to escape footshock. Training was discontinued when a correct arm choice had occurred on three consecutive trials, and retention was measured 2-weeks later by determining the number of trials required to reverse the T-maze habit. When compared to saline-treated controls, Ro 15-1788-treated mice required significantly **fewer trials for acquisition of the T-maze habit**, but significantly **more trials for its reversal** during retention. This finding suggested that Ro 15-1788 had facilitated both learning and memory for the T-maze choice discrimination. In a second experiment, Ro 15-1788 was tested for its ability to protect against amnesia induced by scopolamine in a step-through, passive-avoidance paradigm. Separate groups of mice were injected with either Ro 15-1788 (40 mg/kg) or saline, 20 min prior to a passive avoidance acquisition trial in which entry from the light to the dark chamber of a 2-compartment apparatus was followed by a 5-sec, 2.0 mA footshock. Scopolamine (0.64 mg/kg) or saline was injected 10 min prior to the acquisition trial, and retention was measured by the latency to enter the dark chamber during a 24-h retention test. Mice receiving only scopolamine prior to training showed poorer retention (shorter step-through latencies) when compared with control mice receiving only saline. Ro 15-1788 pre-treated mice trained under scopolamine showed latencies comparable to those of the control group, suggesting that Ro 15-1788 pre-treatment had afforded **protection against scopolamine-induced amnesia.** The enhancement of learning and memory performance by Ro 15-1788 suggests that endogenous ligands for benzodiazepine receptors exert a negative modulatory influence upon brain processes involved in cognitive performance. Those processes involved appear to include the cholinergic system, as Ro 15-1788 afforded protection against memory disruption produced through muscarinic receptor blockade.

Supported by NIH-NIA grant R23-AG06182 (M.J.F.) and NIH-BRSG award S07 RR05879 (T.C.O.M) .

305.6 **HABITUATION AND STIMULUS-SPECIFIC NEURONAL RESPONSES RECORDED FROM THE AMYGDALA OF THE MONKEY**
F.A.W. Wilson & E.T. Rolls. Dept. of Exptl. Psychology, Oxford, U.K.

In order to examine the role of the amygdala in memory function, neuronal activity was recorded in 2 monkeys performing recognition memory and visual discrimination tasks. Typical stimuli were novel and familiar junk objects, familiar foods and faces. In the recognition memory task, lick responses to novel stimuli elicited saline; responses to familiar stimuli elicited fruit juice. Stimuli presented as familiar were shown after 0,2,4,6,8,10,12,14, or 16 other intervening trials. In the visual discrimination task, responses to a circle elicited fruit juice while responses to a square elicited saline. Of 659 neurons recorded, 58% were unresponsive; 35% responded non-specifically; and 2.5% responded differentially in the visual discrimination task. 1.5% responded maximally to novel stimuli, the responses declining with repetition of the stimuli. 'Memory spans' were determined for 5 neurons by plotting responses to familiar stimuli as a function of intervening trials. The magnitude of the differential response decreased with increasing numbers of intervening trials, the memory spans ranging from 2 to 10 trials. The mean latency for the differential responses was 212 ms. Increases in firing rate for familiar stimulus presentations were not observed.

Two groups of selective neurons responded maximally to faces (1.5%), or to foods (1%). The responses of selective neurons were significantly greater to foods or faces than to other stimuli. Selective neurons often responded weakly to ineffective stimuli, but novel stimuli elicited better responses than other ineffective stimuli in certain neurons. Habituation of the selective responses occured in 30% of selective neurons. Recordings sampled the dorsoventral axis of the amygdala, but spared the most anterior, medial and ventral lateral regions. Most responsive neurons were located across the dorsal amygdala, with face-selective neurons located medially in the basal accessory nucleus.

Few task or stimulus-related neurons were observed in this study. This could be due to the specific requirements for activating amygdala neurons and to testing paradigms restricted to the visual modality. However, the activity of the responsive units suggests a contribution by the amygdala to stimulus recognition. The decremental responses indicate a mechanism reflecting the recency of stimulus presentations. The activity of stimulus-selective neurons may encode the presence of highly familiar and emotionally valent stimuli. These results are consistent with a role for the amygdala in behavioral responses to stimuli that are novel, reinforcing and which elicit emotional behavior.

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305.7 AREA POSTREMA LESIONS IN RATS ENHANCE NICOTINE-INDUCED CONDITIONED TASTE AVERSIONS. L. Giugno and K.-P. Ossenkopp. Dept. Psychology, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

The area postrema (AP), a circumventricular organ in the fourth ventricle, has been shown to be a chemoreceptor for blood-borne toxins (Borison, Life Sci., 1974, 14, 1807). Several studies have shown that AP is the central chemoreceptive site for injected toxins used to establish conditioned taste aversions (CTA). AP ablation has been shown to abolish CTAs induced by injections of such toxins as LiCl and scopolamine, but levels of amphetamine or motion sickness-induced CTA are not attenuated by these ablations. The present experiment examined the role of AP in nicotine-induced CTA in rats.

Adult male hooded rats were given thermal cautery lesions of AP or sham lesions. Following a 2 week recovery period the animals were adjusted to a 23½ hr/day water deprivation schedule and on the conditioning day all rats were presented with a 0.15% saccharin solution during the normal drinking period. Immediately after this drinking period half of the rats in the lesioned and sham lesioned groups were injected with nicotine (1 mg/kg, i.p.) and the other rats received isotonic saline injections. Starting 3 days after the conditioning trial the animals were given two-bottle choice tests (saccharin vs. water) on 3 consecutive days. Statistical analyses revealed that the rats injected with saline exhibited strong preferences for saccharin. The sham lesioned rats injected with nicotine showed a weak but significant ($p < .05$) CTA to saccharin. The rats with AP lesions and injected with nicotine displayed a significantly ($p < .05$) larger CTA than the sham lesioned rats given nicotine. Thus, AP lesions enhanced the magnitude of the nicotine-induced CTA. In a subsequent test phase all rats were given chocolate metrecal followed by injections of scopolamine hydrochloride (1 mg/kg, i.p.). In a two bottle choice test the AP lesioned rats exhibited significantly ($p < .01$) greater preference for the chocolate taste than the sham lesioned rats. Thus, AP lesions attenuated the magnitude of scopolamine-induced CTA. The present results clearly suggest that nicotine and scopolamine do not act at the same neural site in producing a CTA.

(Supported by a Natural Science and Engineering Research Council grant, A1239, to KPO.)

305.8 DEVELOPMENT OF AN ALL-OR-NONE DEPOLARIZING POTENTIAL AFTER BURSTING ACTIVITY IN PIRIFORM CORTEX: A POSSIBLE MODEL FOR THE KINDLING PHENOMENON. W.H. Hoffman* and L.B. Haberly. Neurosci. Training Prog. and Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

Intracellular recordings were obtained from layer II pyramidal cells in slices of rat piriform cortex maintained in vitro. The normal response to stimulation of afferent fibers consists of a single EPSP and Cl^- and K^+ mediated IPSPs (Tseng & Haberly, Neur. Abs. 12:667). Removal of Mg^{++} from the bathing medium resulted in evoked and spontaneous bursting activity; upon return to normal bathing medium the bursting subsided but a second depolarizing potential (2-12mv; 25-125ms duration) developed following the monosynpatic EPSP at a variable latency in all cells examined in 19 slices. This new potential occurred in an all-or-none fashion at low stimulus strengths and could trigger action potential generation. The potential persisted with no apparent decrement for as long as impalements were maintained (up to 10 hr). Its frequency of occurrence was not affected by intracellular current injection, suggesting that it is an EPSP rather than an endogenously mediated potential. It did not appear to result from blockage of inhibitory processes since both Cl^- and K^+ mediated IPSPs were present. Although 10μM APV and 10μM ketamine (NMDA antagonists) blocked bursting in 0 Mg^{++} and the subsequent development of the second depolarizing potential, neither agent at 100μM blocked the second potential once it was established (6 of 6 slices). When bursting activity was induced by 100μM 4-aminopyridine or low (10%) bath Cl rather than 0 Mg^{++}, the second depolarizing potential was also evoked after return to normal medium. While the potential that developed after low Cl^- often disappeared within 1 hour, in 4 of 11 slices it persisted for as long as impalements were maintained (up to 5 hr). The presence of 10-25μM APV or ketamine decreased the intensity of bursting and blocked development of the second potential in 10 of 16 slices, although in 4 of the 6 slices in which the second potential was generated it lasted for the duration of impalements (up to 3 hr). This result suggests that NMDA receptor activation is not required for development of the second potential but does facilitate its development, perhaps as a consequence of the intensification of bursting activity.

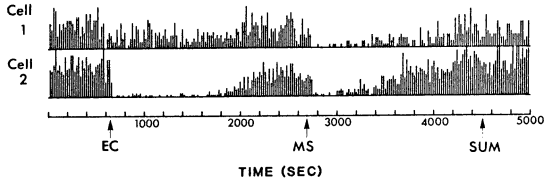
The potential induced by bursting activity in vitro displays a striking resemblance to a second EPSP that appears in pyramidal cells in the piriform cortex of kindled rats in vivo (Sutula & Haberly, Neur. Abs.12:1126). Based on preliminary results obtained by field potential analysis, we postulate that it is mediated by regenerative positive feedback between layer II pyramidal and deep cells following an increase in efficacy of excitatory synapses (see Tseng & Haberly, this vol.). Such a long lasting increase in synaptic efficacy could also have direct relevance to long term memory processes. Supported by NINCDS grant NS19865 to LBH.

305.9 REVERSIBLE INACTIVATION OF HIPPOCAMPAL AFFERENTS SELECTIVELY REDUCES THE SPONTANEOUS DISCHARGE RATE OF DENTATE UNITS. S.J.Y. Mizumori, B. L. McNaughton, C. A. Barnes and K. Fox*. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

This study investigated the consequences of the reversible inactivation of different hippocampal afferents. Exp. 1 assessed the contributions of medial septal (MS), supramammillary (SUM), entorhinal cortical (EC) and contralateral hilar afferents to the activity of units recorded from the hippocampus of anesthetized rats. Exp. 2 examined the relationship between the physiological and mnemonic consequences of selective inactivation of MS or EC in freely behaving rats.

Under Nembutal anesthesia, CA1 complex-spike (CS) cells (N=37) never altered their rate in response to a 0.3-0.5 ul injection of 2% lidocaine (a local anesthetic) into MS, ipsilateral SUM, or ipsilateral EC. In contrast, 45% and 35% of dentate granule cells (N=31) responded to inactivation of MS or EC, respectively, but not SUM, with reduced discharge. A significant increase in granule cell discharge followed injection into the contralateral hilus. Two granule cells that differentially responded to EC, MS and SUM injections are illustrated below. Inactivation of MS and EC, but not SUM, also attenuated firing of some hilar CS cells.

For Exp. 2, rats were implanted with guide cannulae to permit injection of 2% tetracaine (a longer acting anesthetic) and recording "stereotrodes" (McNaughton et al., 1983) for monitoring unit activity while the animals solved a radial maze problem. Five of five CA1 CS cells exhibiting place-specific firing before injection maintained the same specificity in spite of the severe spatial working memory (WM) impairment caused by MS inactivation. In contrast, place fields of four of seven hilar CS cells were silent for the duration of behavioral impairment. Place fields and choice accuracy returned to preinjection levels within about 15 min. Four of five granule cells also showed reduced firing coinciding with the period of behavioral impairment induced by MS inactivation. Ipsilateral EC injection did not affect choice accuracy, yet often resulted in reduced granule cell firing.The main conclusion from these studies to date is that stable place fields can be recorded from CA1 CS cells during the severe spatial WM impairment and large reduction in granule cell activity caused by MS inactivation. Supported by AG05375, AG03376, and NS20331.



305.10 A MULTIPLE REGRESSION ANALYSIS OF BEHAVIORAL CORRELATES OF RAT HIPPOCAMPAL NEURON DISCHARGE. B. Jones Leonard, B.L. McNaughton and C.A. Barnes. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

A number of behavioral variables influence the discharge of hippocampal single units in freely-moving rats. Spatial location is the primary correlate of complex spike (CS) cell discharge, while motion is the primary behavioral correlate of theta cell discharge. However, CS cells also show directional- and velocity-modulated firing (McNaughton et al., Exp. Brain Res., 52:41-49, 1983), and theta cells may exhibit some spatially-selective firing (McNaughton, et al., ibid.; Kubie et al., Soc. Neurosci. Abstr, 11:1231, 1985). Separation of the relative contributions of several variables such as these is a general problem in single unit physiology. For example, in the case of theta cells it has been difficult to distinguish firing correlated with motion from that correlated with spatial location. We have developed an application of multiple regression analysis (MRA) to allow the assessment of the relation of one or several behavioral variables to hippocampal neuron firing while statistically controlling for the effects of other variables.

Single units were recorded from CA1 and the fascia dentata in freely-moving rats. Unit responses and spatial coordinates were collected by a computer while the rat traversed the elevated 8-arm maze. Eight trials of 8-arm traverses were collected and analyzed for each cell. The data for MRA were derived by dividing each arm into 4 space bins. Average neuron firing rates and movement velocities were calculated separately for each bin and radial direction (8 trials x 32 space bins x 2 directions). The 32 spatial locations and 2 directions were represented as binary-valued orthogonal vectors that associated each firing rate with a location, a direction and a velocity. Firing rates were then regressed onto the vectors to obtain the proportion of variance accounted for (Multiple R^2) by spatial location, direction or velocity.

The average R^2 for spatial location for 28 CS cells was 0.261. However, all CS cells showed a significant ($p<.01$) directional component to their spatial firing. When this factor was included, the average R^2 increased to 0.406. In other words, on average 65% of the spatial selectivity was due to the interaction with the directional component. The three-way interaction with space, direction, and velocity increased the R^2 to 0.458. For 12 theta cells 30.4% of the total firing variance was accounted for by a quadratic relationship with velocity. While theta cells showed some spatially selective firing, when the velocity variance was statistically controlled, the individual R^2s for spatial locations and direction were small.

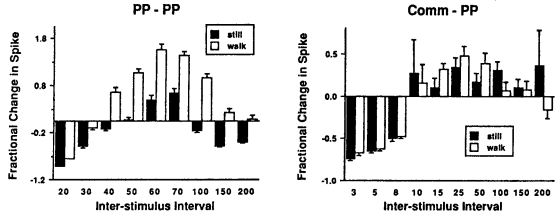
In conclusion, the application of MRA appears to be a useful technique for quantifying behavioral correlations of single unit activity. In the present application it has verified the strong directional component of spatial firing of CS neurons, a component that appears to have been missed in numerous other analyses. Supported by AG-03376 and NS20331.

305.11 EFFECTS OF BEHAVIORAL STATE ON FEEDBACK AND FEEDFORWARD INHIBITION AND FACILITATION IN RAT FASCIA DENTATA. E.J. Green, C.A. Barnes and B.L. McNaughton, Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Prestimulation of the commissural (Comm) or perforant path (PP) afferents shortly prior to stimulation of the PP inhibits the evoked granule cell population spike by activating, respectively, feedforward (Comm-PP) or feedback and feedforward (PP-PP) inhibitory afferents to granule cells. Activation of these pathways at longer latencies can produce an apparent increase in granule cell excitability (spike "facilitation"), the mechanism of which is not well understood. While inhibition and facilitation have been studied in anesthetized preparations, it is not known how they might be altered by the behavioral state of the animal. In these experiments we have evaluated the efficacy of feedback and feedforward inhibition by delivering conditioning shocks to the PP or Comm followed at various intervals by test shocks to the PP while rats were walking slowly on a treadmill or sitting in the same apparatus.

Rats in both behavioral conditions which were given PP prestimulation (PP-PP) exhibited the characteristic pattern of spike inhibition at short intervals and spike facilitation at longer intervals. However, walking rats exhibited a significant right shift in the inhibition/facilitation curve, with markedly smaller amounts of PP-PP inhibition and larger facilitation at some intervals relative to when they were still. These differences could not be accounted for by alterations in the size of the conditioning spike or by the amplitude of the test EPSP. At the stimulus intensities tested thus far, there was no apparent effect of walking on feedforward inhibition produced by commissural prestimulation. There was, however, more spike facilitation at some Comm-PP intervals in walking rats.

These data suggest that the efficacy of inhibitory circuits within the fascia dentata varies with the behavioral state of the animal. Experiments in progress are designed to determine the locus of the behaviorally-dependent alterations within the inhibitory network. Supported by AG05345, AG03376, NS20331.



Examples of PP-PP and Comm-PP interactions as a function of behavioral state for an individual animal.

305.12 BASAL FOREBRAIN GRAFTS INFLUENCE COMPLEX-SPIKE ACTIVITY IN THE HIPPOCAMPUS OF RATS WITH FIMBRIA-FORNIX LESIONS. M.L. Shapiro[1], F.H. Gage[2], and A. Bjorklund[3]. [1]Department of Psychology, Johns Hopkins University, Baltimore, MD, [2]Department of Neurosciences, UCSD, La Jolla, CA, [3]Department of Histology, Lund University, Lund, Sweden.

Complex-spike unit activity from the hippocampus was examined in: (1) normal rats (CON), (2) rats with fimbria-fornix lesions (FF), and (3) rats with fimbria-fornix lesions and intrahippocampal grafts of fetal basal forebrain tissue (GRAFT). The GRAFT rats had previously shown some degree of recovery of spatial memory in the Morris water maze. Single units were recorded while the rats traversed a radial maze during three different types of trials: (1) normal traversal trials, (2) trials in which the maze was covered with cardboard, and (3) trials in which the maze was rotated 90 degrees clockwise. Quantitative analyses of the spatial distribution of activity showed that CON rats had tightly clustered, reliable place-fields that were stable in all trials. In contrast to CON rats, FF rats had more dispersed, less reliable place-fields that were disrupted when the maze was covered or rotated. GRAFT rats had place-fields that were more tightly clustered, more reliable, and more stable when the maze was covered or rotated than the FF rats. Thus, the functioning of hippocampal circuitry was influenced by fetal basal forebrain grafts, and these grafts may have ameliorated the behavioral effects of lesions by restoring, to some degree, critical aspects of neuronal activity in the hippocampus.

305.13 KINDLING OF THE PERFORANT PATH ALTERS DISCRIMINATION-REVERSAL TRAINING OF THE RABBIT NICTITATING MEMBRANE RESPONSE.
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We previously demonstrated that hippocampectomy disrupts reversal learning but not acquisition of the rabbit's nictitating membrane (NM) response to discrimination training (Weikart and Berger, Behav. Brain Res., 1986). In the following study we tested whether similar learning deficits occurred in rabbits with an intact, but neurophysiologically abnormal (i.e. epileptogenic) hippocampus.

Ten male New Zealand White Rabbits were implanted, using electrophysiological control, with stimulation electrodes in the perforant path and a recording electrode in the hippocampal dentate gyrus. Following recovery, 5 rabbits received daily kindling stimulation (2 s, 60 Hz train of 1 ms impulses), at an intensity sufficient to evoke hippocampal epileptiform activity, until 10 class 5 seizures were evoked. The remaining rabbits served as controls. For discrimination training either a tone or a light CS+ was always paired with a corneal airpuff UCS (750 ms ISI), with the other stimulus being the CS- (counterbalanced). A total of 64 CS+ and 64 CS- trials (ITI pseudorandomly varied at 20, 30 or 40 s, averaging 30 s) were given per day (pseudorandom sequence). After reaching a CR rate of >80% to the CS+ and <20% to the CS-, the CS+ and CS- were reversed. Reversal training continued until the same criterion were met, or for a maximum of 28 days.

Kindled animals acquired the initial discrimination significantly ($p<.01$) faster than controls (4.4 vs 13.8 days, respectively). Control animals, however, required significantly ($p<.01$) fewer days (10.0) for reversal learning than the kindled animals (22.2 days), due to the kindled animals continued high response rate to the CS-.

These results demonstrate kindling effects on discrimination-reversal training are more complex than are the effects of hippocampectomy. Although both manipulations disrupt reversal learning, only kindling facilitates acquisition of the initial discrimination. This facilitation may result from a kindling-induced potentiation effect (see Berger, Science, 1984). Kindling also recruits many non-hippocampal structures, a number of which may contribute to either the facilitation or disruption of learning.
Supported by NSF (BNS-8617107)

305.14 HABITUATION OF THE PYRIFORM CORTEX BURST RESPONSE IN VITRO.
J.R. Plant* and D.C. McIntyre. Dept. of Psychology, Carleton Univ., Ottawa, Ont., Canada K1S 5B6.

Habituation, the gradual decrease in amplitude of a behavioral response to repeated presentation of a specific stimulus, is considered to be the most basic and universal form of learning. The habituated response will recover gradually, however, if the same stimulus is withheld or immediately if a different stimulus is presented.

Due to the complexity of the mammalian central nervous system, investigations of habituation have usually involved more simple nervous systems. The few demonstrations of mammalian CNS habituation have involved the disynaptic fexor reflex in the spinal cord (Thompson, R.F. and Spencer, W.A., Psychol. Rev., 173: 73, 1966) and the monosynaptic population response of the hippocampal dentate gyrus in vitro (Teyler, T.J. and Alger, B.E., Brain Res., 115: 413, 1976). In the present study, we demonstrate habituation of the polysynaptic burst response of the rat pyriform cortex in vitro.

Coronal slices of the amygdala-pyriform cortex were taken from male rats as previously described (McIntyre, D.C. and Wong, R.K.S., J. Neurophysiol., 55: 1295, 1986). Provocation of the burst response, recorded intracellularly from neurons in the layer 3 field of the pyriform cortex, was achieved with a bipolar stimulating electrode positioned in the ventral-lateral amygdala. A monophasic anodal pulse (0.1 ms) was passed between the two electrode tips at an intensity suprathreshold for the all-or-none, polysynaptic excitatory burst event. Although this event was always of the same magnitude when elicited at 0.05 Hz using a constant suprathreshold intensity, it gradually habituated to zero when provoked repeatedly at 0.5 Hz. Subsequently withholding the stimulus resulted in the progressive and full recovery of the event within 60 s. In other experiments, after the response was completely habituated at 0.5 Hz, a polarity reversal of the stimulus on the next pulse (cathodal) resulted in an immediate and full recovery of the original burst response. If the next pulse in the series was reversed again to the original polarity (anodal), the burst event was not observed and appeared to be still fully habituated. This result suggests the habituating process has a stimulus specific nature in these cortical circuits.

In intracellular recordings where stimulation resulted in an antidromic response which was observed immediately antecedent to the burst event, no habituation of the antidromic response was observed, while the burst event exhibited the progressive habituation described above. This result suggests that synaptic mechanisms are required for the realization of habituation in the amygdala-pyriform slice preparation.

305.15 TYPE I AND II THETA-LIKE UNIT ACTIVITY IN STRUCTURES OF THE PAPEZ CIRCUIT DURING DIFFERENTIAL AVOIDANCE CONDITIONING IN RABBITS.
M. Mignard, D. Bentzinger*, N. Bender*, and M. Gabriel.
Dept. of Psychol., Univ. of Illinois, Champaign IL 61820.

Rhythmic bursts of neuronal action potentials exhibiting frequencies (4 - 10 Hz) and behavioral relations similar to the the hippocampal theta rhythm occur in the hippocampal formation during differential avoidance conditioning (Gabriel & Saltwick, Physiol. & Behav., 24:303, 1980). In this task, rabbits learn to avoid a shock unconditional stimulus (US) by stepping in an activity wheel in response to a positive conditional stimulus (CS+, a 1 or 8 kHz .5-sec tone) initiated 5 sec before US onset. They also learn to ignore a negative conditional stimulus, a CS- never followed by the US. Trains of rhythmic 7-8 Hz unit bursts following CS onset were similar to type II "immobility" theta (Kramis et al., Exp. Neurol., 49:58, 1975), whereas bursts attaining 10 Hz just before CR initiation, and continuing at high frequencies (8-10 Hz) during locomotion suggested Type I "movement related" theta. Here we report movement related and/or CS related theta-like bursts of action potentials in the posterior cingulate cortex (Brodmann's Area 29b), the anterior ventral (AV) thalamic nucleus, and the medial mammillary (MM) nucleus. Neither the anterior cingulate cortex (Brodmann's Areas 24 and 32), the medial dorsal thalamic nucleus, nor the anterior dorsal (AD) nucleus exhibit such bursts. The CS related bursts were evident in a majority of the approximately 300 Area 29b records obtained since 1983. A similar high prevalence of this pattern has been noted in the dorsal magnocellular region of the AV nucleus just ventral to the AD nuclear border. In 4 MM nuclear recordings to date, each has exhibited CS related bursts. The cortical and thalamic CS related bursts, like immobility theta, were severely attenuated by systemic atropine (25 & 50 mg/Kg) and scopolamine hydrobromide (1, 2, & 4 mg/Kg), but not by scopolamine methylbromide. Clear phase differences between CS+ and CS- elicited burst trains in conditioned rabbits suggested an informational function for the bursts. We have recorded movement related bursts in Area 29b and in the AV nucleus, but at a substantially reduced prevalence relative to CS related bursts. These results implicate the entire circuit of Papez in theta processes. They also support cingulate cortical involvement in these processes (e.g., Holshiemer, Exp. Brain Res, 47:2, 1982), and they indicate that cingulate cortical theta is not volume conducted from the hippocampus as suggested in recent literature. (supported by NIMH Grant 37915 to M.G.)

305.16 NEURONAL AND BEHAVIORAL CORRELATES OF BILATERAL MAMMILLOTHALAMIC TRACT LESIONS DURING AVOIDANCE LEARNING IN RABBITS.
J. Shenker*, Y. Kubota*, M. Mignard, C. Cuppernell*, D. Swanson*, and M. Gabriel. (Spon: E. Donchin). Dept. Psych., Univ. of IL, Champaign, IL, 61820.

Studies of multi-unit activity (MUA) during discriminative avoidance conditioning in rabbits have established the development of massive training-induced excitatory and discriminative neuronal responses to conditional stimuli in the anteroventral (AV) and mediodorsal (MD) thalamic nuclei (Gabriel et al., Information Processing by the Brain, H. Markowitsch (Ed.), in press, 1987). The AV response survives dorsal subicular or posterior cingulate (Area 29) cortical lesions (Gabriel et al., Exp. Brain Res., in press, 1987), suggesting that subcortical afferents are necessary for its development. A critical AV afferent may be the mammillary bodies (MB), which send diencephalic projections exclusively to the anterior thalamus via the mammillothalamic tract (MTT), and damage to which has been related to memory dysfunction in humans (Mair et al., Brain, 102:749, 1979) and monkeys (Aggleton et al., Exp. Brain Res., 58:190, 1985). Here we evaluate the MB contribution to the AV response via bilateral electrolytic MTT lesions (15-30 s., 1.5 mA.) and MUA neuronal recording during conditioning with rabbits. Microelectrodes (10-40 micron tip) were stereotaxically implanted bilaterally in the AV for MUA recording. Rabbits were trained in a running wheel with pure tones (1 and 8 kHz) as stimuli: a positive conditional stimulus (CS+) predicted a footshock (US), delivered 5 sec. after CS onset, while a negative conditional stimulus (CS-) predicted no US. Post-CS+ wheel locomotion (CR) prevented the US. Bilateral MTT lesions ($N=3$) abolished CR acquisition except for a transient burst of CRs early in training, possibly mediated by an intact MD. Lesions of the adjacent hypothalamus and ventral thalamus had no effect on CR acquisition. MTT lesions also eliminated the training-dependent excitation in the homolateral AV response ($N=6$). However, neuronal CS discrimination in AV persisted: the CS+ response did not change but the CS- response decreased with training. This contrasts with AV responses in controls, wherein a training-induced increased CS+ response exceeds an increased CS- response. Thus the exitatory and discriminative components of the AV response may be separately controlled, with MB input to AV necessary for the former but not the latter. It should now be possible to determine which remaining AV afferent(s) are necessary for CS discrimination.
(Supported by NIMH Grant 37915 to M.G.)

305.17 ANTERIOR CINGULATE CORTICAL IBOTENIC ACID LESIONS ENHANCE CONDITIONING-INDUCED UNIT ACTIVITY IN THE MD THALAMIC NUCLEUS IN RABBITS. M. Gabriel, Y. Kubota*, S. Sparenborg, and K. Straube*. Dept. of Psychol., Univ. Illinois, Champaign, IL 61820

This study is part of a project investigating the neural mediation of discriminative avoidance learning, i.e., learning by rabbits to avoid a shock unconditional stimulus (US) by stepping in a running wheel within 5 sec. after the onset of a .5 sec. tone (a positive conditional stimulus or CS+). Also learned is nonresponse to a negative CS (CS-), a different tone not predictive of shock. Asymptotic performance (conditioned responses [CRs] on >80% of CS+ trials and <8% of CS- trials) occurs after an average of 4-5 daily training sessions, each session consisting of 60 trials with each CS in an irregular order. AV and MD thalamic neurons exhibit massive training-induced discharges to the CSs and lesions in these nuclei blocked acquisition (Gabriel et al., in The Hippocampus., IV:1, 1986). Subicular or posterior cingulate cortical (Area 29) lesions increased CS driven AV thalamic unit activity and subicular lesions also increased CR frequency (Gabriel et al., Exp. Brain. Res., in press, 1986). Area 29 lesions decreased CR frequency because, we propose, AV thalamic output must traverse Area 29 to reach motor structures (e.g., the striatum) for CR output. These results suggested that AV thalamic activity triggers CR output. Also, Area 29 neurons controlled by subicular afferents exert a "limiting" influence on AV thalamic activity in intact animals. Here we test the parallel hypothesis that MD activity is limited by afferents from the anterior cingulate cortex (Area 24). To date, acceptable lesions (>60% cell loss) have been made in 5 rabbits by injecting 5 ug/ul of ibotenic acid (IBO) at six Area 24 sites equally-spaced along the midline in each hemisphere. One ul of IBO per mm of tissue was injected as the cannula was raised from the ventral to the dorsal limits of Area 24. Stainless steel microelectrodes (tip lengths: 10-40 microns) were implanted bilaterally in the MD nucleus for chronic unit recording. MD records were obtained in 4 lesion, 4 saline-injected and 5 noninjected rabbits. In support of the hypothesis, the MD unit response to both CSs was enhanced in lesion rabbits during the sessions of the first behavioral discrimination and criterion attainment ($P<.006$). Activity in the two control groups did not differ. The rabbits with lesions required an average of 8.6 sessions to reach criterion compared to 5.0 sessions in saline injected and incompletely lesioned controls ($P<.08$). We suggest that this learning impairment is due to the elimination of MD driven Area 24 output to striatal motor system targets. (Supported by NIMH Grant 37915 to M.G.)

305.18 AD THALAMIC LESIONS, AV THALAMIC AND CINGULATE CORTICAL NEURONAL ACTIVITY, AND AVIODANCE LEARNING IN RABBITS. Y. Kubota*, J. Shenker*, M. Mignard, D. Bentzinger*, and M. Gabriel. (SPON: P.Johnston). Dept. Psychol., Univ. Illinois, Champaign, IL 61820

The anteroventral (AV) thalamic nucleus develops learning-related discriminative neuronal activity in response to auditory conditional stimuli (CSs) during differential avoidance conditioning in rabbits (Gabriel et al., Science, 208:1050, 1980). The anterodorsal (AD) nucleus exhibits learning-related neuronal changes that are reciprocally related to those in the AV nucleus with respect to the stages of behavioral acquisition (Bice et al., Neurosci. Abstr., 12:517, 1986). In the first training session, AD firing frequency peaked whereas AV and conditioned response (CR) frequency were low. These relations were reversed during the criterial session: AD activity decreased, AV activity was at its peak and many CRs occurred. Also, AD activity increased significantly in response to a novel stimulus. These findings suggest that the AD provides the source of synaptic drive that limits AV activity when unexpected environmental events call for response suppression. It follows that lesions in the AD nucleus should increase AV activity and CR frequency in sessions in which novel training contingencies are experienced.

Bilateral electrolytic or chemical (ibotenic acid) lesions were made in 12 rabbits. Histological examination revealed bilateral damage in the AD nucleus in 4 of these subjects. The remaining subjects had small lesions in the hippocampus or in the cortex. Rabbits were given standard conditioning, followed by extinction (procedures described in adjacent abstract). As predicted the rabbits with AD lesions made significantly more CRs than controls and those with hippocampal or cortical lesions in the first acquisition session and in the first extinction session ($P<.001$). In the first extinction session, the CS-elicited AV activity of rabbits with the AD lesions was greater than that in the control rabbits ($P<.001$). No statistically significant difference was found in the first acquisition session, but there was a trend in the expected direction ($P<.12$). Area 29 activity was enhanced in the same sessions after damage in the AD nucleus ($P<.01$ for both sessions). Increased activity after AD lesions also appeared in Area 24, only in the first acquisition session ($P<.05$). The similarity between these effects and the effects of subicular lesions (Gabriel et al., Exp. Brain Res., in press, 1987) suggests that the subiculum and the AD nucleus cooperate in the limiting of AV activity and behavior in response to unexpected training contingencies. (Supported by NIMH Grant 37915 to M.G.)

NEUROENDOCRINE CONTROLS: OTHER II

306.1 A COMBINED ROLE FOR ESTROGEN AND PROGESTERONE IN THE CYCLING OF OPIATE RECEPTORS IN THE RAT MEDIAL PREOPTIC AREA. M. Hijazi* and R. P. Hammer, Jr. (SPON: H. Gillary). Depts. of Physiology and Anatomy & Reproductive Biology, University of Hawaii School of Medicine, Honolulu, HI 96822.

Opiate receptor distribution in the medial preoptic area (MPOA) is known to be sexually dimorphic and gonadal-steroid dependent. We examined [^{3}H]naloxone binding in autoradiographs of sections containing caudal levels of the MPOA in adult rats. Females were ovariectomized and treated with the following hormonal regimens: 1) acute estrogen (AE) group - subcutaneous injection of 25 ug estradiol benzoate (E) in sesame oil vehicle 2 hrs before decapitation; 2) delayed estrogen (DE) group - subcutaneous injection of 10 ug E at 0 and 24 hrs, followed by decapitation at 72 hrs; 3) estrogen-progesterone (EP) group - subcutaneous injection of 10 ug E at 0 and 24 hrs, 2.5 ug progesterone (P) at 69 hrs, and decapitation at 72 hrs. Males received no surgical or hormonal treatment. Following decapitation, brains were removed, frozen, and sectioned. Sections were incubated for 60 min in 2.5 nM [^{3}H]naloxone in 50 mM Tris (pH 7.4) and 100 nM NaCl at 0°C. These conditions favor binding to the mu-subtype opiate receptor. After incubation, tissue was fixed in paraformaldehyde vapors, defatted in xylene, and apposed to ^{3}H sensitive film in X-ray cassettes. Autoradiographs were analyzed using computer-assisted densitometry and calibrated using autoradiographic standards in units of dpm/mg protein. MPOA [^{3}H]naloxone binding density in males was significantly less ($p<0.01$) than in all hormone-treated female groups. [^{3}H]Naloxone binding in the EP group was significantly greater than in the AE ($p<0.001$) and the DE group ($p<0.01$). MPOA opiate receptor density in the AE and DE groups were not significantly different. Therefore, exposure to P increased MPOA opiate receptor concentration in E-primed females, whereas E alone produced lower MPOA opiate receptor levels. These results confirm earlier studies of MPOA opiate binding during pregnancy (Hammer and Bridges, Brain Res., in press). Further studies using enhanced resolution methods and additional control groups are underway in order to determine the precise neuroanatomical correlate of the gonadal steroid-dependent MPOA opiate receptor region. These data suggest that the cyclical alteration of MPOA opiate receptor density is dependent on the presence of P after sufficient E priming. These gonadal steroid hormones probably act in concert to alter MPOA opiate receptor content. (Supported by UHPHS Awards HD19951, RR08125 and NS01161 to R.P.H.)

306.2 ALPHA-METHYL-PARATYROSINE (αMPT): A TOOL TO EXAMINE THE INTERACTION BETWEEN ESTROGEN, CATECHOLAMINES AND α1 RECEPTORS. M.A. Sortino, N.G. Weiland and P.M. Wise. Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201.

α MPT is a competitive inhibitor of tyrosine hydroxylase, the rate limiting enzyme in catecholamine biosynthesis. It has been used to block the LH surge which occurs on proestrous afternoon and in several studies relating catecholamine turnover to reproductive function. As estrogen decreases α1-adrenergic receptor densitities in the medial preoptic nucleus, we wanted to determine whether this effect was related to increased norepinephrine (NE) turnover. To assess the relationship between NE and α1 receptor densities, we wished to block the diurnal increase in NE turnover by administering αMPT. It was therefore essential to establish whether αMPT had a direct effect on ^{3}H-Prazosin binding in vitro or in vivo.

In the first experiment slide-mounted, 20 µM thick brain sections were incubated for 30 min with different concentrations of αMPT ($1x10^{-4}$ to $1.28x10^{-2}$ M) in the presence of 2 nM ^{3}H-Prazosin. After 3 subsequent washes, the brain slices were removed with glass fiber filters and counted. αMPT inhibited ^{3}H-Prazosin binding to α1 receptors in a dose-related manner: the first significant inhibition was detected at 400 µM and half maximal inhibition occurred at about 6 mM. Scatchard analysis was performed using ^{3}H-Prazosin in the presence or absence of 3 different concentrations of αMPT. ^{3}H-Prazosin bound to a single class of binding sites with a Kd of 0.6 nM. αMPT exhibited a simple competitive interaction with the ^{3}H-Prazosin binding sites evidenced by a progressive increase in the apparent Kd for ^{3}H-Prazosin in the presence of increasing concentrations of the drug. To establish whether the effect of αMPT on ^{3}H-Prazosin binding is reversible in vitro, brain slices were preincubated with 5 mM αMPT for 30 min, washed and then incubated with ^{3}H-Prazosin for 30 min in the absence of the drug. No inhibition was observed, suggesting that the inhibitory effect of αMPT is fully reversible in vitro. In a final experiment we tested whether αMPT treatment in vivo affected ^{3}H-Prazosin binding in vitro. Rats were treated with αMPT (400 mg/kg, i.p.) and were killed 45 min later. Brains were removed and prepared as described above. α1 receptor densities are unaffected by prior exposure to αMPT in vivo. The data indicate that αMPT inhibits the binding of ^{3}H- Prazosin to α1 receptors in whole brain slices. This effect is reversible and not observed when αMPT is administered in vivo. Therefore αMPT can be used as a tool to inhibit NE activity and study the relationship between catecholamine release and α1 receptor densities. (Supported by NIH grants AG-02224, AG-00168, HD-15955, AG-05357).

306.3 IMMUNOCYTOCHEMICAL LOCALIZATION OF PROGESTIN RECEPTORS IN GUINEA PIG BRAIN USING MONOCLONAL ANTIBODIES. J.D. Blaustein, J.C. King and J. Turcotte*. Neuroscience and Behavior Program and Psychology Department, University of Massachusetts, Amherst, MA 01003.

A variety of studies has implicated intracellular neural progestin receptors (PRs) in the mediation of progesterone's effects on sexual behavior in female rodents. Most studies of PRs have used techniques which require binding of radioactively-labeled ligands to the PR. Recently, the feasibility of reliable immunocytochemical detection of PRs in guinea pig brain has been demonstrated (M. Warembourg et al., Brain Research, 384, 121, 1986).

We used monoclonal antibodies directed against either purified chick oviduct PRs or rabbit uterine PRs in an unlabeled immunocytochemical technique. In estradiol-primed ovariectomized guinea pigs (10 ug estradiol benzoate x 3 days), PR-immunoreactive neurons were observed in heaviest concentration in the arcuate nucleus. Other areas of high density are the ventromedial nucleus of the hypothalamus, in particular, the ventrolateral aspect, the periventricular hypothalamus, the periventricular preoptic area, the medial preoptic area and the suprachiasmatic preoptic area. Few PR-positive cells have been detected outside this region. These results are in substantial agreement with previous work using [^{3}H]progesterone autoradiography (S. Sar and W. Stumpf, Science, 183, 1266, 1973).

Confirming a variety of recent reports on the subcellular localization of PRs, immunoreactivity was intranuclear, with little if any reaction product in the soma. No immunoreactivity was seen in ovariectomized animals in the absence of estradiol-priming. Progesterone injection had no effect on the intracellular localization. A single, behaviorally-effective injection of estradiol benzoate (10 ug) 2 days prior to sacrifice was sufficient to induce immunoreactivity, although the intensity was lighter than in animals receiving repeated injections.

A similar pattern of immunoreactivity was seen with monoclonal antibodies to chick or rabbit progestin receptors, although the rabbit antibody resulted in a darker reaction product. In preliminary experiments, we have observed a small degree of colocalization of tyrosine hydroxylase-immunoreactivity (TH) in PR-immunoreactive neurons in the arcuate, but not the periventricular nucleus. In addition, in a limited number of cases, we have observed TH-immunoreactive varicosities closely abutting PR-immunoreactive neurons in the arcuate nucleus.
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306.4 CHLORDECONE EFFECTS ON CNS ESTROGEN AND PROGESTERONE RECEPTORS. J. Williams*, K. Eckols*, and L. Uphouse. Department of Biology, Texas Woman's University, Denton, Texas, 76204.

The chlorinated pesticide, chlordecone, mimics estrogen on a variety of parameters, including increased uterine growth, uterine fluid retention and vaginal cornification. In mammalian uterus and chick oviduct, such estrogenicity has been attributed to the pesticide's interaction with the intracellular estradiol receptor. In neural tissue, the similarities between estrogen and chlordecone are less evident. In female rodents, estrogen regulates neuroendocrine function and is essential for the expression of female reproductive behavior. Although chlordecone mimics estradiol's negative feedback on LH (but not FSH) release, it fails to facilitate the LH surge . Similarly, the pesticide fails to mimic estradiol's facilitation of female rat sexual receptivity. Furthermore, chlordecone can attenuate estradiol's effect on both the preovulatory LH surge and sexual receptivity. It is generally assumed that an interaction between estradiol and its intracellular receptor is required to prime the nervous system for an LH surge and for sexual behavior. CNS estrogen receptor replenishment and elevation of the progesterone receptor depend upon estrogen's interaction with its CNS receptor. Consequently, estradiol's effect on CNS steroid receptors is required for progesterone's facilitation of the preovulatory LH surge and sexual receptivity.

In the following studies, chlordecone's interaction with the CNS estradiol receptor and its ability to elevate progesterone receptors were examined in neural and uterine tissues. In both tissues, chlordecone showed evidence of interaction with the intracellular estrogen receptor. The pesticide competed *in vitro* for binding to the receptor and *in vivo*, led to nuclear retention of the estrogen receptor. Chlordecone's potency in competiting for ^{3}H-estradiol binding was relatively low with an IC_{50} about 10^{-4} to 10^{-5}. Nuclear retention duration and time course were also significantly different for chlordecone than for estradiol but there was a definite estrogen receptor elevation in both estradiol and chlordecone treated females.

The apparent estrogenicity of the pesticide at the CNS estrogen receptor was in sharp contrast to its behavior regarding the progesterone receptor. There was no evidence of competition between chlordecone and ^{3}H-R5020 for the progesterone receptor. Female rats receiving chlordecone *in vivo* exhibited levels of CNS progesterone receptors that were identical to control females, while progesterone receptors were significantly elevated by estradiol. The neural effects were clearly distinct from the uterine effects of the pesticide. In uterine tissue, progesterone receptors were significantly elevated by the *in vivo* exposure.

The present results confirm prior suggestions that the similarities between chlordecone and estradiol are more prevalent at peripheral than at neural tissues. It is suggested that the failure of the pesticide to mimic molecular responses to estradiol's interaction with its CNS receptor accounts for the pesticide's failure to reproduce the entire spectrum of neural events characteristic of estrogen.

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306.5 ESTROGEN PRIMING AFFECTED MEDIAL AMYGDALA NEURON EXCITABILITY RECORDED INTRACELLULARLY. Mya C. Schiess, Marian Joëls, Patricia Shinnick-Gallagher, UTMB, Dept. of Pharmacology, Galveston, Texas, 77550

The medial nucleus of the rat amygdala (MNA) is a sexually dimorphic structure and a target tissue for estrogen binding. Recently Nabekura et al using an in vitro preparation of the medial amygdala showed that superfusion of 17-beta estradiol had a direct membrane effect, causing a membrane hyperpolarization and a decrease in input resistance (Ri). This effect was thought to be mediated by a decrease in K^+ permeability. In this project, we tested whether estrogen priming (>24 hours) had a long-term or genomic effect on cell activity.

Adult female Spraque-Dawley rats were ovariectomized (OVX) at 10 to 12 weeks. 500 micron coronal slices of the right amygdala including the medial nucleus (-2.3 to -3.3 mm Bregma) were obtained from OVX non-primed (N=20) and OVX estrogen (EB) primed rats (N=24). Estrogen priming involved administration of 100 micrograms of β-estradiol subcutaneously to the nape of the neck 24 hours prior to the experiment. Intracellular recording techniques were employed and discerned that there was no significant difference between the resting membrane potential (RMP) and Ri of the OVX and EB primed cells; OVX RMP=-71 mv/EB RMP=-69 mV, OVX Ri=71 mΩ/EB Ri=68.5Ωm .

Active membrane properties differed significantly between neurons from the OVX non-primed rats and the EB primed rats. Neurons from EB primed rats had prominent voltage dependent depolarizing after potentials (DAP's). Neurons from EB primed rats were spontaneously active with frequent EPSP's (70%), while the OVX non-primed neurons were rarely spontaneous (10%). Intracellular injection of small cathodal pulses of 5 ms and 100 to 300 pA elicited volleys of 3 to 4 spikes (N=22/24), whereas OVX non-primed neurons fired once and then shut off irrespective of the duration or amplitude of current injected (N=20/20). When long cathodal pulses of 500 ms and 100 to 600 pA were passed through the recording electrode 60% of the EB primed neurons did not accommodate, while 95% of the neurons from OVX non-primed rats fired once then shut off.

These results suggest that long-term exposure to estrogen at an estrogen sensitive target tissue such as the MNA increases synaptic activity and enhances neuronal excitability at an intracellular level.

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Nabekura, J; Oomura, Y.; Minami, T; Mizuno, Y; Fukuda, A Science, 23: 226-228, 1986.

306.6 **PITUITARY-ADRENAL AND THYROID EFFECTS ON THE CONTENT OF MELATONIN IN THE PINEAL GLAND OF THE RAT.** **M. Bauer; R. Poland*; P. Whybrow and A. Frazer, Depart. of Psychiatry, Univ. of Pennsylvania and Vet. Adm. Hospital, Philadelphia, PA, 19104 and Dept. of Psychiatry, Harbor-UCLA Medical Center, Torrance, CA 90509.**

Clinical studies have shown that decreased nocturnal levels of melatonin in serum occur frequently in persons with major depressive disorder. This phenomenon is often accompanied by evidence of increased pituitary-adrenal activity. Similarly, thyroid abnormalities are seen in a subset of depressives. In preclinical studies, both glucocorticoids and thyroid hormones have been shown to alter adrenergic function, which plays an important role in the control of melatonin production. We therefore hypothesized that alterations in the pituitary-adrenal or thyroid axes could alter the nocturnal rise of the content of melatonin in the pineal gland of the rat.

Male Sprague-Dawley rats (200-225 g), housed under a 12:12 light:dark cycle, were used in both experiments. In the first experiment, three groups of rats were treated with once daily s.c. injections of either ACTH(1-24), 10 units; corticosterone, 7.5 mg; or sesame oil vehicle. All injections were given at 1600 hrs and were continued for 9 days. Rats were then sacrificed 2 to 23 hrs after the final injection. Trunk blood was taken for determination of corticosterone in serum. The pineal was removed for measurement of its content of melatonin. Both corticosterone and melatonin were analyzed by radioimmunoassay. Administration of either corticosterone or ACTH caused a significant increase in serum concentrations of corticosterone between 1730-0100 hrs, compared to that measured in controls. Despite this, treated rats did not differ from control rats in either the day- or night-time content of melatonin in the pineal gland.

In the second experiment, animals were obtained 3 days after thyroid-parathyroidectomy (TPx) or sham operation. After 7 days of acclimitization, TPx rats and one group of sham operated animals received for 9 days daily subcutaneous injections of saline (hypothyroid and euthyroid groups, respectively), and one group of sham operated animals received daily injections of 15 ug of triiodothyronine (hyperthyroid group). Both experimental groups showed significant differences from control rats in heart weight-to-body weight ratios, as expected in hypo- and hyperthyroidism. Again, no significant difference was observed in either daytime levels or the nocturnal content of melatonin of the pineal gland in the hypo- or hyperthyroid rats in comparison to that in euthyroid controls.

The results of these two studies indicate that the amplitude of the nocturnal rise of pineal melatonin content is not readily affected by alteration in the pituitary-adrenal or thyroid axes. Alterations in these two neuroendocrine systems may not be responsible for the blunted nocturnal rise in melatonin observed in major depressive disorders. **(Supported by research funds from the Vet. Adm. and USPHS grants MH 29094 and MH 14654).**

306.7 EVIDENCE THAT DARK RELATED CHANGES IN NORADRENERGIC INPUT COULD BE THE ORIGIN OF THE 24 HOUR RHYTHM IN RAT PINEAL BETA-ADRENERGIC RECEPTOR DENSITY. A. Gonzalez-Brito*, R.J. Reiter, D.J. Jones, J.M. Guerrero* and M. Puig-Domingo*. (SPON: T. Mikiten). Dept of Cell. & Struct. Biology and Dept. of Anesthesiology. UTHSCSA, San Antonio, TX 78284-7762.

It has been reported repeatedly that there exists an increase in rat pineal norepinephrine (NE) content and/or turnover during the dark phase; however, studies concerning a 24 h variation in NE release from the sympathetic pineal nerve endings has not been reported. We have recently reported a 24 h variation in rat pineal beta-adrenoreceptor density using a simple procedure that permits measurement of [125I]Iodopindolol ([125I]IPIN) binding to individual pineal glands (Gonzalez-Brito et al, submitted). Since NE interacts with postsynaptics beta-receptors on pinealocytes to regulate the melatonin rhythm, the purpose of the present study was to define the alterations in pineal beta-receptors during the light-dark cycle and to determine if the beta-agonist isoproterenol differentially alters receptor regulation.

Adult male Sprague-Dawley rats maintained in a 14:10 LD cycle (lights on at 0600 h) showed an increase in [125I]IPIN binding sites during the first half of the night which peaked at 0200 h and decreased to lowest values before lights on. We now report that in animals maintained on the same lighting schedule the decrease in [125I]IPIN binding detected late in the dark phase was prevented by moving the animals to light at 0200 h. However, a single subcutaneous (s.c.) injection of isoproterenol (0.5 mg per kg of body weight, dissolved in saline) at 0200 h in light exposed animals induced a decrease in beta-adrenoreceptor density that paralleled and simulated that normally seen in control LD animals at this time. Animals that were kept in light from the onset of the normal dark period (2000 h) exhibited a sustained increase in density of [125I]IPIN binding sites. In these animals a s.c. 0.5 mg/kg isoproterenol injection at 2000 h induced, several hours later, an increase in beta-adrenoreceptor density.

The present results show that both the increase and subsequent decrease in pineal beta-adrenoreceptor density that occurs at night can be modified by changing the lighting environment conditions and can be simulated by the beta-adrenergic agonist isoproterenol. We conclude that the genesis of the 24 h rhythm in pineal beta-adrenoreceptor binding sites previously reported seems to be determined by mechanisms of up and down regulation of the receptors and that such mechanisms are a consequence of photoperiodic induced changes in noradrenergic input to the pineal gland. However, we can not preclude a role for other putative modulating factors.

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306.8 POSSIBLE SUPRACHIASMATIC NUCLEUS ---> SPINAL CORD PATHWAYS. J.W. Patrickson, and T.E. Smith*. Department of Anatomy, Schools of Medicine and Dentistry, Loma Linda University, Loma Linda CA 92350.

The secretion of melatonin by the pineal gland is under the control of the circadian rhythm generator, the suprachiasmatic nucleus (SCN) of the hypothalamus. Impulses generated by the SCN ultimately reach the pineal gland via postganglionic sympathetic fibers of the superior cervical ganglia. The objective of this study was to determine the possible SCN - spinal cord pathways. This was accomplished by comparing the efferent projection sites of the SCN (group 1) to those neuronal sites that project to the C8 - T2 segments of the spinal cord (group 2) in Sprague Dawley rats (275 - 325gm). The spinal segments were determined by localizing the distribution of the preganglionics that project to the superior cervical ganglion using the HRP neuron tracing technique.

The efferent projections of SCN were determined by injecting .05µl of 4% WGA-HRP into the nucleus and localizing the anterogradely transported product. Spinal projecting neurons were identified by localizing HRP-positive neurons subsequent to the injection of 0.5µl of 4% WGA-HRP into the spinal cord (C8-T2). Both groups of animals were anesthetized with pentobarbital (50mg/kg). The animals were re-anesthetized and perfused 48hrs post HRP injection, and 30µm frozen sections were made and processed using tetramethylbenzidine as the chromagen.

The sites common to both groups are: the paraventricular nucleus of the hypothalamus (periventricular, dorsal cap, medial parvocellular) and the dorsal hypothalamic area. These sites were also confirmed using double labeling techniques. In addition, spinal projecting neurons were identified within the retrochiasmatic area.

We conclude that these sites represent the most probable pathways by which the SCN relays its circadian generated rhythm to the preganglionic sympathetic neurons of the spinal cord.

306.9 THE EFFECTS OF PINEALECTOMY ON HIGH VOLTAGE CORTICAL ACTIVITY AND AFTERDISCHARGE THRESHOLD AND DURATION FROM THE AMYGDALA IN THE RAT. J.H. Peck, K. Soria, and L.J. Grota. Dept. of Psychology, Ithaca College, Ithaca, NY 14850.

The pineal gland and its hormone melatonin, have been implicated in a variety of disorders including epilepsy, parkinson's disease and seasonal affective disorders. A number of investigators have shown behavioral and EEG effects of pinealectomy. Subsequent injections of pineal concentrate or melatonin have reversed these effects. Our past research has attempted to replicate findings on the pineal's role in epileptic-like abnormal brain EEG. We have used antibody to melatonin and pinealectomy in both acute and chronic preparations without success. This study investigated the role of pinealectomy in afterdischarge production by the amygdala.

Twenty male rats were either pinealectomized or sham operated using a suction technique. One pair of stainless steel bone screws served as cortical electrodes on each hemisphere. Depth electrodes were also implanted in the amygdala and hippocampus contralateral to each other. The rats recovered for one week and EEG was recorded during the light and dark phases of their daily cycle. No efforts were made to prevent the rats from sleeping and hence generating low frequency high voltage outbursts or "sleep spindles". Next, the rats were given an ascending series of constant current shocks from 30 to 1000 microamps, every 5 mins., to the amygdala, during the dark phase of their daily cycle, and afterdischarge was recorded. Only one rat reached a stage 5 seizure and was stopped before reaching the maximum stimulation.

There was significantly more high voltage cortical activity during the day than during the night. But there was no effect of pinealectomy - sham surgery or an interaction. There was a marginal statistical difference in afterdischarge threshold ($.075 > p > .05$), with pinealectomized rats having a lower threshold than shams. There was no significant difference in duration of afterdischarge, but pinealectomized rats had a significantly higher % of trials with amygdala afterdischarge than shams.

These data support the idea that the pineal gland may play a role in some disease states. The role of melatonin should not be taken for granted, however, since most recent evidence indicates that the pineal gland may have direct connections with the brain and hence modify the brain through synaptic connections rather than hormonally.

306.10 NADOLOL OR PROPRANOLOL PREVENTS SHORT PHOTOPERIOD-INDUCED BUT NOT MELATONIN-INDUCED GONADAL REGRESSION IN MALE SYRIAN HAMSTERS. T.H. Champney. Department of Medical Anatomy, Texas A&M University, College Station, TX 77843.

Beta adrenergic control of pineal melatonin (MEL) synthesis is responsible for the nocturnal increase in rat pineal MEL production. Regulation of Syrian hamster MEL synthesis also appears to be regulated by beta adrenergic receptors with a powerful reuptake system. This mechanism prevents pharmacologic stimulation of MEL synthesis unless the reuptake system is blocked or disabled. However, beta adrenergic antagonists are capable of blocking pineal MEL production in Syrian hamsters. The present study examined the ability of two beta adrenergic antagonists, propranolol (PROP) and nadolol (NAD), to block pineal MEL production on a daily basis and, thereby, disrupt the pineal's influence on short photoperiod-induced and MEL-induced gonadal regression. Adult male Syrian hamsters were placed in either long (14L:10D, lights on at 0500h) or short (10L:14D, lights on at 0700h) photoperiods. Short photoperiod (SP) groups consisted of a saline-injected (SAL) group, three PROP-injected groups (500 µg, 250 µg or 125 µg PROP-injected) and a NAD-injected group (250 µg). These hamsters were injected (0.1 ml s.c.) every day for nine weeks at 1630h. Long photoperiod (LP) groups consisted of a SAL group, a PROP group (250 µg), a NAD group (250 µg), a MEL group (25 µg), a PROP + MEL group and a NAD + MEL group. These hamsters were injected (0.1 ml s.c.) every day for nine weeks at 1645h. At the end of the experimental period, the hamsters were killed by decapitation and their testes weights and serum were collected. Serum testosterone was determined by radioimmunoassay kit (Diagnostic Products, Inc.). Both testicular weights and testosterone levels were depressed in SP/SAL, LP/MEL, LP/PROP + MEL and LP/NAD + MEL treated groups ($p<0.001$ vs. LP/SAL). PROP and NAD prevented SP-induced gonadal regression. PROP is a general beta adrenergic antagonist which can cross the blood brain barrier, while NAD is unable to cross the blood brain barrier in appreciable amounts. Therefore, NAD blocks peripheral beta adrenergic receptors without affecting central beta adrenergic function. Since the pineal is located outside of the blood brain barrier, it appears that NAD and PROP are preventing SP-induced gonadal regression by blocking pineal neurotransmission and not by altering central beta adrenergic receptors. Interestingly, PROP and NAD are unable to prevent MEL-induced gonadal regression, even though intact pineal MEL synthesis has been implicated in MEL-induced regression. The present results indicate that the endogenous MEL peak may not be necessary for MEL-induced testicular inhibition. In conclusion, PROP and NAD are capable of preventing SP-induced, pineal mediated gonadal depression, while they are incapable of preventing MEL-induced testicular regression. Supported by NIH grant #RR05814.

306.11 EFFECT OF LIGHT AND ISOPROTERENOL ON THE NOCTURNAL RISE OF TYPE-II THYROXINE 5'-DEIODINASE IN RAT FRONTAL CORTEX. J.M. Guerrero*, M. Puig-Domingo*, R.J. Reiter, A. Gonzalez-Brito* and A. Menendez-Pelaez*. Dept. of Cellular & Structural Biology, UTHSCSA, San Antonio, TX 78284.

We have previously described the existence of a diurnal variation of type-II thyroxine 5'-deiodinase (5'-D) activity in rat frontal cortex. This enzyme exhibits the lowest activity at daytime, increasing progressively after the onset of the dark period with a peak at 0500 h, just prior the lights on. We have also described that maintaining the rats under continuous light enhances the 5'-D activity. We now report, for the first time, the effect of isoproterenol and/or light on rat frontal cortex 5'-D activity. Adult male Sprague-Dawley rats were housed under a 14:10 LD cycle with lights off at 2000 h. A group of animals were injected (s.c.) with a single dose of isoproterenol (0.5 mg/kg in saline) either at 0200 or 0400 h, and 5'-D activity determined at 0400 and 0600 h, respectively. In another experiment, animals were transferred to the light either at 0130 or at 0330 h, then injected with isoproterenol (0200 or 0400 h) and 5'-D activity determined two hours later. 5'-D activity was determined by a radioenzymatic method measuring the release of 125I from [3',5'-125I]T4 and results expressed as fmol 125I released/mg prot/h. As previously described, 5'-D activity increased progressively during the dark period (4.4 ± 0.9 at 0200 h; 7.5 ± 2.2 at 0400 h; 10.1 ± 2.3 at 0600 h). A single injection of isoproterenol at 0200 h elicited an increase of 5'-D activity at0400 h (15.0 ± 2.3; $p<0.01$) but injection at 0400 h was ineffective in increasing the 5'-D activity at 0600 h. When animals were transferred to the light at 0130 h an increase of 5'-D activity was observed both at 0200 h (9.6 ± 3.0; $p<0.05$) and 0400 h (11.9 ± 1.8; $p<0.05$). Similarly, when animals were tranferred to the light at 0300 h, 5'-D activity also increased at 0400 h (23.7 ± 2.8; $p<0.01$); at 0600 the increase was much lower (16.6 ± 2.3; $p<0.05$). A single injection of isoproterenol at 0200 h increased the 5'-D activity at 0400 h (19.6 ± 3.0; $p<0.05$) but the injection at 0400 h had no effect (15.6 ± 2.6). In conclusion, these results confirm that light exposure during the dark period enhances 5'-D activity in rat frontal cortex, being the effect less evident at the end of darkness. Isoproterenol also enhances the 5'-D activity in both control rats and rats transferred to light at night, but only at 0200-0400 h. If the effect of the light is also mediated by an adrenergic input remains to be studied; but it is clear that 5'-D activity is sensitive to both light exposure and isoproterenol only in the middle of the dark period, being insensitive to the same two stimuli just prior the lights on.

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306.12 INTERACTIONS BETWEEN DOPAMINE AND ANGIOTENSIN II IN THE REGULATION OF ALDOSTERONE PRODUCTION. P.Liberini*, C.Missale, M.Memo, M.O.Carruba*, P.F.Spano.(SPON:L.Valzelli) Inst Pharm Exp Ther, School of Medicine Univ of Brescia, Brescia, Italy

Aldosterone (ALD) secretion is subject to both stimulatory and inhibitory control. Angiotensin II (AII) is the primary stimulator of ALD production and an inhibitory role of dopamine (DA) has been recently suggested. On this line, we found that there are both D-1 receptors coupled with stimulation of adenylate cyclase (AC) and D-2 receptors associated with inhibition of AC in rat adrenal glomerulosa.

We now investigated the interactions between DA and AII in the intracellular events leading to ALD production. The intracellular mechanisms by which AII stimulates ALD production appears to involve several molecular events in the plasma membrane of glomerulosa cells including stimulation of polyphosphoinositide turnover. By measuring ALD secretion and cAMP formation in isolated adrenal glomerulosa cells we obtained evidence that AII induced a sustained stimulation of ALD secretion and a rapid and transient increase in cAMP formation. The early effect is an increase in cAMP with a maximum at 10 min with ALD secretion beginning later (at 20 min) and linearly increasing up to 120 min. The pharmacological characterization with AII analogs and AII receptor antagonists is consistent with a role of cAMP in AII steroidogenesis, suggesting a dual messenger function in the action of AII. DA, over the range of 10 nM to 1 uM, inhibited both ALD secretion and cAMP formation induced by submaximal concentrations of AII. DA agonists selectively acting on D-2 receptors such as LY 171555, bromocriptine or dihydroergotoxine, while inactive under basal conditions, were more effective than DA in inhibiting the glomerulosa cell responses to AII. In contrast, the selective D-1 agonist SKF 82526 was virtually inactive. The pharmacological characterization of these effects with DA antagonists is also in line with a role of D-2 receptor inhibiting cAMP formation in the action of DA. As a matter of fact, DA lost its inhibitory effects in rat glomerulosa cells exposed to pertussis toxin (100 ng/ml) for 24 h. In conclusion these data are consistent with a role of cAMP in AII stimulated ALD production and suggest that the inhibitory effect of DA is mediated by inhibition of AII-induced enhancement of cAMP formation through interaction with D-2 receptors.

306.13 SEX DIFFERENCE IN NUCLEAR UPTAKE OF LOW DOSE 3H-CORTICOSTERONE BY LIMBIC STRUCTURES IN RAT. B.B. Turner, E.D. Baker*, and M.S. Ansari*. Dept. of Physiology, College of Medicine, East Tennessee State Univ., Johnson City, TN 37614

We have previously shown that the binding capacity of the hippocampus (HC) for ^{3}H-corticosterone (CORT) is greater in female than male rats, but that binding affinity is less in the female (Kd). The existence of a dual corticoid receptor system has been described in rat brain (Type I, high affinity for CORT; Type II, classical glucocorticoid receptor). We asked whether differences in soluble receptor binding reflect differences in the in vivo uptake of CORT by HC and other limbic structures. We predicted that at low dose the in vivo nuclear uptake of ^{3}H-CORT, reflecting binding to Type I receptors, would be greater in males.

We compared the cell nuclear uptake of a tracer dose of ^{3}H-CORT in several brain regions of male and female rats. In each of ten experiments, two sexually mature, gonadectomized rats were adrenalectomized 12 h before i.v. injection with ^{3}H-CORT (3.5 nmol/kg, 85.8 Ci/mmol). Rats were killed by cardiac perfusion with cold dextran-saline and the following tissues were taken: HC, hypothalamus, cortex, amygdala, pituitary and liver. Purified cell nuclei were prepared by centrifugation of crude pellet through dense sucrose; the radioactivity was then extracted and counted. Samples were assayed for protein and DNA content. The amount of ^{3}H-CORT injected into both sexes was comparable (3.49 nmols/kg for males, and 3.42 for females. Plasma levels of radioactivity at the time of sacrifice were not significantly different (35 pmols/dl for males, and 28 pmols for females). The two tissus showing the highest binding, HC and AMG, also showed a sex difference, with males having greater nuclear uptake: HC, 125 ± 7 vs. 72 ± 4 fmols/mg DNA), $p<0.001$; amygdala, 20 ± 1 vs. 14 ± 2 fmols/ mg DNA $p<0.025$. Nuclear uptake in hypothalamus, cortex, and pituitary was markedly lower; liver nuclear uptake was negligible. A similar pattern of nuclear uptake was obtained when binding was calculated as fmols/mg protein. We suggest that this nuclear uptake represents binding to Type I receptors since nuclear uptake did not occur in the liver (Type II receptors), dosage was extremely low, and high concentrations of Type I receptors have been reported in HC and other limbic structures.

In a separate set of experiments, we determined the binding parameters of ^{3}H-CORT (112 Ci/mmol) to Type I soluble receptors in HC cytosols from male and female rats. Our data indicate that males do not have more Type I receptors in this tissue (68 ± 8 vs. 92 ± 4 fmols/mg protein). However, the dissociation constant (Kd) is considerably lower in cytosols from males as compared to females (0.22 ± 0.01 nM vs. 0.52 ± 0.01 nM, $p<0.01$). This difference in affinity appears sufficient to explain the sex difference in nuclear uptake. Supported by NIH grant NS-22158 to BBT.

306.14 VIP EFFECTS ON ADRENAL STEROIDOGENESIS IN VITRO. L. A. Cunningham* and M. A. Holzwarth. Department of Anatomical Sciences, University of Illinois, Urbana, IL 61801.

The rat adrenal cortex contains a plexus of VIP-immunoreactive nerve fibers which arborize primarily in the capsule and zona glomerulosa region (J. Auton. Nerv. Syst., 11:269). The close apposition of nerve fibers to adrenocortical cells (Cell Tissue Res., 241:139) together with observations of diminished VIP-immunoreactivity following acute stress and increased VIP-immunoreactivity in rats chronically on high dietary potassium suggest that this innervation may be important in the regulation of adrenal function. Therefore, we have tested the effect of VIP on adrenocortical steroidogenesis in normal rat adrenal cortex in vitro.

Capsular portions of rat adrenals which comprise the capsule, zona glomerulosa and a small portion of the zona fasciculata were placed in 200 µl perifusion chambers and continuously perifused with modified Krebs Henseleit buffer containing 0.25% BSA, 0.20% glucose and 3.6 mM K^+, saturated with 95% O_2/5% CO_2 at 37°C (flow rate = 0.3-0.4 ml/minute). Test substances were infused for 20 minutes and five-minute fractions were collected continuously and subsequently assayed for aldosterone and corticosterone content by radioimmunoassay. Mean basal aldosterone and corticosterone secretion rates were approximately 0.1 ± .04 and 1.1 ± 0.23 ng/minute/ adrenal, respectively. ACTH infusion (10^{-12}, 10^{-11}, 10^{-10}, 10^{-9} and 10^{-8}M) increased mean total aldosterone secreted over 50 minutes approximately 1.4, 3, 17, 45 and 60 fold over baseline and increased mean corticosterone 1.3, 2, 4, 9 and 12 fold over baseline (n=4-6 per dose). Angiotensin II infusion (10^{-8}, 10^{-7}, 10^{-6} and 10^{-5}M) stimulated mean aldosterone secretion 1.2, 2, 3 and 8 fold (n=3-6 per dose). VIP infusion (10^{-6}, 10^{-5} and 10^{-4}M) increased mean aldosterone secretion 1.3, 5 and 17 fold over baseline while corticosterone secretion increased 1.9, 4 and 10 fold (n=3-9). Whether the high dose of VIP required to elicit steroidogenesis is the consequence of the perifusion system or a result of low affinity VIP receptors is not known. The structurally related peptide, glucagon (10^{-5} and 10^{-4}M), had no effect on aldosterone secretion which suggests that the effect is specific for VIP. On the other hand, stimulation with $5x10^{-5}$M (Ac-Tyr^1, D-Phe^2)-GRF(1-29)-NH_2, a peptide reported to inhibit VIP binding to high affinity VIP sites in pancreatic tissue (Endocrinology, 116:2653), did not alter basal secretion or VIP-stimulated (10^{-5}M) steroidogenesis. In summary, the presence of VIP-immunoreactive nerve fibers closely associated with adrenocortical cells and the ability of VIP to stimulate steroidogenesis in vitro suggests that this peptide may contribute to the regulation of adrenal steroid secretion in vivo.

306.15 CHARACTERIZATION OF A CORTICOSTERONE-SENSITIVE CYTOSOLIC PHOSPHOPROTEIN FROM RAT HIPPOCAMPUS. L.K. Schlatter* and L.A. Dokas (SPON: H. Waller) Departments of Biochemistry and Neurology, Medical College of Ohio, C.S. 10008, Toledo, OH 43699.

It has been proposed that endogenous glucocorticoids are a cause of cell degeneration in the rat hippocampus as the animal goes through natural aging. Administration of exogenous glucocorticoids can mimic this process, producing first, down-regulation of glucocorticoid receptors in the hippocampus, followed by irreversible cell loss. Phosphorylation of a cytosolic protein band with an apparent molecular weight of 68,000 from the rat hippocampus is increased following subcutaneous injections with 5 mg. of corticosterone at 24 hour intervals. This dosage produces prolonged circulating plasma levels of corticosterone that mimic those observed during stress. The phosphorylation of this protein is also increased in some untreated and sham-treated rats, possibly due to endogenous stress responses in these animals. There are no reproducible phosphorylation changes seen in the crude nuclear, mitochondrial-synaptosomal, or membrane fractions. Using two-dimensional gel electrophoresis, the 68,000 band is split into multiple phosphorylated spots with isoelectric points ranging from 6.6 to 6.2. Phosphorylation of this protein is optimal at pH 6.5. It is not present in the liver cytosolic fraction. The phosphorylation pattern observed with short-term injection (4 hours prior to sacrifice) is similar to that seen in the control animal groups for the longer injection times. However, four hour injections with this same dosage do cause an apparent increase in synthesis of a protein with a molecular weight of approximately 35,000 as indicated by ^{35}S-methionine labeling. No change in ^{35}S-labeling of a protein with a molecular weight of 68,000 is seen. The identity of either protein is at present unknown. However, these changes may be biochemical correlates of a glucocorticoid-induced alteration of hippocampal function. Supported by a grant from the Dorothy VanNess-Thompson Foundation.

306.16 CENTRAL MECHANISMS OF ETHANOL-INDUCED ADRENOCORTICAL RESPONSE IN SELECTIVELY BRED LINES OF MICE. J.M. Zgombick and V.G. Erwin. University of Colorado, School of Pharmacy, Alcohol Research Center, Boulder, Colorado 80309

The stimulatory effects of acute ethanol on the hypothalamo-pituitary-adrenal (HPA) axis were investigated in the selectively bred long-sleep (LS) and short-sleep (SS) mice. Ethanol (0.2-4.5 g/kg ip), CRF (0.1-10 ug iv), and ACTH (0.1-10 ug iv) produced greater dose-dependent elevations in plasma corticosterone (C) in LS than SS mice. However, the differential adrenocortical activation elicited by the peptides was much less pronounced than observed with acute ethanol. Passive immunoneutralization of circulating corticotropin-releasing factor (CRF) completely abolished ethanol-induced elevation in plasma corticosterone in LS mice, suggesting that the response is mediated through an enhanced secretion of hypothalamic CRF. Additionally, these mice were infused intracerebroventricularly (icv) with noradrenergic and cholinergic compounds to ascertain whether these substances modulate the differential adrenocortical response elicited by acute ethanol (2.5 g/kg). Clonidine, an alpha-2 adrenergic agonist, blocked ethanol-induced elevations in plasma C in a dose-dependent manner (1 and 10 ug icv) in LS mice; however, only the 10 ug dose of clonidine effectively antagonized this response in SS mice. Yohimbine (1 ug icv), an alpha-2 adrenergic antagonist, reversed the inhibitory effect of clonidine in ethanol-treated LS and SS mice. Other adrenergic agonists and antagonists were without effect on this stimulatory response. Carbachol (1 ug icv), a nonspecific cholinergic agonist, potentiated ethanol-induced elevation in plasma C in both lines of mice. Cholinergic antagonists did not attenuate ethanol-induced adrenocortical activation. These results suggest that differential adrenocortical response to ethanol exhibited by LS and SS mice (1) is primarily due to differential hypothalamic CRF release, (2) may be mediated through an alpha-2 adrenergic mechanism, and (3) that differences in central noradrenergic systems may account for differential ethanol-induced elevation in plasma C exhibited by LS and SS mice.

306.17 DISTRIBUTION OF A RENIN-RELEASING FACTOR IN RAT BRAIN AND PERIPHERAL TISSUE. J.H. Urban, M.S. Brownfield+ and L.D. Van de Kar. Dept. Pharmacol., Loyola Univ., Maywood, IL 60153 and +Univ. Wis. Sch. Vet. Med., Madison, WI 53706.

We have partially characterized a renin-releasing factor (RRF; Hypertension 9, 1987). The RRF is a peptide with a molecular weight between 5,000-10,000, and is found in both plasma and hypothalamic extracts. Extracts of rat hypothalami produce dose dependent increases in renin release from rat renal cortical slices. The equivalent of the content from one hypothalamus produces a maximal stimulation of renin release in vitro. The present studies were designed to determine the distribution of the RRF in brain and peripheral tissues. To test for the distribution of RRF, rat brains and tissues were dissected, homogenized in boiling water, and then boiled for 20 minutes and centrifuged. The supernatant was added to the kidney slices in a volume that is equal to the content of one hypothalamus. Addition of adrenal extract produced a decrease in renin release from kidney slices. Addition of kidney extract increased the amount of angiotensin I (AI) detected by radioimmunoassay, either by increasing renin release from the slices or by a residual amount of AI that was present within the kidney extract. Extracts of spleen, liver and skeletal muscle did not alter renin release from the kidney slices. A number of brain areas were found to have significant renin releasing activity: cerebellum > medulla oblongata > cerebral cortex > hypothalamus. No significant renin releasing activity was present in the pituitary, pons, thalamus, caudate, hippocampus, midbrain or amygdala. In order to differentiate between RRF contained in cell bodies and RRF in nerve terminals, a group of rats were given intracerebroventricular (icv) injections of colchicine to stop axonal transport of peptides from the cell body. After colchicine treatment, the hypothalamus was the only brain area that exhibited significant renin-releasing activity and this value was increased over the previous (control) value. The renin releasing activity that was previously observed in the cerebral cortex and medulla oblongata was significantly reduced after colchicine treatment. The RRF activity in the cerebellum completely disappeared. These studies suggest that the RRF cell bodies are localized within the hypothalamus and that the renin releasing activity present in other brain areas is likely located in nerve terminals. (Supported in part by the Heart Research Foundation and by Sigma Xi).

306.18 COMPARISON OF ANGIOTENSIN AND ACETYLCHOLINE ON ADRENAL CATECHOLAMINE SECRETION IN DOGS. W.C. Engeland, M.P. Lilly*, P. Miller* and D.S. Gann. Dept. of Surgery, Section of Neurobiology, Brown University/R.I. Hospital, Providence, R.I. 02902

There is evidence that angiotensin (AII) contributes to increased adrenal secretion of epinephrine (E) and norepinephrine (NE) after hemorrhage. AII may act directly on the adrenal or its effect may be centrally mediated. To assess the relative sensitivity of the adrenal medulla to AII compared to that of acetylcholine (ACh) alone, and to coincident presentation of AII and ACh, a preparation was developed for direct arterial injection into the adrenal that permitted measurement of adrenal hormone secretory rates. Also, since circulating AII is a potent stimulus for aldosterone secretion from the adrenal cortex, the effectiveness of AII to stimulate catecholamine secretion was compared to its effectiveness to stimulate aldosterone secretion. Mongrel dogs (n=8) were prepared with catheters in the femoral artery for hemodynamic monitoring, in the lumboadrenal vein for monitoring adrenal blood flow and secretory rates and in the lumboadrenal and phrenicoabdominal arteries for adrenal injections. Forty-eight hours after surgery, dogs were anesthetized with pentobarbital, artificially respired and given supplemental oxygen and iv fluids. Dogs received in random order adrenal injections (200ul) of AII (2, 20 and 200 pmol), of ACh (0.2, 2, 20, 200 nmol), of AII (20 and 200 pmol) with ACh (2 nmol) and of diluent. Adrenal venous samples were collected prior to and at 1, 5 and 10 min after injection with injections separated by 20 min. Catecholamines and aldosterone were measured by HPLC with EC detection and by RIA, respectively. Peak changes in catecholamines (1 min) or in aldosterone (5 min) were analyzed by ANOVA after log transformation to reduce variance. Injection either of ACh or of AII resulted in log dose-dependent increases ($p<0.01$) in E and NE. The range of secretory responses to ACh for E were 98±23 - 2000±379 ng/min and for NE were 25±7 - 408±52 ng/min. The range of secretory responses to AII for E were 111±33- 294±62 ng/min and for NE were 28±8 - 56±16 ng/min. However, AII was 100X as potent as ACh on a molar basis. The secretory responses to 20 and 200 pmol AII was equivalent to those of 2 and 20 nmol ACh, respectively. Neither ACh or AII produced a change in the secretory ratio of E to NE. The responses to AII occurred despite a decrease ($p<0.05$) in adrenal blood flow. No additional hemodynamic effects resulted from AII injection suggesting that the action of AII was restricted to the adrenal gland. When AII was given coincident with ACh, the effect on catecholamine secretion was neither additive nor synergistic; the response to each dose of AII with ACh was equivalent to the response to each dose of AII alone. Injection of AII resulted in a log dose-dependent increase ($p<0.01$) in aldosterone secretion. The range of responses were 5±1 - 26±7 ng/min. However, the minimal dose (2 pmol) of AII that increased ($p<0.05$) aldosterone secretion did not increase E or NE secretion, suggesting that the adrenal cortex is more sensitive to AII than the adrenal medulla. The data show that AII is more potent than ACh as a secretogogue for adrenal catecholamine secretion. However, AII does not augment the catecholamine response to ACh or stimulate catecholamine secretion at a dose that is effective in stimulating adrenocortical secretion. A physiological role for circulating AII in direct activation of adrenomedullary secretion remains to be defined. (Supported in part by NIH grant AM 26381 and GM 27946.

306.19 ANTIBODIES TO CHROMOGRANINS. J. Qian*, R.Y. Xu*, E. Ling*, and R. Hogue Angeletti. Neuropathology, Univ. of Pennsylvania Medical School, Philadelphia, PA 19104-6079.

Specificities of monoclonal antibodies to chromogranins were compared by two dimensional immunoblotting and by immunohistochemical distribution. A polyclonal antibody to the peptide PL26, located near the carboxy terminus of chromogranin A, was used as a standard. Although the function of chromogranin A is not yet known, it appears to be a pan-endocrine marker. It is also found in many neurons, but is not found in exocrine cells. One antibody, 6F2, displayed all of the properties of chromogranin A. Another antibody 3G11, stained with the specificity of chromogranin B. The immunohistochemical distribution of these two proteins was overlapping, but not coextensive. The most unusual antibody was 3C9, which reacted with secretory vesicles in both endocrine and exocrine cells. A final polyclonal antibody to peptide SF24, specific to the carboxy terminal region of the endocrine carboxypeptidase, was also tested. This antibody demonstrated an even wider distribution than antibodies to chromogranin. (NS-22697)

306.20 **REDUCED INSULIN BINDING IN RAT HYPOTHALAMUS AFTER 6-hydroxydopamine (6-OHDA) LESION.** B.J. Wilcox, A.M. Matsumoto*, D.M. Dorsa and D.G. Baskin. Veterans Administration Medical Center and University of Washington, Seattle, WA 98108.

High densities of insulin binding sites have been localized by quantitative autoradiography (QAR) to the arcuate (ARC) nucleus of the hypothalamus. However, the types of cells bearing these binding sites remain unknown. Since biochemical studies on whole hypothalamus suggest that insulin may have a modulatory influence on catecholamine (CA) turnover, we have hypothesized that insulin binding sites may be associated with catecholamine terminals in the ARC nucleus. To test this hypothesis, we measured insulin binding in the ARC by QAR after lesioning catecholamine terminals with 6-OHDA. Male Sprague/Dawley rats were injected stereotaxically with 75 µg (icv) of 6-OHDA. Control rats received only saline vehicle. The animals were sacrificed 7 days after injection. Brains used for biochemical measurement of CA levels were removed, dissected and processed using high performance liquid chromatography with electrochemical detection. Brains from the remaining animals were removed and processed for QAR of insulin binding. Ten micron slide-mounted coronal sections through the hypothalamus were incubated in the presence of 0.1 nM human ^{125}I-insulin for 18 hours at 4°C, rinsed and dried. Non-specific binding was determined by addition of 10 nM unlabeled porcine insulin. The labeled sections were apposed to LKB Ultrofilm for 5 days. Computerized image analysis was used to measure the amount of binding within the ARC and ventromedial (VMN) nuclei, by conversion of optical density of autoradiographic images to radioactivity bound per sq. mm. Treatment with 6-OHDA caused a 65% reduction in NE content in the hypothalamus when compared to saline treated controls (control: 23.6 ± 2.5 ng/mg, n=11; 6-OHDA: 8.25 ± .77 ng/mg, n=12, $p < 0.01$). QAR results from 3 separate experiments show a 20-30% decline in insulin binding in the ARC of the lesioned animals (n=10) when compared to controls ($n=7, p < 0.01$). Data from a typical experiment were: 79 ± 5 dpm/mm^2 vs. 58 ± 7 dpm/mm^2. No change in insulin binding was observed for the VMN. Reduction of insulin binding in the ARC after 6-OHDA lesion supports the hypothesis that insulin binding sites are located pre-synaptically on CA terminals in this area. The results suggest that insulin may modulate neural systems regulated by catecholamines in the ARC.

NEUROTRANSMITTERS: UPTAKE, STORAGE AND SECRETION II

307.1 UPTAKE AND RELEASE OF ENDOGENOUS DOPAMINE FROM RAT STRIATAL SLICES: INTERACTION OF METAPHIT WITH PCP AND NOMIFENSINE. L.P. Dwoskin*, G.A. Gerhardt, C.J. Drebing*, A.E. Jacobson*, R.A. Lessor*, K.C. Rice* and N.R. Zahniser (SPON: L. Gronke). Depts. Pharmacol. and Psychiat., Univ. Co. Hlth. Sci. Ctr., Denver, CO 80262 and NIADDK, Bethesda, MD 20892.

Metaphit is a phencyclidine (PCP) analog which specifically acylates PCP binding sites in vitro (Rafferty et al., FEBS Lett. 181:318, 1985) and antagonizes the behavioral and electrophysiological effects of PCP (Contreras et al., JPET 238:1101, 1986). Also, Metaphit irreversibly inactivates cocaine binding sites in rat striatum (Berger et al., Neuropharm. 25:931, 1986) possibly associated with the dopamine (DA) uptake site. We examined the effect of Metaphit on presynaptic dopaminergic function and its ability to antagonize the effect of PCP and a reversible DA uptake blocker, nomifensine (NOMI). Striatal slices (0.5 mm thick) were incubated for 30 min in Krebs' buffer and for an additional 30 min either in the absence (control) or presence of Metaphit (100 uM). A single control or Metaphit-treated slice was transferred to each chamber and was superfused at 1 ml/min with buffer in the absence or presence of 10 uM PCP or 10 uM NOMI. Doses were chosen based on maximal effects in dose-response curves. After 60 min, one-min samples were collected. Stimulation (1 Hz, 60 pulses) occurred at 62 min. Endogenous DA and dihydroxyphenylacetic acid (DOPAC) in superfusate were measured using HPLC-EC. Ascorbic acid oxidase was added and 50 ul were assayed. The detection limit for both DA and DOPAC was 1 pg/ml/mg tissue. In the absence of drugs, spontaneous outflow of DA and DOPAC were detected (1.4 and 44 pg/ml/mg, respectively) and were increased above basal (2.6 and 290 pg/ml/mg, respectively) over the ten-min period after stimulation (overflow). In control slices, PCP did not change DOPAC or DA outflow. NOMI also did not change the DOPAC outflow, but increased (3.6 pg/ml/mg) DA outflow. PCP and NOMI increased DA overflow (12 and 35 pg/ml/mg, respectively) and decreased DOPAC overflow (64 and 87 pg/ml/mg, respectively). Therefore, PCP appears to act as a DA uptake blocker in this system. Metaphit did not alter DA outflow, but slightly increased DOPAC outflow (56 pg/ml/mg). DA and DOPAC overflow were greatly decreased (0.43 and 12 pg/ml/mg, respectively) by Metaphit. PCP and NOMI did not alter the Metaphit-induced increase in DOPAC outflow. Unexpectedly, the effect of the combinations of Metaphit and PCP or Metaphit and NOMI on DA outflow were greater than the effect of either drugs alone. Metaphit blocked the increase in DA overflow produced by both PCP and NOMI. Therefore, Metaphit may interact with the DA uptake site by acylating a closely associated PCP recognition site. Supported by USPHS NS09199 and AG06434.

307.2 COMPARISON OF THE EFFECTS OF PCP, COCAINE, AMPHETAMINE AND NOMIFENSINE ON ELECTRICALLY-EVOKED ENDOGENOUS DOPAMINE RELEASE AND RE-UPTAKE FROM RAT STRIATAL SLICES. C.C. WILCOX*, L.P. DWOSKIN*, G.A. GERHARDT, N.R. ZAHNISER (SPON: M. Luttges). Depts. Pharmacol. and Psychiat. Univ. Co. Hlth. Sci. Ctr., DENVER, CO 80262

Phencyclidine (PCP) may act to block the re-uptake of dopamine (DA) and/or stimulate the release of DA. The effect of PCP on dopaminergic function was compared to that of cocaine and nomifensine (uptake blockers) and to amphetamine (presumed releaser).

Endogenous DA and dihydroxyphenylacetic acid (DOPAC) were measured in the superfusate from rat striatal slices using HPLC-EC. Each chamber contained a single (0.5 mm thick) slice superfused at 1 ml/min with Kreb's buffer containing a single dose (.01-100 uM) of drug. After 60 min of superfusion, 12 one-min samples were collected. The slices were stimulated (60 pulses, 1Hz) at 62 min. Ascorbic acid oxidase was added to each sample and 50 ul were assayed. The limit of detection was 1 pg/ml/mg tissue. In superfusate from control samples (absence of drug) DA and DOPAC were detected (1.4 and 35 pg/ml/mg, respectively) prior to stimulation (outflow) and were increased to 5.0 and 190 pg/ml/mg, respectively, upon electrical stimulation (overflow). Only high doses of cocaine (10 uM) increased DA outflow, but had no effect on DOPAC outflow. Only high doses of nomifensine (100 uM) increased both DA and DOPAC outflow. Amphetamine (10-100 uM) increased DA outflow while decreasing DOPAC outflow. PCP had no effect on DA or DOPAC outflow. In general, DA overflow and DOPAC overflow were increased and decreased, respectively, by all the drugs examined. The efficacy of the increased DA overflow was nomifensine >> cocaine = PCP = amphetamine. These drugs decreased DOPAC overflow to approximately 12% of control. Increased levels of DA in superfusate reflect enhanced DA release and/or blockade of re-uptake. If a drug acts only to release DA, then DOPAC would also be expected to increase in superfusate. However, if a drug acts only to block re-uptake, then DOPAC would be expected to decrease. Therefore, under the conditions of these experiments (following a 60-min exposure to drug) all of the drugs appear to act primarily via blockade of re-uptake. Supported by USPHS NS09199, GM07635, DA04216 and AG06434.

307.3 RELEASE OF CORTICOTROPIN-RELEASING FACTOR (CRF) FROM RAT PREFRONTAL CORTEX IN VITRO. M.J. Owens, B. Maynor* and C.B. Nemeroff, Depts. Pharmacol. & Psychiat., Duke University Medical Center, Durham, North Carolina 27710.

Corticotropin-releasing factor (CRF), a 41 amino acid-containing peptide, is the major physiological regulator of ACTH secretion from the anterior pituitary. In addition, CRF possesses many of the requisite criteria for classification as a neurotransmitter in the CNS. These include: a heterogenous distribution throughout the CNS and specific, high affinity binding sites, putative receptors. In previous studies by our group (Smith et al., Endocrinol. 118:1997, 1986) and others another requisite neurotransmitter criterion for CRF was demonstrated, calcium-dependent, potassium induced release from hypothalamus, amygdala, midbrain and striatum. In the present study we studied release of CRF from rat prefrontal cortex in vitro.

Male Sprague-Dawley rats (21 days old) were decapitated and the prefrontal cortex was sliced into thin (0.75 mm) sections and immediately placed on a superfusion chamber containing a modified Kreb's-Henseleit buffer which was gassed with 95% O_2/5% CO_2; the pH was adjusted to 7.4 at 35°C. At ten minute intervals the buffer was removed and replaced with either fresh buffer in the case of basal release or buffer containing a depolarizing concentration of potassium (40 mM). In addition, other putative secretagogues were studied. Basal release was measured for three consecutive ten minute intervals, and then a test substance was added during the 30-40 minute period. Basal release was continued between 40-60 minutes and then buffer containing 40 mM KCl was added during the 60-70 minute epoch.

The radioimmunoassay for CRF utilizes [^{125}I]-rat/human CRF as a tracer, and has a sensitivity of 1.25 pg/tube. Addition of 40 mM KCl induced a 136% increase (n=16) in CRF release compared to basal levels of CRF release. In an attempt to characterize the neurotransmitter control of cortical CRF release, the effects of d-amphetamine (10 mM) were studied. This psychostimulant produced a small but significant increase in CRF release. Subsequent studies have shown that this response does not appear to be mediated by increased availability of 5-HT because concentrations of this indoleamine of 10^{-8} and 10^{-9} M had no effect on cortical CRF release.

The presence of CRF and CRF receptors in the cerebral cortex and its release by depolarizing concentrations of potassium, taken together, are concordant with the view that this peptide acts as a neurotransmitter in the cerebral cortex. (Supported by NIMH MH-42088 and NIEHS ES-07031.)

307.4 SPECIFICITY OF [^{3}H]1-METHYL-4-PHENYLPYRIDINIUM ION UPTAKE IN STRIATAL SYNAPTOSOMES E.A. Debler*, A. Hashim*, A. Lajtha, and H. Sershen* (SPON: N. Marks). Center for Neurochemistry, N.S. Kline Institute, Ward's Island, New York, NY 10035.

The neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is attributed to the selective uptake of its metabolite 1-methyl-4-phenylpyridine (MPP^+) into dopamine terminals with subsequent terminal destruction. MPTP neurotoxicity can be prevented by the administration of monoamine oxidase-B specific inhibitors, which prevent the metabolism of MPTP to MPP^+, or by blocking the uptake of MPP^+ into dopamine terminals. Several compounds were evaluated, in vitro, with respect to their ability to block the transport of [^{3}H]MPP^+ (final concentration of 0.1 μM) into striatal synaptosomes. The classical dopamine uptake blockers GBR 12909, cocaine, and mazindol strongly inhibited striatal MPP^+ uptake, exhibiting IC_{50} values of approximately 0.1 μM. Nicotine and its metabolites, compounds potentially involved in the reported reduced incidence of Parkinson's disease in smokers, proved to be weak inhibitors (IC_{50} = 1 mM). The pyridine derivative 4-phenylpyridine (4-PP), recently shown to attenuate MPTP neurotoxicity in vivo, inhibited MPP^+ uptake, exhibiting an IC_{50} of 100 μM. Ascorbic acid, previously shown in our laboratory to attenuate MPTP neurotoxicity in vivo (Neuropharmacology 24:1257, 1985), was also seen to inhibit MPP^+ transport with an IC_{50} value of 100 μM. Kinetic analysis of [^{3}H]MPP^+ uptake in the presence of ascorbic acid indicated that ascorbic acid is a non-competitive inhibitor of MPP^+ uptake. The K_m and V_{max} values are 673 nM and 89 pmol/mg protein per min in the control condition and 654 nM and 31 pmol/mg per min in the presence of ascorbic acid. This effect was not due to conversion by ascorbic acid of [^{3}H]MPP^+ to a metabolite that is not transported, i.e., MPTP. HPLC analysis of [^{3}H]MPP^+ incubated with ascorbic acid and membranes indicated that MPP^+ was not metabolized during the incubation. The IC_{50} values of the above compounds were the same for inhibition of [^{3}H]MPP^+ uptake and for inhibition of [^{3}H]dopamine uptake into striatal synaptosomes, consonant with the idea that MPP^+ is transported by the dopamine carrier.

Supported by a grant from the Council for Tobacco Research, Inc.-U.S.A. 1630A).

307.5 EFFECTS OF PHENCYCLIDINE ON EXTRACELLULAR DOPAMINE: AN IN-VIVO VOLTAMMETRY STUDY. F.M. Petracca*, R.A. Gazzara, and S.G. Howard. MRRC, BRI and Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.

Phencyclidine (PCP) has been demonstrated to influence a number of different neurochemical systems, including serotonergic, noradrenergic, and cholinergic systems. Although PCP has no direct interaction with dopamine (DA) receptors, behavioral, electrophysiological, and neurochemical data indicate that this drug exerts an effect on the dopaminergic system. Using the technique of in-vivo voltammetry, this laboratory has previously demonstrated a long-lasting decrease in extracellular DA in the striatum after treatment with PCP. It was suggested that this decrease may be due to a non-specific anesthetic property of PCP. The present study addresses this issue by comparing the effects of PCP with those of ketamine (KET), a structurally related anesthetic, on extracellular DA in rat brain. Additionally, it has been hypothesized that one of the specific actions of PCP is to inhibit DA uptake. To address this postulated specific effect, PCP was evaluated in a paradigm which has been used to study known DA uptake inhibitors. It has been shown that nomifensine and other uptake inhibitors antagonize amphetamine(AMP)-induced DA release. In the present study, amphetamine-induced DA release was examined in rats pre-treated with PCP.

Male Sprague-Dawley rats were anesthetized with urethane (1.5 g/kg), and semidifferential voltammetry was performed according to methods previously described (Takeda, H. et.al., Neuropharmacol. 25(12):134, 1986). The doses of PCP and ketamine administered were 0.1, 1.0, and 10.0 mg/kg. D-AMP (1 mg/kg) was administered 60 minutes after PCP treatment for evaluation of uptake inhibition.

KET and PCP induced similar dose-related decreases in extracellular DA in the neostriatum. These results support the hypothesis that the PCP-induced decrease in DA release in anethetized animals is due to the anesthetic properties of PCP. In addition, high doses of PCP attenuated the AMP-induced increase in DA release normally found when AMP is administered alone. These data suggest that inhibition of DA uptake may be one of the mechanisms through which PCP exerts its CNS effects. Taken together, these results support the notion that PCP may have multiple actions in the CNS.

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307.6 THE ANTAGONISM OF AMPHETAMINE-INDUCED DOPAMINE RELEASE BY DOPAMINE UPTAKE INHIBITORS: A DEVELOPMENTAL IN-VIVO VOLTAMMETRY STUDY. R.A. Gazzara and S.G. Howard. MRRC, BRI and Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA, 90024.

CNS motor stimulants have been categorized as belonging to either an amphetamine(AMP)-like class (antagonized by alpha-methyl-p-tyrosine) or a methylphenidate class (antagonized by reserpine). Studies have shown that AMP-like agents act by releasing dopamine (DA) and that methylphenidate-like agents act through the inhibition of DA uptake (e.g.,Ross,Acta Pharmacol.et Toxicol.,41: 392,1977). In addition, compounds which inhibit DA reuptake have been found to antagonize AMP-induced hyperactivity in reserpinized mice (Ross, Life Sciences, 24:159,1979). These data suggest that DA uptake inhibitors antagonize AMP-induced DA release. The experiments presented here were designed to determine the effect of DA uptake inhibitors on AMP-induced changes in DA release in the neostriatum of 21-22-day-old rat pups and adult rats as measured by in-vivo voltammetry.

We have previously reported (Gazzara et al.,Dev.Brain Res.,28: 213,1986) that AMP(1.0 mg/kg, s.c.) produces a rapid increase in extracellular DA concentration in the neostriatum of adult rats, and a slight increase followed immediately by a prolonged decrease in extracellular DA concentration in the neostriatum of 21-22-day-old rat pups. In both cases we postulated that the results were due to an AMP-induced increase in DA release. In the case of the 21-22-day-old pups, however, we postulated that an increase in DA release in the substantia nigra depressed the firing rate of the nigrostriatal dopaminergic neurons, and resulted in a net decrease in DA release in the neostriatum.

In the experiments reported here, the following inhibitors of DA uptake were injected 60 min prior to AMP in order to test their effect on AMP-induced DA release: methylphenidate, nomifensine, mazindol and cocaine. All four of these compounds abolished the increase in DA release seen when AMP was administered alone to adult rats. In addition, both methylphenidate and nomifensine attentuated the prolonged decrease in extracellular DA concentration found when AMP alone is administered to 21-22-day-old rat pups. These data support the hypothesis that inhibitors of DA uptake antagonize AMP-induced DA release in the neostriatum of adult rats. Furthermore, these data suggest that DA uptake inhibitors may also antagonize AMP-induced DA release in the substantia nigra of 21-22-day-old rat pups as evidenced by the attenuation of the AMP-induced decrease in extracellular DA concentration in the neostriatum. The mechanism of this AMP antagonism remains to be elucidated, however it has been postulated that AMP-induced DA release may require a functional DA uptake carrier mechanism.

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307.7 COCAINE ACTION ON STRIATAL DOPAMINE TRANSMISSION STUDIED BY MICRODIALYSIS. Yasmin Hurd* and Urban Ungerstedt* (SPON: C. Hildebrand). Dept. of Pharmacology, Karolinska Institute, Box 60 400, S-104 01 Stockholm, Sweden.

A microdialysis probe (Carnegie Medicin, Stockholm) was implanted into the caudate nucleus to study the neuronal mechanism of cocaine on transmitter release and metabolism. Cocaine, a reuptake inhibitor of dopamine (DA) was administered intravenously (IV) or via the dialysis probe into the caudate nucleus. IV administration of cocaine (1.0, 1.5, and 2.0mg/kg) caused a dose dependent transient increase of DA with a slight decrease in extracellular DOPAC. The maximum increase of DA appeared in the first 10 min perfusate and subsequently declined to baseline within 30 mins. This time pattern fits the fast subjective euphoric "high" that is characteristic for cocaine. To ascertain whether any acute sensitivity to prior cocaine exposure had occured a second dose of cocaine was administered after stable baseline was achieved. The DA and DA metabolite response pattern was similar to that following the first administration.

Via the perfusing medium cocaine was directly administered to the caudate nucleus at different concentrations. A dose dependent increase of DA was observed. Low concentrations (10^{-5} and 10^{-6}M) produced a stable increased level of DA with no change of the metabolites while at the highest dose (10^{-4}M) there was a sharp increase of DA in the second 10 min sample which subsequently declined to a level higher than that produced by the two lower concentrations. Although the DOPAC level declined at the highest concentration similar to that of specific uptake blockers, such as lu19005 and lu17133, the increased stable DA level induced after cocaine did not fit the gradual increasing DA level generally obtained with the other uptake blockers.

The microdialysis probe allows the possibility of not only monitoring endogenous neurochemicals in the brain but also the manipulation of essential intrinisic ions. Thus a Ca^{+2}-free perfusion medium abolished the DA response to intravenously administered cocaine. This indicates that the increased DA release originates from a vesicular pool of DA.

307.8 DISSIMILAR EFFECTS OF HALIDES ON [^{3}H]METHYLPHENIDATE BINDING AND SYNAPTOSOMAL DOPAMINE TRANSPORT. M.M. Schweri* (Sponsor: C.H. Hockman). Mercer U. School of Medicine, Macon, GA 31207.

Many characteristics associated with the binding site for the stimulant drug [^{3}H]methylphenidate ([^{3}H]MP)in striatal tissue suggest that this site may be physically or functionally related to the dopamine (DA) carrier complex in nigrostriatal dopaminergic nerve terminals. Possible anionic influences on both [^{3}H]MP binding and [^{3}H]DA transport were investigated by surveying the effects of varying concentrations of the halide salts on these two functions. When binding of 8-10 nM [^{3}H]MP to rat striatal synaptosomal membranes was studied in the presence of 10-500 mM NaF, -Cl, -Br, and -I, a qualitatively similar pattern of binding was observed in the presence of all of the halides except iodide. Peak binding of a similar magnitude occurred when the assay buffer contained 50-100 mM Naf, -Cl, or -Br, while maximal binding in the presence of NaI occurred at 250 mM and was more than three times that obtained in the presence of the other halide salts. Scatchard analysis of [^{3}H] MP binding in the presence of 100 mM NaCl and NaI demonstrated that the increase in binding observed with the latter was attributable to an increase in the affinity of the radioligand for its binding site rather than a change in receptor number (Kd's = 109 nM when NaCl was added and 46 nM when NaI was used). This increase in affinity was shown by kinetic analysis to be due mainly to iodide's ability to decrease the off-rate of [^{3}H]MP. Interestingly, I- did not enhance DA's affinity for the [^{3}H]MP binding site, even though DA competitively inhibits binding of [^{3}H]MP. The effects of the halides on synaptosomal accumulation of 30 nM [^{3}H]DA differed markedly from their effects on [^{3}H]MP binding. Maximum transport of [^{3}H]DA was observed in the presence of 120 mM NaCl. The same rank order ($Cl^- > Br^- > I^- > F^-$) of halide modulation of [^{3}H]DA transport was observed at all salt concentrations tested (10-150 mM; osmolality was maintained by addition of sucrose to the assay buffer). When [^{3}H] DA transport was compared in synaptosomes suspended in Krebs-phosphate buffer containing equimolar amounts of NaCl or NaI, the K_m in the presence of iodide was found to be almost three-fold greater than in Cl^- containing buffer (496 nM ± 50 vs 168 ± 8, respectively), while the V_{max} was only marginally affected. As predicted from the [^{3}H]MP binding studies, however, MP was found to be more potent in blocking [^{3}H]DA uptake in buffer containing I^- compared to equimolar amounts of Cl^- (K_i in I^- = 124 nM vs 207 nM in Cl^-). This data suggests that separate but overlapping domains with different sensitivity to halides may exist for the binding of MP and DA to the [^{3}H]MP binding site. Although the effect of the halides on MP binding is predictive of the influence of the halides on the potency of MP to inhibit DA transport, it is not predictive of their effect on DA transport itself. Supported by NIH Grant NS-22546

307.9 THE RELEASE OF LABELED AND UNLABELED CATECHOLAMINES FROM RABBIT CAROTID BODY. G.-F. Cheng*, B. Dinger and S.J. Fidone. Dept. of Physiol., Sch. of Med., Univ. of Utah, Salt Lake City, Utah 84108.

The arterial chemosensory tissue of the mammalian carotid body contains concentrated stores of catecholamines (CA), primarily dopamine (DA), in type I parenchymal cells. Previous studies in our laboratory utilized radioisotope techniques to study the release of ^{3}H-DA evoked by hypoxia from rabbit and cat carotid bodies. In the present study we have used our standard radioisotope methods in combination with HPLC and coulometric electrochemical detection to compare the release of tritiated and unlabeled DA from the rabbit carotid body.

Carotid bodies were pooled (3 to 8 per experiment) and incubated for 2 h at 37°C in Tyrode's solution equilibrated with 100% O_2, which contained ^{3}H-tyrosine (23.6 µM; 34 Ci/mmole). The tissue was washed for 90 min in 4 changes of 100% O_2-media (37°C). Superfusion media were collected during 10 min control, 5 stimulus and 6 successive 10 min post-stimulus periods. DA release was evoked by Tyrode media containing 60 mM K^+ (equimolar Na^+ reduction). Media samples and perchloric acid (PCA) extracts of tissue were processed in acid alumina and eluted with 1N PCA. CAs were separated and quantified using reverse phase HPLC with coulometric electrochemical detection (ESA Coulochem 5100; 5021 Conditioning Cell; 5011 Analytical Cell; 10 pg sensitivity). Eluted peaks were quantified on a Waters Data Module 730 and collected for determination of tritium in a Packard Model 1500 liquid scintillation analyzer. The total radioactivity recovered from each eluted sample routinely exceeded 95%. DA release (measured as the sum of DA plus 3,4-dihydroxyphenylacetic acid, DOPAC) is expressed as the percent of tissue content for each method of CA determination.

Less than 10% of the CA stores of the carotid body were labeled during the 2 h incubation in ^{3}H-tyrosine, thus providing two distinct pools of CAs in the tissue. The mean basal release of DA (0.21%/10 min) was sharply elevated by high K^+ superfusion media (9.28%/5 min), and release rapidly returned to basal levels in the succeeding 2 post-stimulus collection periods. The percentage of DA released from the radiolabeled stores during control, stimulus and post-stimulus periods corresponded closely to the release from the total DA stores (labeled plus unlabeled) determined electrochemically. Preliminary experiments in which carotid bodies were stimulated in media equilibrated with 10% O_2 show a similar correspondence between labeled and total release.

Our findings demonstrate the feasibility of utilizing a highly sensitive electrochemical method for determination of CA efflux from the carotid body and, in addition, they show that unlabeled and labeled (newly synthesized) DA is released from the carotid body in identical proportion during stimulus and resting conditions. Supported by USPHS grants NS12636, NS07938.

307.10 VIP RELEASE FROM MOUSE CEREBRAL CORTICAL SLICES : EFFECT OF CALCIUM CHANNEL BLOCKERS AND INVOLVEMENT OF ARACHIDONIC ACID METABOLITES. J.-L. Martin and P.J. Magistretti, Dept. of Pharmacology, CMU, 1211 - GENEVA 4, Switzerland.

The mechanisms of VIP (Vasoactive Intestinal Peptide) release were investigated in mouse cerebral cortical slices. Slices (250 x 250 µm) were preincubated for 90 min at 37° in oxygenated Krebs-Ringer bicarbonate buffer pH 7.4 containing (mM) : NaCl 120; KCl 3; $CaCl_2$ 2.6; $MgSO_4$ 0.67; KH_2PO_4 1.2; $NaHCO_3$ 27.5 and glucose 3 mM. After this period of time, slices were incubated for 6 min at 37° in the same buffer. At the end of incubation, tubes were centrifuged and released VIP was assayed by RIA in the supernatant. VIP tissue content was assayed in the pellet after extraction with 1 M CH_3COOH. The effect of various neuroactive agents on basal VIP release (BVR) was examined. Generally BVR was comprised between 0.2 to 0.4 % of VIP tissue content corresponding to 6-15 pg/mg prot/6 min. Ca^{++} channel blockers such Co^{++} 2.5 mM, Ni^{++} 0.1 mM and Mn^{++} 1 mM inhibited BVR by 50.98 ± 2.61 %, 26.2 ± 4.71 % and 21.32 ± 5.42 % respectively. In contrast Cd^{++} 20 µM, diltiazem 20 µM and nifedipine 10 µM did not affect BVR. The Ca^{++} channel agonist Bay K 8644 10 µM was similarly without effect on BVR. TTX at 2 µM slightly decreased (16.18 ± 3.53 %) BVR. 4-Aminopyridine (4-AP) stimulated in a concentration-dependent manner VIP release, reaching 567.27 ± 25.01 % of BVR at 1 mM. Effects of 4-AP were already detectable at 50 µM (203.15 ± 16.1 % increase over BVR). VIP release evoked by 4-AP 1 mM was completely abolished by Co^{++} 2.5 mM, but partially inhibited (67.78 ± 1.65 %) by TTX 2 µM. Indomethacin, which prevents the formation of arachidonic acid metabolites, decreases at 0.1 mM the effect of 4-AP 1 mM by 43.29 ± 1.2 %. The effect of 4-AP 1 mM was similarly reduced (75.94 ± 4.18 %) by TMB-8 0.1 mM, an inhibitor of Ca^{++} mobilization from intracellular stores. K^+ channel blockers such as Ba^{++} 1 mM, Cs^+ 1 mM and apamin at 1 nM and 1 µM were without effect on BVR. In contrast TEA 10 mM increased BVR by 163.86 ± 20.89 %. The possible involvement of adenylate cyclase and protein kinase C in VIP release was also investigated by testing forskolin (20 µM), TPA and PMA (1 µM) respectively. All these agents, alone or in combination, were without effect on BVR. VIP release was evoked in a concentration-dependent manner by K^+ with significant effects observed already at 15 mM (251.62 ± 25.77 % increase); at $[K^+]_o$ of 20 mM and 25 mM, increases over BVR of 380.91 ± 30.14 % and 677.76 ± 59.21 % respectively were reached. The K^+-evoked release of VIP was insensitive to TTX 2 µM even at $[K^+]_o$ of 50 mM. In contrast, the effect of K^+ 20 mM was antagonized by Co^{++} 2.5 mM (complete antagonism), Mn^{++} 1 mM (89.01 ± 6.2 %) and Ni^{++} 0.1 mM (39.19 ± 4.91 %) but not by Cd^{++} 20 µM, and was potentiated by TEA 10 mM (364.72 ± 16 %), Ba^{++} 1 mM (199.96 ± 27.24 %) and Cs^+ 1 mM (165.49 ± 5.1 %). Other depolarizing stimuli such as veratridine at 15 µM and ouabain at 100 µM evoked a VIP release of 725.55 ± 46.76 % and 460.6 ± 45.3 % respectively. The ouabain effect was partially blocked (42.57 ± 4.21 %) by TTX at 2 µM. Initial results indicate that phosphatidylserine at 100 µM stimulates VIP release by 161.09 ± 13.61 %.

307.11 THE IN VITRO RELEASE OF AMINO ACIDS, PEPTIDES AND CATECHOLAMINES FROM THE KINDLED RAT BRAIN AND THE HUMAN EPILEPTIC FOCUS. P Stoufflet,* NS Nadi, (SPON: Niles Bernick). Rice Univ Sch of Medicine, Houston, TX. and NINCDS, Bethesda, MD 20892.

The study of peptide and amino acid content in the kindled rat brain has shown an increase in the levels of somatostatin (ST) and β-endorphin but no alterations occurred in the content of amino acids when compared to sham-operated rats. The human epileptic (spiking region) focus had an increase in ST, neuropeptide Y (NPY), glutamate (GLU), glycine (GLY), and catecholamines (CA) when compared with non-focal tissue (non-spiking region) from the same patients. Studies of the enzymes for CA have shown an increase in tyrosine hydroxylase in rat and human brain indicating a possible acceleration in the turnover rate. We investigated the alteration in release of neurotransmitters and neuropeptides after depolarization by high K^+ from the kindled rat hippocampus and cortex as well as focal and non focal temporal cortex surgically removed from epileptic patients. The tissue was chopped into 400 um thick cubes and superfused successively with Krebs-Ringer buffer containing 3mM K^+, 60mM K^+, and 0mM Ca^{++}, 60mM K^+. The superfusates were collected at 0.5 min intervals and analyzed for amino acids, CA, and neuropeptides. In the kindled rat hippocampus and cortex the K^+-stimulated release of GLU, aspartate, (ASP) and GLY was 1.5, 1.6 and 2.0-fold higher respectively, when compared with sham-operated rats. The release of ST was also increased 1.9-fold when compared with the sham-operated animals. The release pattern of CA was not changed when compared to sham-operated rats in kindled rat brains collected one day following the last seizure. In the human brain treated in a manner similar to the kindled brain, preliminary studies showed that the release of CA, GLU, GLY, ST, and NPY was increased in the focal region when compared with the non-focal region by 1.5, 1.6, 1.4, 1.7, 1.9, and 2.1-fold, respectively.

The findings that GLU and ASP release were increased in both the kindled model and human cortex may indicate that these amino acids are involved in the excitability of the epileptic brain. The release of GLY was unexpected, but may now be explained in light of recent findings that GLY may activate NMDA receptors. The role of ST in the epileptic focus remains unclear, however, recent evidence demonstrating an increase in the potency of acetylcholine by this peptide may implicate it as a molecule regulating excitability in the focus. The increase of CA in the focus may be a secondary phenomenon, i.e., an increased inhibitory tone to stabilize a depolarized zone. The data will be discussed in view of the interactions of the above mentioned molecules in relation to epilepsy.

307.12 SYMPATHO-ADRENAL CO-STORAGE, RELEASE AND SYNTHESIS OF ENKEPHALINS AND CATECHOLAMINES INDUCED BY STRESS. R. L. Klein, R. W. Duncan*, T. J. Selva*, W. L. West* and A. Thureson-Klein. Dept. of Pharmacol. and Toxicol., Univ. Miss. Med. Ctr., Jackson, MS 39216.

Enkephalins (ENKs) occur at similar molarities and ratios to co-stored catecholamine (CA) in adrenal chromaffin granules and large dense cored vesicles (LDVs) of noradrenergic terminals. Amounts of ENKs tend to increase with animal size and in proportion to LDV populations in certain terminals. Exocytotic release occurs primarily during stress. In pig, ENK-CA co-storage in chromaffin cells and middle cerebral, coronary and mesenteric arteries is relatively high.

In response to 10 min CNS ischemia (CNS-I), there is intense sympatho-adrenal stimulation causing immediate rise in mean arterial pressure and in circulating (femoral vein, adrenal vein outflow) CAs and ENKs. Epinephrine especially is rapidly released from adrenal cells and compensated by resynthesis. Proportional release of ENKs occurs with 40% depletion of met-ENK, but not leu-ENK, even though acute precursor processing is induced. Norepinephrine is moderately depleted (20-30%) from terminals in blood vessels, while met-ENK is depleted 80-95% and leu-ENK 25-60%.

Reserpinization, while depleting CAs to various degrees, induces acute peptide precursor processing in chromaffin cells and in sympathetic terminals to blood vessels, increasing ENK contents 2 to 4-fold. After a lag period, *de novo* synthesis/processing is enhanced to achieve a 6 to 8-fold increase of ENK stores in chromaffin cells by 2 to 4 weeks, while contents in terminals of blood vessels tend to return to control levels. *In vivo*, reserpine causes blunting of CNS sympathetic outflow, so that usual intense stimulation by CNS-I is abolished. Upon recovery from reserpine, CNS-I response to increase blood pressure, and circulating CAs and ENKs gradually returns. Now adrenal release of ENKs reflects maintained reserpine-induced increase in stores. Partially recovered blood vessel innervation shows resistance to CA depletion after CNS-I and both ENKs increase 50-200% above control due to acutely induced precursor peptide processing.

It can be concluded that induction of precursor peptide processing and new synthesis due to cardiovascular stress (CNS-I) occurs in noradrenergic terminals to blood vessels as well as in adrenal chromaffin cells, but with a different pattern of response. Further, upon recovery from reserpine treatment, the noradrenergic innervation and the chromaffin cells undergo homeostatic adjustments which tend to minimize the enkephalin depleting effects of CNS-I. (AHA-Miss. Affil., BRSG RR05386.)

307.13 DIFFERENTIAL EFFECTS OF RESERPINE ON NOREPINEPHRINE AND NEUROPEPTIDES IN PERIPHERAL NERVE FIBERS AND THE ADRENAL MEDULLA OF DOMESTIC PIGS. J. Y. Kong*, A. Thureson-Klein, R. L. Klein and W. West*. Dept. of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS 39216.

Pigs (Sus scrofa) weighing 25-50 kg were treated with reserpine, using a relatively low loading dose of 0.08 mg/kg followed by 0.05 mg/kg every other day for 3 to 14 days and the effects on catecholamine(s) and neuropeptide(s) believed to be costored in peripheral nerve fibers and adrenal medullary cells examined. In other species, eg. guinea pig, a high single dose (5 mg/kg) reserpine causes tissue-specific neuropeptide Y (NPY) depletion along with norepinephrine (NE) from nerve fibers (Lundberg et al. Acta Physiol. Scand. 123:363, 1985) and in rats, adrenal NPY is also rapidly depleted followed by a slow recovery (de Quidt and Emson, Neuroscience 19:1011, 1986).

The pig differs from rodents by its high number of large (75-95 nm) dense cored vesicles (LDV) in the noradrenergic terminals. This should facilitate studies on neuropeptide storage and release because analyses of purified LDV fractions indicate that only this type of vesicles contains NPY and/or opioid peptide, putatively co-stored with NE. Small (45-65 nm) vesicles, that constitute 95% of the total vesicle population in rodents, appear to store only NE and some ATP.

Three to seven days of reserpine increased met-enkephalin 2-3 fold (RIA) in several blood vessels but did not change the levels in vasa deferentia. A decrease of 80% NE in perivascular fibers was paralleled by a loss of small vesicle contents and histofluorescence. NPY immunofluorescent fibers were of similar density as met-enkephalin reactive varicosities but had a somewhat different distribution, particularly in the vas deferens. Control and reserpinized pig had NPY reactive fibers but no reactive chromaffin cells in the adrenal medulla, probably accounting for the low tissue levels.

The reserpine dosage did not destroy the LDV synthesis and storage capability of NE as shown by a rapid recovery of tissue contents and glyoxylic acid reactive fibers while most small vesicles showed low chromaffin reactivity. The results suggest that small doses of reserpine affect NPY and enkephalin differently from co-stored catecholamines. However, the mechanisms involved remain obscure. While reserpine interferes with the NE storage in small and large vesicles there is no evidence that the neuropeptides, prepacked into LDVs in the perikaryon, leak out of the vesicles. Reserpine (0.5 mg/kg) affects cellular calcium levels and can impair the energy metabolism of secretory cells in rodents (Müller et al. Acta Physiol Scand. 123, 1985). Similar effects on the neuron may interfere with exocytosis.

307.14 WHOLE-BODY AUTORADIOGRAPHIC ANALYSIS OF THE TISSUE DISTRIBUTION OF THE CALCIUM CHANNEL ANTAGONIST CI-951 IN RATS. W.P. MCNALLY AND T. CHANG,* Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Whole-body autoradiography and video-densitometry have been used to examine the distribution and fate of ^{14}C-labeled CI-951 (1,4-dihydro-2-methyl-5-(1-methylethoxy)-4-[2-trifluoromethyl)-phenyl]-1,6-naphthyridine-3-carboxylic acid, ethyl ester). CI-951, a calcium channel antagonist, is a new drug under development which has shown high specificity for cerebral vasculature. CI-951 is considerably more potent than nimodipine in dilation of cerebral arteries, and does not affect calcium channels in myocardial cells at therapeutic doses. These present studies were undertaken to examine the tissue distribution of the compound with special emphasis on the uptake and regional localization in the brain.

Methods: ^{14}C-CI-951 was synthesized at Goedecke AG, Freiburg, West Germany. Drug was administered in PEG solution to male Wistar rats via tail vein injection at a dose of 4 mg (514 uCi)/Kg. Animals were sacrificed by halothane anestesia at 0.25, 1, 4, or 12 hours post dose and prepared for whole-body sectioning and autoradiography. ^{14}C methacrylate standards were exposed simultaneously with sections.

Results and conclusions: CI-951 radioactivity was widely distributed to tissues following an IV dose. Activity in the liver and GI tract suggests significant biliary excretion while uptake in the gastric mucosa may reflect some degree of direct secretion into the gut. There is also evidence of urinary excretion. Radioequivalents in most tissues declined markedly while radioactivity in body fat appeared to increase at early time points and maintained activity over the course of these experiments. There was rapid penetration of considerable radioactivity into the brain with localization into specific areas, notably inferior colliculus, cerebral cortex, mammillary body and pontine nucleus. However, this distributional pattern was not persisent as most brain areas showed significant decrease in activity at 4 hours post dose. At this interval, areas of white matter such as the corpus callosum and spinal white demonstrated a considerable increase in activity compared to other regions. This enhanced uptake remained apparent at 12 hours post dose. CI-951 appeared to have an affinity for tissues rich in lipid, suggesting the possibility of a deep reservoir for the compound, which may have significance in assessing its pharmacologic activity.

307.15 EFFECT OF IN VITRO ADDITION AND IN VIVO TREATMENT WITH VARIOUS CALCIUM ANTAGONISTS ON MET-ENKEPHALIN RELEASE FROM RAT STRIATAL SLICES. S. Di Giovine*, S. Govoni, F. Battaini, M.S. Magnoni* and M. Trabucchi^. (SPON.: V. Olgiati) Institute of Pharmacological Sciences, University of Milan and ^Chair of Toxicology, 2nd University of Rome, Italy.

The brain has a fairly high density of binding sites for calcium entry blockers, which appear to be located on neurons. However, the first studies on synaptosomes were unable to show an action of these drugs on calcium movements and on neurotransmitter release, which represents the prototype of calcium dependent processes in the central nervous system (CNS).

The discovery of various types of neuronal calcium channels (Nowycky et al., Nature 316:440,1985) among which only one is sensitive to calcium antagonist may explain this discrepancy.

In addition, recent data indicate that in opportune experimental models calcium antagonists may indeed inhibit neurotransmitter release. These experiments include studies on cultured PC12, on neuroblastoma-glioma cells, and on brain slices.

Along this line we have studied the effect of in vitro addition and in vivo treatment with various calcium antagonists on the release of met-enkephalin immunoreactive material (ME-IR) from rat striatal slices.

For release experiments striatal slices (300 x 300 microns) were obtained through a McIlwain tissue chopper and incubated in oxygenated Krebs Ringer buffer. Met-enkephalin released in the medium was measured according to Pasinetti et al. (Brain Res. 293:364, 1984).

The results indicate that flunarizine, verapamil and nifedipine at micromolar concentrations are able to inhibit the potassium stimulated ME-IR release (42, 60 and 41% respectively using a 10 micromolar concentration). The stimulated release of ME-IR was inhibited also by the in vivo treatment with the three drugs (flunarizine 40 mg/Kg, 4 hrs; verapamil 40 mg/Kg, 1 hr; nifedipine 10 mg/Kg, 1 hr).

The powerful effect exerted by the acute in vivo treatment with calcium antagonists on ME-IR release is interesting since literature data indicate the existence of an interaction between calcium antagonists and the opiate system. In particular, nimodipine, a dihydropyridine calcium antagonist, inhibits the opiate and ethanol withdrawal syndrome and the chronic treatment with morphine increases the number of brain DHP binding sites.

Whether the effect observed in our experiments is entirely due to an action on calcium ion movements has to be established. On the other hand, the results support the concept that the treatment with calcium antagonist may directly interfere with brain neurotransmission.

307.16 Na^+ TRANSPORT: A REQUIREMENT FOR COPPER AMPLIFICATION OF PROSTAGLANDIN E_2 STIMULATION OF LHRH RELEASE FROM MEDIAN EMINENCE (ME) EXPLANTS. A. Barnea, M. Colombani-Vidal*, D.E. Hartter, and G. Cho*, Depts. OB/GYN & Physiol, The Green Ctr., UTHSCD, Dallas, Tx. 75235

We have previously shown that copper complexed to histidine (Cu) modulates LHRH release from ME explants in 2 ways. Way 1, Cu^+ stimulates LHRH release by a process requiring Na^+ transport, the driving force of which is the Na/K ATPase-generated $[Na^+]$ gradient. This process is inhibited by Li^+ or ouabain (Ouab) but not by tetrodotoxin (TTX) nor amiloride (Amil). Way 2, Cu amplifies PGE_2 stimulation of LHRH release. The question arises: Is Na^+ required also for amplification of PGE_2 action and if so, is it required for Cu uptake or Cu action? ME of adult male rats were incubated at 37 C for 5 min with 150μM Cu and then for 15 min with 10μM PGE_2 (Cu/PGE_2), with Cu alone, or with PGE_2 alone. LHRH release was (mean ± SE):

INHIBITOR	N	L H R H Basal	R E L E A S E ; pg/15min/ME Cu	Cu/PGE_2	Cu/PGE_2-Cu
None	34	4.4±0.4	20.0±1.6	48.0±1.6	28.0
127mM Li^+	9	5.2±0.4	18.8±2.0	24.0±2.4	5.2
0.5mM Ouab	8	29.2±4.0	35.6±2.0	47.6±3.2	12.0
10μM TTX	11	4.0±4.0	21.6±2.4	46.4±6.0	24.8
0.1mM Amil	9	4.4±1.6	27.2±5.2	53.2±4.4	26.0

Only Li^+ and Ouab inhibited (80% and 60%) amplified release (Cu/PGE_2-Cu); the elevated basal release induced by Ouab indicates inhibition of Na/K ATPase. PGE_2-stimulated release was 4.3±.4(15) pg, or 15% of amplified release, and it was not inhibited by Li^+ or Ouab. Cu uptake was measured 15 min after incubation with 100μM 67-copper complexed to histidine. Cu uptake was not effected by Li^+ or Ouab: with 140 mM Na^+ (regular buffer) uptake was 7.6 ± .4; with 127mM Li^+ it was 7.0 ± .2; and with 0.5mM Ouab uptake was 6.8 ± .3 nmol/15'/mg P [mean ± SE; N=10]. Thus, Cu-amplified PGE_2 stimulation of LHRH release requires transport of Na^+ driven by Na/K ATPase, whereas Cu uptake does not require Na^+ transport. In conclusion, Na^+ transport is required for both stimulation of LHRH release and amplification of PGE_2 stimulation of LHRH release, and the Na^+ requirement is for copper action and not for copper uptake.

307.17 LOCALIZATION OF THE DOPAMINE UPTAKE SITE IN RAT BRAIN USING [^{3}H]-GBR 12935: EFFECTS OF LESIONS. J.K. Wamsley, T.M. Dawson, F. Filloux.* Depts. of Psych., Pharmacol., Neurol., Univ. of Utah School of Medicine, SLC, UT 84132.

A number of radioactive ligands including [^{3}H]-mazindol, [^{3}H]-nomifensine, [^{3}H]-cocaine and [^{3}H]-methylphenidate have been shown to label the dopamine (DA) uptake site. [^{3}H]-GBR 12935 has recently been proposed as a more selective ligand for the DA uptake complex (Janowsky et al., J. Neurochem. 46:1272, 1986), and conditions allowing for localization of this uptake site using quantitative autoradiography have been reported (Dawson et al., Eur. J. Pharmacol. 126:171, 1986). In order to further investigate the utility of [^{3}H]-GBR 12935 as a selective ligand for the DA uptake site, the effects of neurotoxin lesions on the autoradiographic distribution of [^{3}H]-GBR 12935 binding have been examined.

Unilateral stereotaxic lesions of the substantia nigra (SN) or of the caudate-putamen (CPu) were performed using ibotenic acid (IA) or 6-hydroxydopamine (6-OHDA). The former toxin destroys local neurons, sparing fibers of passage, while 6-OHDA selectively damages catecholaminergic neurons. Control animals received injections of vehicle alone. Ten micron, slide-mounted serial tissue sections of rat forebrain were incubated in 0.5 nM [^{3}H]-GBR 12935 as described elsewhere (Dawson et al., Eur. J. Pharmacol. 126:171, 1986). Blanks were generated by the addition of unlabeled GBR 12909. Autoradiograms were produced by apposing the labeled tissue sections and brain mash tritium standards to tritium-sensitive LKB Ultrofilm. Quantitation was performed using computerized microdensitometry.

6-OHDA and IA lesions of the SN produced up to 50% loss of [^{3}H]-GBR 12935 binding throughout the ipsilateral CPu and in the substantia nigra compacta (SNC). Significant, but smaller reductions in binding were also seen in the entopeduncular nucleus and the substantia nigra reticulata (SNR), but no losses occurred in the pre-frontal cortex, nucleus accumbens, claustrum and globus pallidus. 6-OHDA lesions of the CPu also resulted in marked loss of [^{3}H]-GBR 12935 in the CPu, but the loss was restricted to the lesioned area (central and anterior CPu) in comparison to the prominent loss of binding seen in the posterolateral CPu of animals with SNC lesions. IA lesions of the CPu resulted in variable (statistically nonsignificant) effects: the most extensive lesion substantially reduced [^{3}H]-GBR 12935 binding in the damaged area, while more restricted CPu lesions failed to do so.

These results provide further evidence that [^{3}H]-GBR 12935 labels the DA uptake site. The regional loss of binding following SNC lesions suggests that DA fibers project not only to the CPu, but also to the ipsilateral entopeduncular nucleus and SNR.

307.18 INFLUX OF CHOLINE ANALOG INTO DOG BRAIN MEASURED BY POSITRON EMISSION TOMOGRAPHY (PET). C. Redies, M. Diksic*, B. Collier, A. Gjedde*, S. Gauthier*, C.J. Thompson*, and W.H. Feindel*. Brain Imaging Center, Montreal Neurological Institute and Hospital, and Department of Neurology and Neurosurgery, McGill University, Montréal, Québec H3A 2B4.

The usefulness of the choline analog pyrrolidinocholine as a tracer for acetylcholine turnover in brain was evaluated by PET. In vitro experiments showed that pyrrolidinocholine is transported by the high-affinity choline uptake system; it is, however, not substrate for acetylcholine esterase (Fisher, A., Hanin, I., Life Sci. 27:1615, 1980).

The brain uptake of C-11 labeled pyrrolidinocholine was measured in 3 dogs by rapid PET imaging (4 s frames) and rapid arterial plasma sampling during the first minutes after the intravenous injection of 5 to 15 mCi tracer (specific activity at end of synthesis 1000-2000 Ci/mmol). Synthesis of the tracer was described previously (Diksic, M., Jolly, D., J. Label. Compds. Radiopharm. 13:987, 1986).

Data were analyzed by plotting the apparent distribution volume of tracer as a function of exposure time to tracer (Gjedde, A., J. Neurochem. 36:1463, 1981). During the first 1.5 min after tracer injection, brain uptake of tracer was found to be unidirectional. For brain tissue, the initial volume of distribution V_0 of pyrrolidinocholine was 0.08 ml/g ± 0.03 SD. The clearance K_{in} was 0.017 ml/g/min ± 0.008 SD; assuming a brain plasma flow of 0.3 ml/g/min, this yields a brain pyrrolidinocholine extraction of 6%. This value is at least 5 times higher than the value expected from simple diffusion of tracer across the blood-brain barrier (BBB) (Cornford, E.M., et al., J. Neurochem. 30:299, 1978). For the carotid artery, K_{in} was 0.07 ± 0.05 ml/g/min, and, for the jugular vein, 0.11 ± 0.09 ml/g/min. Therefore, the endothelium of the capillaries perfusing the walls of major blood vessels is more permeable to tracer than the BBB.

The short initial period of unidirectional uptake of pyrrolidinocholine into brain was followed by rapid clearance of radioactivity from brain and plasma (Friedland, R.P., et al., J. Nucl. Med. 24:812, 1983). Pyrrolidinocholine cannot be used to image the metabolism of choline because of its relatively low extraction in brain, its short plasma half-life, and because it may be incorporated into phospholipids.

This study is the first to measure transport of a choline analog across the BBB in vivo by PET. Such measurements may shed light on the availability of blood choline to brain and other tissues in degenerative diseases and aging in man (Cornford, E.M., et al., J. Neurochem. 30:299, 1978).

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308.1 FURTHER CHARACTERIZATION OF A CELL SURFACE CARBOHYDRATE DISTRIBUTED IN A DORSOVENTRAL GRADIENT IN PERINATAL RAT RETINA.
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Interest in the coding of positional information in retina has led to the identification of a cell surface molecule which labels with monoclonal antibody JONES. This antigen is distributed in embryonic and early postnatal rat retina in a dorsoventral gradient and persists in the adult as only a punctate band in the outer plexiform layer(Nature 324:459,1986). Analysis has revealed that the gradient in part reflects differing proportions of cells bearing the JONES antigen rather than the amount expressed per cell. Biochemical characterization has indicated that the JONES antigen in retina is a modified ganglioside, 9-O-acetyl GD3. Here we describe an examination in retina of the 1) relationship between JONES and GD3 expression 2) cell-type distribution of JONES antigen .

Monolayer cultures of neural retinae from rat pups (E18-PN2) were grown on collagen or laminin in supplemented MEM at $2x10^5$cells/ml. Freshly dissociated neural retinal cells (PN1-4) were fixed in suspension with 1-2% paraformaldehyde and attached to poly-lysine coated coverslips. GD3 was detected with specific antibody R24 (gift of K. Lloyd) and cell types were identified with previously described monoclonal antibodies.

At PN1 92% of the freshly dissociated cells stained positively for R24 but only 68% were JONES labeled. Virtually all of the JONES positive cells also stained for R24: 1% of the cells were R24 negative being JONES positive. 7% of the cells did not label with either antibody. By PN4 the frequency of JONES positive cells decreased to 41%, while the proportion of R24 labeled cells only declined to 85%.

Immunofluorescent staining of fixed cultures with JONES antibody revealed that both the flat cells, presumably glia and the small round process bearing neuronal-like cells were labeled. Subsequent analysis of freshly dissociated PN2 retinal cells revealed that 53% of ganglion cells, as identified by prior retrograde labeling with the fluorescent dye Fluoro-Gold, were also labeled with JONES. By PN4 the proportion was 39%. At PN2, 43% of rod photoreceptors, as detected with antibody RET-P1, were also labeled with JONES. At PN4 30% of these cells expressed JONES. Amacrine cells, as distinguished with antibody HPC-1, also express the JONES antigen. At PN4 67% of HPC-1 positive cells were labeled with JONES.

We conclude that 1) differences in the percentages of JONES and R24 positive cells and in particular the different rate at which JONES and R24 staining declined with age, indicate that the expression of the JONES epitope is regulated with some independence from its possible precursor GD3 2) JONES is expressed on a variety of retinal cells, both neurons and glia 3) at a given postnatal age the percent of JONES labeled cells varies with the cell-type 4) from PN2-4 there is approximately a 30% decline in number of photoreceptor and amacrine cells expressing the JONES antigen .

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308.2 THROMBOSPONDIN IN THE DEVELOPING CEREBELLAR CORTEX
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Thrombospondin (TSP) is a trimeric glycoprotein of Mr 400,000 which is synthesized and secreted by a variety of cells including: endothelial cells, smooth muscle cells and glial cells. The secreted TSP is incorporated into the extracellular matrix of these cells. Matrix TSP has been implicated in a number of cell migratory and proliferative events in vitro. To date, however, there are no reports of its presence in any developing tissue, including the CNS.

To examine the possible role of TSP in the cell migrations involved in CNS development, we have mapped the differential deposition of TSP during cerebellar cortex formation during: active division of the external granule cells (postnatal day 1; PN1), active inward migration (PN10), and after migration was completed (PN61) in male, CD-1 strain mice.

Blocks of cerebellar cortex were removed from the region of the forming primary fissure and fresh frozen in OCT embedding compound in hexane cooled over acetone/dry ice. Transverse sections were cut at 8 micrometers, washed in PBS and exposed to normal goat serum (1:20) followed by the primary antibody (1:20, 2 h, room temperature). Sections were washed, then exposed to goat anti-rabbit IgG-FITC (1:50,30 min, room temperature), washed extensively, coverslipped, viewed and photographed in a Leitz Dialux Orthoplan photomicroscope.

On PN1, TSP surrounded the external granule cells and was densely deposited in the superficial portion of the Purkinje cell layer. By PN10 during active granule cell migration, TSP still surrounded granule cells but was particularly densely deposited on postmitotic, pre-migratory granule cells. The forming molecular layer stained intensely as did the Purkinje cell layer. In PN61 cortex in which cell migration was complete, there was little staining of the molecular layer, although Purkinje cell and internal granule cell layers stained intensely. White matter was negative for TSP, as were processes of the Golgi epithelial cells.

Presence of TSP on granule cells and in the molecular layer during active granule cell migration suggest that TSP may play a role in granule cell migration during cerebellar histogenesis. Current investigations are in progress to extend these observations to the ultrastructural level.

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308.3 A CELL ADHESION ASSAY FOR TRANSMITTER-IDENTIFIED NEURONS.
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Several laboratories have shown that developing neurons interact specifically with astrocytes. We have previously measured the extent to which astrocytes promote neurite outgrowth and stimulate the activity of a transmitter producing enzyme. The fact that astrocytes accelerate the development of these morphological and biochemical aspects of the nervous system raises the question of whether a specific binding may exist between neurons and glia to mediate developmental processes.

We modified the binding assay of McClay et al. (PNAS, 78:4975-4979, 1981) to quantify the specificity of binding between transmitter-identified neurons and glial substrates as compared with binding of neurons to fibroblast substrates. Purified postnatal rat astrocytes or Normal Rat Kidney fibroblasts were maintained in 250ml culture flasks. To measure adhesion, these substrate cells were suspended in a low concentration of trypsin (0.03%) in EDTA (Versene 1:5000) and either plated onto poly-D-lysine coated 96-well flexible assay plates and allowed to grow to confluency overnight, or spun as a confluent monolayer onto poly-D-lysine and glutaraldehyde coated plates just before binding was assayed. Raphe nuclei were dissected from E14 rat embryos. Developing serotonergic (5-HT) neurons of the raphe were labeled by high affinity uptake of ^{3}H-5-HT. The specificity of this labeling was determined by combined autoradiography/immunocytochemistry (^{3}H-5-HT, a-5-HT), and competition with unlabeled fluoxetine. Tissue was dissociated by trituration after incubation in EDTA.

Probe neurons were spun onto monolayers at 20 x g. To measure binding, samples were inverted and spun at 120 x g. The amount of radioactivity remaining bound to the substrate was compared to the radioactivity dislodged to give the percentage of cells bound.

Using high affinity uptake to label probe neurons, the binding properties of virtually any neuronal subclass can theoretically be measured using this assay. PHS Grant #NS21840.

308.4 SELECTIVE *IN VITRO* REASSOCIATION OF EARLY VERSUS LATE POSTMITOTIC NEURONS FROM THE RAT FOREBRAIN.
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The development of pattern in the central nervous system involves cell proliferation, migration and adhesion. Neurons of the patch compartment of the mammalian striatum leave the mitotic cycle earlier in development than neurons of the striatal matrix compartment. Previously we have shown that patch (but not matrix) cells from the embryonic striatum preferentially adhere to each other when allowed to migrate on an artificial substrate *in vitro* (Krushel et al. 1986, Soc. Neurosci. Abstr. 12:238). This selective adherence may underlie striatal compartmentalization. Cell suspension reaggregate cultures have now been employed to allow for greater experimental reassociation of embryonic rat forebrain cells.We asked if telencephalic neurons which become postmitotic at the same embryonic time, selectively adhere to one another. That is,when removed embryonically, dissociated and reaggregated *in vitro* , will cells with similar birthdates selectively reassociate with each other? Timed pregnant Wistar albino rats were injected with 1mCi ^{3}H-thymidine on embryonic day (E) 13 or 18. The E13 injection primarily labeled patch cells in the striatum and deep layer cells in the cortex. The E18 injection primarily labeled striatal matrix neurons and the neurons of the superficial layers in the cortex. Two or seven days later, the striatum and cortex were separately dissected, dissociated into single cells, and each plated at a cell density of approximately $1 X 10^7$ per 60mm dish. The cell suspensions were placed on a shaker at 70 rpm. After five days in culture, the aggregates were fixed in 4% paraformaldehyde in PBS and embedded in paraffin. 5 um sections were cut, defatted, and dipped in Kodak NTB2 nuclear track emulsion. After a two week exposure, the emulsion was developed and the tissue dehydrated and counterstained with cresyl violet.

Both dissociated striatal and cortical cultures formed multicellular aggregates. The largest aggregates were produced by the tissue removed earliest from the embryo (E15). Within their separate aggregates, both striatal and cortical cells labeled on E13 with ^{3}H-thymidine were found to be preferentially located near to one another in small clumps of 3-5 labeled cells. This contrasted with the results from striatal and cortical reaggregates labeled with ^{3}H-thymidine on E18. These cultures exhibited labeled cells which were randomly dispersed throughout the aggregates. The results suggest that the patch cells of the striatum and the deep layer neurons of the cortex possess adhesive factor(s) which allow them to organize *in vivo* . This initial adhesion of early postmitotic neurons may create a template, upon which pattern is formed in the striatum and cortex through the migration of later born and non self-adhesive neurons.

308.5 BINDING OF GRANULE NEURONS TO THE MOLECULAR LAYER IS DEVELOPMENTALLY REGULATED L. M. Bolin and R. V. Rouse*. Department of Pathology, Stanford Univ. Sch. of Med., Stanford, CA 94305

During histogenesis of the mammalian cerebellum, cell-cell interactions involving surface molecules are necessary for pattern formation. In the neonatal mouse, post-mitotic granule neurons traverse the developing molecular layer guided by Bergmann glia fibers. We have devised a binding assay to investigate these heterotypic neuron-glia interactions *in situ*. Single cell suspensions of live granule neurons applied to tissue sections of developing brain will bind only to cerebellum and hippocampus. Within the cerebellum, cells from ten-day-old (P10) mice (the time of presumed maximal contact guidance) adhere preferentially to the molecular layer. With increasing age of substrate tissue, these cells will also bind internal granular layer. This cell-tissue interaction is calcium and magnesium independent. The granule cell surface molecules involved are not trypsin sensitive. To confirm the specificity of this adherence, a variety of other cell types have been applied. While mouse fibroblast cell lines adhere in a non specific manner to all areas of the brain only the mouse neuroblastoma, N2A, binds cerebellum in a pattern similar to migrating granule cells.

The granule cell binding is developmentally regulated. Substrate tissue under one week of age is only bound by granule cells near P10 in age; younger and older cells do not bind to young tissue. With increasing tissue age, binding of granule cells of progressively younger and older ages is seen. This data is compatible with a single receptor-ligand mechanism in which both elements are developmentally controlled. The granule cell component (receptor) must increase in number or affinity from birth to age P10 but decline after that age. P10 represents the peak of migratory activity at which time the necessary receptor would be maximally expressed and/or present in its high affinity form. The tissue substrate ligand must increase in number and/or affinity with postnatal age. These developmentally regulated changes would explain the increase in binding between progressively younger tissue and cells of increasing age until P10. After this age, increasingly older substrate tissues expressing increasingly more ligand are necessary for this interaction with cells whose receptor number or affinity is declining. Cells younger than P10, expressing few or low affinity receptors would require older tissue with high amounts of ligand as substrate.

The system defined by this assay may involve components that participate in phenomena described by others *in vitro*. The binding patterns observed exhibit the same anatomic and temporal characteristics as *in vivo* cerebellar granule cell migration during the postnatal period. It allows for an analysis of a single receptor-ligand system in the complex context of tissue section and predicts independent patterns of regulation of its components.

308.6 NEURON-GLIAL INTERACTION CONTROLS CEREBELLAR GRANULE CELL MATURATION WITH GLUTAMATE-TAURINE INTERPLAY E. Trenkner, Department of Pharmacology, New York University Medical Center, 550 First Avenue, New York, NY 10016

Cell migration is a fundamental process of development. In CNS development, the pathway of neuronal migration determines the general topography of brain regions and the cellular location within regions. In the cerebellum granule cell migration is a timed process leading these cells through different cellular environments (1).

A dynamic relationship between neuron and glia has been documented based on neurotransmission. Glutamate is released in a Ca^{++} dependent manner from cultured glutamatergic cerebella granule cells. Taurine, 2-aminoethane sulfonic acid, is by far the most abundant free amino acid particularly in the cerebellum, exceeded only by glutamate. Viewed as an inhibition amino acid transmitter, taurine has received clinical attention for its anti-convulsant properties. This study focuses on the role of taurine and glutamate since their essential role in cerebellar development appears evident.

Neurological mutations in the mouse provide powerful tools for anlayzing cell migration and assembly. The neurological mutant weaver (wv/wv) is particularly relevant to the analysis of glia-neuron interaction during cell migration and assembly. The defect of weaver is inherent to granule cells (2) Weaver granule cells fail to interact with normal (+/+) astroglia whereas normal granule cells react with astrocytes of wv/wv (3). Biochemical analysis of developing wv/wv cerebellum revealed that in contrast to +/+ the concentrations of glutamate and taurine were reduced during the period of granule cell migration (4).

This study demonstrates that in microwell cultures (5) of wv/wv cerebellum, fiber formation and cell migration is impaired. Whereas normal cerebellar cells subsequently extend cables consisting of fiberbundles between cellular reaggregates, very few such cables were formed by reaggregated weaver cells. In addition the absence of migrating granule cells was characteristic for wv/wv in vitro. These perturbed functions were restored, however, in the presence of 10^{-2}M taurine. In the absence of glutamate cell patterns of both +/+ and wv/wv looked alike, but the pattern was restored in the presence of taurine. Taurine uptake blockers blocked cell migration considerably, independent of glutamate. Taurine was released from glia cell populations when glutamate (10^{-4}M) or enriched granule cell populations were added to ^{3}H-taurine loaded glial cells. In summary, the results reported here support the hypothesis that glutamate stimulates astroglial cells to release taurine. Extracellular (released) taurine, independent of glutamate induces granule cells to sustain their viability and function.

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308.7 DEVELOPMENT OF AFFERENTS TO MOUSE SOMATOSENSORY CORTEX S. Senft* and T. Woolsey. Departments of Neurology and Neuro-surgery & McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO. 63110. (Supported by NIH grant 17763)

An in vitro slice preparation (Bernardo, 1986) which makes use of horseradish peroxidase (HRP) and an oblique plane of section to label defined regions of the adult mouse whisker pathway has been adapted for use on neonates. Afferents to the developing "barrel" cortex, from day 1 (the day of birth) to day 7, have been labeled.

Animals were ice-anesthetized and perfused with cold artificial cerebro-spinal fluid (ACSF). After decapitation their brains were removed, bisected, and placed in chilled ACSF. Slices (~1500 mu.) taken 45 degrees from midline were stained with methylene blue to show the ventrobasal nucleus of the thalamus (VB) and the thalamic radiations. Chips of HRP/ethylene glycol were positioned in these slices, which were then kept in warm (36-37 degrees C.) oxygenated ACSF for 4 to 8 hours. They were next fixed, frozen-sectioned at 75 mu. and reacted (after Adams). Sections were carefully mounted to avoid cracks; alternate sections were counter-stained for Nissl. Selected fibers were traced at 800x to 2000x using a camera lucida aided by a par-focal video camera to enhance visual contrast.

Placing HRP either in VB or in the thalamic radiations labeled many presumed thalamocortical afferents (TCA). Fibers were judged "thalamocortical" if orthograde label greatly dominated retrograde label in cortex and somata were retrogradely filled in VB. Enough candidate arbors were followed to invite distinct hypotheses about the strategy of TCA ingrowth, particularly in relation to emerging laminar and barrel boundaries as delineated by Nissl stains. For example, it is clear that TCA's cluster in barrel-like formations before a pattern can be observed in cell somata. Also, this class of fiber is seen to reach and traverse the cortical plate as early as post-natal day one: several days earlier than expected. Another peculiar characteristic of this projection pattern is that fibers from older animals run more perpendicularly to the pia than those from younger animals.

We hypothesize that thalamocortical afferents invade the cortex in several stages. Upon leaving white matter, afferents proceed along fairly direct paths towards the pia, but with an apparently random pitch. At various points along the trajectory, they emit collaterals which rise more rapidly towards the pia. All fibers arborize more when they encounter the cortical plate, so a single afferent can sample a wide area of cortex whereas afferents from a local thalamic zone should overlap and colonize the cortex as a Gaussian around an average center-point. If neighboring thalamic areas correlate highly, in either intrinsic activity or sensory input, and if Hebbian-like mechanisms operate in neonatal cortex, then reinforcement will be most probable at peaks of arbor overlap Transformation to a barrel pattern could then occur: for example via competitive thresholding, with or without fiber retraction.

308.8 THE EARLY POSTNATAL ONTOGENESIS OF VIP/PHI AND CCK-IMMUNOREACTIVE STRUCTURES IN THE CAT VISUAL CORTEX. P.Wahle* and G.Meyer* (SPON:K. Albus).MPI Biophysik.Chemie,Dept.Neurobiology,3400 Göttingen, FRG, and Dept.Anatomy, Fac.Medicine,Univ. La Laguna, Tenerife, Spain.

Immunohistochemistry was performed in kittens aged from postnatal day (P)0 to P50.Neurons were classified on the basis of axonal criteria. Several cell types are only seen in the immature cortex.This means, that they were transient, and immunohistochemistry reveals degeneration and death of these neurons and they were no longer observed in the adult cortex. VIP/PHI 1.Transient cells: Pseudo-Horsetail cells possess axonal bundles descending to and terminating in layer VI, start differentiation around P4, peak from P14 to P20 and become eliminated until P30. Neurons with columnar dendritic fields of layers IV,V and axons projecting to layer VI appear at P7 and disappear at P20. Bipolar cells of layers IV-VI project to the white matter, differentiate around P4, peak from P10 to P16, and become eliminated until P25. Concurrently, the vertical fiber architecture issued by these cell types disappears. 2.Persisting cells: Bipolar cells with short dendrites and intralaminar axons appear in layers II-IV around P12. The main VIP/PHI-ir cell types are neurons with descending axons giving horizontal collaterals (appearing at P 15) and local axon basket cells (appearing at P30) in layers II-IV. The innervation of deep layers largely disappears and only terminal strata in superficial layers persist. Thus, the early postnatal phase ist characterized by transient cell types and innervation pattern which are progressively replaced by the persisting ones. CCK 1.Transient cells: Neurons with columnar dendritic fields (P2 to P14) of layers IV,V project to layer VI. 2.Persisting cells: Multipolar neurons of layer VI, bitufted to multipolar neurons of layer V and III project to and terminate in layer VI, where they establish long horozontal connections. Many neurons of these types (10%) possess two axons. Neurons with intralaminar axons of layer I derive from the marginal zone and appear at P2. Neurons with horizontal axons in layer II and those with descending axons giving horizontal collaterals in layers II-IV appear during the third week. Small local axon cells and neurons with short axonal bundles appear at P30 in these layers. A medium-sized local basket cell with horizontal collaterals appear at P40 in layer IV in area 17. The adult innervation pattern of area 17 comprises a densely innervated upper layer I, small patches of 100 µm in diameter at the layer I/II border, continuous terminal-strata in lower layer III/upper layer IV, in layer IVc and in layer VI. In area 18 the terminalstrata in layer IV and the local basket cells are absent.Thus, in contrast to VIP/PHI, the CCK-innervation pattern is progressively formed during the first month and is completed during the second postnatal month. We do not observe dramatic changes in the innervationpattern and only one transient cell type.We thank Drs. J.M.Polak and M.Tohyama for supply with antisera.

308.9 NEONATAL LESIONS OF THE INFERIOR COLLICULUS REDUCE TRANSIENT ACETYLCHOLINESTERASE ACTIVITY IN DEVELOPING RAT AUDITORY CORTEX. Richard T. Robertson, Fraidoon Mostamand* and Jen Yu. Departments of Anatomy and Neurobiology and of Physicial Medicine and Rehabilitation, College of Medicine, University of California, Irvine, CA 92717.

The presence of transient acetylcholinesterase (AChE) activity is characteristic of some developing thalamocortical systems, including the medial geniculate-auditory cortex projection of the rat. The location and appearance of the AChE histochemical reaction product suggest that the transient AChE is found within terminals of geniculocortical neurons. The purpose of the present study was to determine the effects of neonatal lesions of the inferior colliculus on development of the transient pattern of AChE in auditory cortex.

Subjects were laboratory born Sprague-Dawley rats of 0-30 postnatal days (PND) of age. Animals were anesthetized by hypothermia on PND 0 and one or both inferior colliculi were aspirated under visual control. Animals were sacrificed by aldehyde perfusion on PND 6-30. Frozen sections of 64um were cut in the transverse plane or parallel to the pial surface. AChE activity was detected histochemically by a modified version of the technique of Koelle.

AChE activity in cortical area 41 (primary auditory cortex) of normal animals appears at about PND 6 as a fine fiber-like plexus in deep layer III and layer IV. AChE reaction product reaches peak intensity in animals sacrificed at PND 10-12 and then declines until adult levels are reached by PND 18. The area and laminar extent of the transient AChE plexus correspond precisely to the terminal field of geniculocortical projections.

Neonatal removal of the inferior colliculus results in a marked loss of transient AChE in layer III-IV of cortical area 41 ipsilateral to the lesion. No effects of the lesion were detected on transient AChE in other cortical areas of the lesioned hemisphere or in the hemisphere contralateral to the lesion. Results from animals sacrificed at various ages demonstrate that the normal transient pattern of AChE in cortical area 41 never develops.

These data indicate that transient AChE activity normally found in developing rat auditory cortex is dependent on normal afferent innervation and/or stimulation. These data closely parallel the results reported previously for the visual system (Robertson et al., Dev. Brain Res., 1987).

308.10 INTRAOCULAR INJECTIONS OF TETRODOTOXIN REDUCE TRANSIENT ACETYLCHOLINESTERASE ACTIVITY IN DEVELOPING RAT VISUAL CORTEX. Rajeev K. Ambe*, Richard T. Robertson, and Jen Yu. (SPON: E.A. Davis). Departments of Anatomy and Neurobiology and of Physicial Medicine and Rehabilitation, College of Medicine, University of California, Irvine, CA 92717.

Transient acetylcholinesterase (AChE) activity is characteristic of the geniculocortical projection system of developing rats. The location and appearance of the transient AChE, along with its disappearance following lesions of the lateral geniculate body, suggest that the AChE is found in terminals of geniculocortical axons. Previous work from this laboratory (Robertson et al., Dev. Brain Res., '87) demonstrated that neonatal enucleation disrupts the normal development of transient AChE. The purpose of this study was to determine the effects of repeated intraocular injections of tetrodotoxin (TTX) on development of transient patterns of AChE.

Subjects were laboratory born Sprague-Dawley rats of 0-12 postnatal days (PND) of age. Each animal was anesthetized and received monocular injections of TTX (0.5 ul; 10^{-4}M in saline) on PND 3,5,7,9 and 11. On PND 12, animals were sacrificed by aldehyde perfusion. Frozen sections were cut in the transverse plane or parallel to the pial surface. AChE activity was detected histochemically by a modified version of the technique of Koelle.

AChE activity in cortical area 17 of normal infant rats appears as a fine fiber-like plexus in deep layer III and layer IV. Monocular injections of TTX result in a marked loss of AChE staining in the medial sector of layers III-IV of cortical area 17 contralateral to the injected eye. AChE staining in the lateral sector of the contralateral hemisphere and in the hemisphere ipsilateral to the injected eye appear normal. The cortical region sustaining the loss of AChE corresponds to the monocular segment of area 17. Some animals received injections of HRP along with TTX on PND 11. These cases demonstrated that the optic nerve of the injected eye was still capable of axonal transport, suggesting that the reduction in AChE activity was not attributable to damage to the optic nerve. Other animals were monocularly enucleated at PND 3 and injected with TTX on the standard schedule. Analysis of these cases revealed that monocular enucleation results in a more drastic reduction of transient AChE activity in layer III-IV of cortical area 17 than does the series of TTX injections.

These data indicate the transient pattern of AChE activity normally found in developing rat visual cortex is dependent on normal afferent stimulation.

308.11 PRENATAL DEVELOPMENT OF THE SPINOCEREBELLAR FIBERS IN THE RAT. H. Yaginuma*, (Spon: N. Okado) Dept. of Anatomy, Univ. of Tsukuba, Ibaraki 305, Japan.

To clarify the temporal and spatial relationship between developing cerebellar afferent fibers and their target, the development of the spinocerebellar fibers in the prenatal rats was examined using the anterograde wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP) technique. Pregnant rats were anesthetized with pentobarbital. After the uterus was incised, WGA-HRP was injected into the lower thoracic or upper lumbar spinal cord of embryos ranging from embryonic day (E) 16 to E21 (the day of sperm positivity was counted as E1). Following 9-12 hour survivals, the embryos were removed from the uterus, and fixed by perfusion. The sections of the brain stem and the spinal cord were reacted with tetramethylbenzidine. At E16.5, no labeled fibers were found in the cerebellar plate, although unidentified labeled fibers were found in the ventrolateral superficial part of the lower medulla oblongata. Labeled fibers were found first in the cerebellar plate at E17.5. These fibers passed through the ventrolateral superficial part of the medulla oblongata and the superior cerebellar peduncle (SCP), suggesting that these fibers belong to the ventral spinocerebellar tract. They reached the rostral part of the superficial fibrous layer (SF) of the cerebellar plate and ran medially for a short distance. Few labeled fibers reached the cerebellar plate through the inferior cerebellar peduncle (ICP). At E18.5, many labeled fibers, which passed through SCP, were found in the rostral part of SF. They reached the midline region and some of them crossed the midline. A few labeled fibers, which passed through ICP, were seen in the caudal part of SF. At this stage, the external germinal layer (EGL) had not reached the rostral portion of the cerebellar plate which contains labeled fibers. At E19.5, many labeled fibers entered the primitive cerebellum through both SCP and ICP. The fibers which passed through SCP were located in the rostral part of SF, while the fibers which passed through ICP were located in the caudal part of SF. Most of them crossed the midline to enter the contralateral side of the cerebellum. At this stage, the SF containing the labeled fibers was covered with EGL. The Purkinje cell layer (PCL) had not formed between EGL and the labeled fibers. However, a cluster of longitudinally elongated cells, presumably migrating Purkinje cells, was seen immediately beneath the labeled fibers. At E20.5 and E21.5, labeled fibers were present in the rostral part of the anterior lobe; the PCL was formed between EGL and the labeled fibers.

These findings suggest that the spinocerebellar fibers reach the rostral part of the cerebellum before the cellular components (EGL and PCL) of the primitive cerebellar cortex are formed, and that fibers of the ventral spinocerebellar tract reach the cerebellum earlier than those of the dorsal spinocerebellar tract.

308.12 PRENATAL UNILATERAL FRONTAL CORTEX ABLATION IN CATS. B.L. Shook, J.R. Villablanca and D.A. Hovda. Mental Retardation Research Center, Departments of Anatomy and Psychiatry, School of Medicine, University of California, Los Angeles, CA 90024.

Neonatal cerebral hemispherectomy or unilateral frontal cortex ablation in cats results in greater behavioral sparing than similar lesions in adults. The enhanced sparing is accompanied by exhuberant bilateral projections from the intact hemisphere and reduced thalamic-brain stem degeneration (Refs. in Brain Res., 420:219, '87). We sought to determine whether the early lesion effect could be extended to the prenatal period. Towards this aim, the left frontal cortex was ablated in two fetuses on either embryonic day 41 or 43. As adults these cats received multiple 0.02 μl injections of WGA-HRP in the intact (right) pericruciate cortex. Three days postinjection cats were perfused transcardially, the brains frozen sectioned at 50 μ, and alternate sections reacted for HRP or thionin stained.

Gross Anatomy: Lesions were restricted to the sigmoid gyri with subtotal removal of areas 4 and 6. Gyral and sulcal patterns were grossly abnormal throughout the entire injured hemisphere but the cytoarchitecture appeared normal. The ipsilateral thalamus and telencephalon were reduced in area (measured in 5 coronal planes) by, $\bar{X}$ = 13.5% - 24.4% and 8.3% - 10.8%, respectively. The decrease in size covaried with the extent of the lesion.

Cortical Connectivity: Tracer injections were restricted to areas 4 and 6 with minor involvement of area 3a in one animal. The intact pericruciate exhibited normal ipsilateral subcortical connections. As in neonatally lesioned cats, terminal label (minimal) was found in the contralateral thalamic intralaminar, ventral and ventrobasal nuclei. More dense, homotopically distributed, label was evident in the contralateral red nucleus and the crossed corticocaudate projection was abnormally widespread with reaction product distributed over much of the mediolateral extent of the head of the nucleus. Sparse anterograde label was also evident in the ipsilateral n. gracilis and dorsal horn of the cervical cord. The medullary crossing of the pyramidal tract was apparently normal.

Behavior: In contrast to neonatally frontal lesioned cats (Exp. Neurol., 61:615, '78), tactile, visual and chin contact components of the contralateral limb placing responses failed to develop in the present cats.

It is concluded that in utero unilateral pericruciate ablation can induce widespread changes in the gross structure of the injured hemisphere as well as anomalous sparse crossed projections from the intact frontal cortex. However, unlike neonatal lesions, embryonic frontal cortex injury may prevent the development of various aspects of the contralateral limb placing responses. Grants USPHS HD-05958 and HD-04612.

308.13 ABNORMALITIES OF GRANULE CELL DENDRITES AND AXONS IN THE DENTATE GYRUS OF THE NZB/BINJ MOUSE.
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In the dentate gyrus of NZB/BINJ mice there are ectopic granule cells in the molecular layer. We have studied the dendritic and axonal processes of ectopic and normally positioned granule cells in the dentate gyrus using the Golgi method.

The ectopic granule cells of the molecular layer and also the granule cells situated at the border of the molecular and granule cell layers have abnormal dendritic trees. The ectopic granule cells have three different dendritic morphologies: inverted, horizontal, and large-angled. The inverted granule cells are characterized by one or more primary dendrites extending from the soma down towards the granule cell layer. These dendrites usually turn toward the pial surface before reaching the granule cell layer and then arborize in a seemingly normal fashion. Many of the ectopic granule cells are only partially inverted in that they have primary dendrites extending both towards and away from the pial surface. The horizontal granule cells have primary dendrites which extend parallel to the granule cell layer. The large-angled granule cells have primary dendrites extending at a large obtuse angle towards the pial surface. The distal dendrites of both the horizontal and large-angled granule cell turn towards the pial surface and arborize normally as they pass through the molecular layer. The granule cells located at the border of the molecular and granular cell layers are usually of the large-angle type, and, in general, they have a less atypical appearance than the ectopic granule cells. The dendritic morphology of the granule cells within the granule cell layer appears to be normal.

Many of the ectopic granule cells have an axon that emerges from a primary dendrite and project horizontally or sometimes upward through the molecular layer. This indicates that heterotypical (granule cell to granule cell) synapses may be present.

The fact that the ectopic granule cells in the dentate gyrus of NZB/BINJ mice have abnormal dendritic and axonal morphologies, whereas normally positioned granule cells have normal dendritic morphologies is in marked contrast to the situation in the dreher mutant mouse (Nowakowski et al., 1987, Anat. Rec. 218:99A) in which both ectopically and normally positioned granule cells have abnormal dendritic and axonal arborization patterns. It is likely that the dendritic abnormalities of the granule cells in NZB/BINJ mice are secondary to disruptions of neuronal migration that produced their ectopic position.

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308.14 DENDRITIC AND AXONAL ARBORIZATION OF ECTOPIC PYRAMIDAL CELLS IN THE HIPPOCAMPUS OF THE DREHER MUTANT MOUSE.
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Dreher (gene symbol: dr, also known as shaker short-tail) is an autosomal recessive mutation located on chromosome 1. Among the various abnormalities typical of homozygous dreher (dr/dr) mice is the presence of numerous ectopic pyramidal cells in the stratum radiatum in area CA3 of the hippocampus (Nowakowski and Wahlsten, '85, Anat. Rec., 211: 140A). We have studied the morphology of these ectopic pyramidal cells with the Golgi method.

The most striking difference between the dendrites of ectopic pyramidal cells and those of normally positioned pyramidal cells is that the dendritic trees of the ectopic cells almost always lack a radially-oriented, apically extending dendrite. Instead, their primary dendrites exit from the soma at a large-angle and can extend in any direction. However, they invariably have at least one primary dendrite which emerges from the lateral or basal surface of the soma and which extends down through the pyramidal cell layer into the stratum oriens. Those dendrites which pass through the pyramidal cell layer usually have small dendritic excresences indicative of contact with mossy fibers; usually there are smaller and fewer excrecences than on normally positioned pyramidal calls.

The axon of normally positioned pyramidal cells typically emerges from the base of the soma and extends downward into the stratum oriens. In contrast, the axon of ectopic pyramidal cells often emerges from a primary dendrite or a lateral surface of the soma and then extends upwards into the stratum radiatum or parallel to the pyramidal cell layer. The impregnated axons were not seen to enter the alveus, but, in general, they could not be followed very far because they left the plane of section.

Thus, the ectopic pyramidal cells in the dreher hippocampus which are located in the stratum radiatum make synapses with mossy fibers. They lose, however, the radially directed, apical orienta tion of their dendrites, and the initial direction of projection of their axon is abnormal. These characteristics are markedly different from the Hld mutant mouse in which there are ectopic pyramidal cells located at the border of the stratum oriens that retain their radially directed, apical orientated dendrites. We believe these differences are the result of different types of disruptions in neuronal migration.

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308.15 HETEROZYGOUS DREHER MICE HAVE ABNORMALITIES IN THE HIPPOCAMPAL FORMATION.
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Dreher (gene symbol: dr) is an autosomal recessive mutation located on chromosome 1. Homozygous dreher (dr/dr) mice are ataxic, have a white belly spot, a short-tail and a variety of CNS abnormalities, including disruptions of cerebellar foliation and cytoarchitecture of the hippocampal formation, tectum, olfactory bulb and neocortex. Spontaneous mutations at the dreher locus have occurred in several laboratories, and at least five different alleles exist. Recently, in our dreher colony (the dr^{sst-J} allele on a B6C3Fe background) we noticed mice which were not ataxic and which had a normal length tail but which had one or more white belly spots typical of dr/dr mice. Wild-type mice (+/+) of the same genetic background do not have similar belly spots. We hypothesized that these were dr/+ mice and matings of the putative heterozygotes were set up. Of the 31 progeny produced so far, 5 clearly had the typical dr/dr phenotype, 15 had white belly spots but no other overt dr/dr traits, and 11 were wild-type. Chi-square tests were consistent with the interpretation that the non-ataxic animals with white belly spots and normal tail length were heterozygote dreher (dr/+) mice.

Histological examination of the putative heterozygotes revealed clear abnormalities in the cytoarchitecture of the hippocampus and dentate gyrus. The observed abnormalities are similar to those that occur in homozygous dreher mice, but are less severe. Most frequently, there is an increase in the number of pyramidal cells in CA3 and a marked thickening of the pyramidal cell layer. Sometimes, the additional pyramidal cells extend out into either or both, the stratum oriens and the stratum radiatum and may occur either unilaterally or bilaterally. In the dentate gyrus, there are numerous ectopic granule cells in the molecular layer above the suprapyrmidal limb.

Thus, we conclude that a new mutation at the dreher locus may have occured in our breeding colony and further experiments are in progress to test this idea. The major difference between this new allele (tentative gene symbol: dr^{Now}) and the other dreher alleles is that its effects are observable in heterozygotes. Homozygous dr^{Now}/dr^{Now} mice do not appear to be more severely affected than either homozygous dr^{sst}/dr^{sst} or dr^{sst-J}/dr^{sst-J} mice. The existence of an allele at the dreher locus that adversely affects the development of the CNS in heterozygotes may be useful in identifying the primary developmental defect in dreher mice.

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308.16 FASCICULAR ORGANIZATION OF THE RADIAL GLIAL FIBERS IN DEVELOPING MURINE NEOCORTEX; A IMMUNOCYTOCHEMICAL STUDY COMBINED WITH 3H-THYMIDINE AUTORADIOGRAPHY.
J.F. Gadisseux*, Ph. Evrard*, J.P. Misson*, V.S. Caviness, Jr. E.K.Shriver Center, Waltham, MA 02254.

Migrating neurons destined for the cerebral cortex and elsewhere in the CNS are guided in their ascent by following the surfaces of radial glial cells (RGC). The bipolar RGC which serve as migratory guides in the embryonic cerebrum are ordered into fascicles which vary in their organization at different depths in the cerebral wall (Gadisseux and Evrard, Dev. Neurosci. 7:12 1985, Levitt and Rakic, JCN. 193:815 1980). In particular, compact fascicular arrays characteristic of the trans-intermediate zone span appear to "defasciculate" within the trans-cortical span. The present analysis, based upon RGC staining with a sensitive and selective monoclonal antibody marker, RC2 (Misson et al,Soc. Neurosci Abstr. 13, 1987), provides a precise characterization of the relation of radial glial fiber fascicles (RGFF) organization to the separate strata of the developing cortex and the relation of migrating neurons to the RGFF as they ascend through the separate strata. Cohorts of migrating neurons are defined by prior in utero labeling with 3H-thymidine. Defasciculation of the transcortical fibers advances progressively in the interval E14-P0. The change in fascicle order is initiated precisely at the intermediate zone (IZ) interface with the external sagittal stratum (ESS), a fiber stratum including the thalamic projection which marks reliably the inferior margin of the future layer VI. A spatial correction is also achieved in the trans-ESS segment of the fascicles in that the glial fibers are deflected tangentially as they cross the ESS into the base of their "target" cortical region. Thus, as they ascend from the ESS into the cortical subplate (future layers V/VI), they are re-deflected to a true radial ascent across they full width of the cortex. In layers V/VI, from E14 to P0, RGFF are progressively reduced to several fibers at most; within the final segment spanning the cortical plate (CP), single fibers are fully separated at the end of the neuronal migration. The relation of migrating cells in their ascent through the successive levels of defasciculation is as one might predicted. Thus, in the IZ and ESS spans, the migrating neuron is in contact with multiple fibers. This ratio of fibers to neurons is markedly reduced in the cortical subplate. Within the CP at E17, the young neuron appears to contact only isolated glial fibers. These systematic changes in the organization of the fibers may represent cortical strata-specific differences in the affinity of the fibers for each other. Alternatively, they may reflect changes in neuron to fiber affinities which result in greater fiber dissection by the migrating neurons, as it ascends through the successive cortical strata. [Supported by NIH grant NS12005 and the Princess Marie-Christine Foundation].

308.17 PARASAGITTAL ZONATION IN THE CEREBELLAR CORTEX DEVELOPS INDEPENDENT OF AFFERENT INPUT. N. Leclerc*, C. Gravel, R. Hawkes* (SPON: M. Colonnier), Dept. Biochemistry and Lab. of Neurobiology, Laval University, Quebec, Canada G1J 1Z4.

Monoclonal antibody mabQ113 recognizes a polypeptide antigen that, in the adult cerebellum, is confined to a subset of the Purkinje cells distributed in parasagittal zones. By immunocytochemistry, peroxidase reaction product is found throughout the cell, including the dendritic tree, the soma and the axon. The mature pattern of expression of the mabQ113 antigen appears during postnatal development in three stages. Until P5, all Purkinje cells are mabQ113$^-$. In the following week mabQ113 antigen expression spreads throughout the cerebellar cortex until at P12, all Purkinje cells are mabQ113$^+$. Subsequently, immunoreactivity is suppressed in a subset of the Purkinje cells leaving behind the adult pattern of parasagittal antigenic bands. There is a close correlation between the parasagittal bands and patches detected in the adult with mabQ113 and the topography of the olivocerebellar projection. Therefore, selected lesions of the cerebellar afferent projections were made to explore the interplay of cerebellar hodology and mabQ113 expression. Lesions were made in the adult to test the role of afferent input in the maintenance of antigen expression, and in the newborn, prior to synaptic contact between afferents and Purkinje cells, to explore the role of afferent input in antigen induction.

The olivocerebellar projection was ablated bilaterally by using 3-acetylpyridine in the adult. Animals were sacrified either 3 or 30 days after treatment. In the newborn rat, the inferior olivary complex was destroyed by electrolytic lesion. Animals were sacrified 30 days post-lesion. Mossy fibers inputs were disturbed both in adult and in newborn rats by unilateral electrolytic lesion of either the spinal trigeminal nerve interpositus nucleus or the gigantocellularis reticular nucleus, or by surgical ablation of dorsal and ventral spinocerebellar tracts. Finally, the consequences of total blockage of vibrissal and hind-limb inputs by peripheral nerve sectioning were also explored in both adults and neonates. None of these precedures led to a modification in the pattern of mabQ113 epitope expression in the adult cerebellar cortex nor did they alter the developmental timetable. Taken together, these results suggest that the mabQ113$^+$/mabQ113$^-$ band display develops independently of postnatal afferent input.

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308.18 PATTERNS OF ASTROCYTIC DEVELOPMENT IN REAGGREGATE CEREBELLAR TRANSPLANTS. E. B. Ezerman. Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405

Cerebellar (CB) reaggregate transplants in the rat have been shown (Ezerman, E. and L.F. Kromer, Dev. Brain Res. 23:287, 1986) to develop a mature cytoarchitectural organization similar to that of intact CB transplants. Thus, although initial cell associations are disrupted in reaggregate transplants, the substrates for development of the mature cytoarchitectural pattern are present. Using antibodies to the intermediate filaments vimentin and glial fibrillary acidic protein (GFA), the present study examines the structure and relationships of astrocytes to other components of the transplant during development of the mature cytoarchitectural pattern.

During transplant development, changes are seen in the structure and regional patterns of the astrocytes. At one week survival, transplants show modest GFA staining including slender, long processes running in numerous directions with relation to the plane of section. No particular distribution has been noted although processes may be oriented along blood vessels and encircling cell bodies. Staining with antibody to vimentin shows a similar pattern: astrocytic processes are seen extending radially with respect to blood vessels or pial surfaces, determined by staining with antibody to laminin. By 2 weeks survival, there is increased staining, regional patterns are beginning to emerge, and there are numerous processes along or abutting blood vessels or pia. Often large cell somas are outlined, a long astrocytic process extending away from the encircled cell body. Central regions of the transplant appear relatively devoid of astrocytes in comparison with cortical areas. Stellate astrocytes often appear in the central areas; the cortical areas have a preponderance of astrocytes with longer processes often oriented perpendicular to blood vessels. Staining for GFA in 6 week survival transplants confirms that Bergmann (B.) glia orient themselves in reaggregate transplants as in intact transplants (and in vivo). Areas can be seen in which parallel arrays of B. glial fibers traverse the molecular layer from the Purkinje cell layer to a blood vessel or pial membrane. The regional pattern is better defined in the 6 week transplant: in deep nuclear regions, small, stellate GFA-positive astrocytes; in cortical regions either the typical B. glial arrangement or, near the edge of the transplant, numerous "reactive" astrocytes, dense with thick and irregular processes. These features also are seen in mature intact transplants, as described previously (Bjorklund, H. et. al., Exp. Brain Res. 55:7, 1984). Clearly, inductive and/or environmental cues for B. glial fiber orientation are present in reaggregate transplants. Thus, the same scaffolding is present for migration of granule cells as in vivo and in intact transplants. More detailed relationships among astrocytes, neurons, and blood vessels will be presented.

308.19 THE INFLUENCE OF CELL INTERACTIONS ON THE DEVELOPMENT OF THE DENDRITIC ARBORS OF HIPPOCAMPAL NEURONS IN CULTURE. A.B. Waxman and G.A. Banker. Department of Anatomy, Albany Medical College, Albany, NY 12208

We have analyzed the influence of cell interactions on dendritic development by preparing cultures of hippocampal neurons at different plating densities, ranging from 1,000 to 32,000 cells/cm^2. At the higher densities the density of axonal processes, and therefore the opportunity for contacts between axons and developing dendrites, was greatly increased. After development for 7 or 24 days in culture dendrites were selectively stained using an antibody against Microtubule Associated Protein 2. Dendritic trees were reconstructed using a computer-based cell analysis system and the length and diameter of each segment was determined.

At higher plating densities there was a 2.5 fold increase in the frequency of dendritic branching, but no change in the area occupied by the dendritic tree. As a result the average interval between branch points decreased by about 50% and the total dendritic length increased by about 50%. The changes in frequency of branching and segment length were already apparent after 7 days in culture.

Many parameters of dendritic diameter did not change during development between 7 and 24 days, and were not influenced by cell density. These included stem diameter, terminal diameter, and the branch power relationship, which consistently followed the 3/2's power rule. At each plating density there was a linear relationship between stem diameter and the extent of branching; as plating density increased, the slope of this line approached that seen in situ. Intrasegment taper, which was particularly prominent in terminal segments, decreased at higher plating densities.

These results suggest that stem diameter can be thought of as setting an upper limit on the extent of dendritic branching. The decrease in diameter between stem and terminal segments can occur either at branch points or by taper within terminal segments. As the opportunity for cell interactions increases there is more branching and consequently less taper.

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308.20 REGIONAL EFFECTS OF OPIOID RECEPTOR BLOCKADE ON CORTICAL THICKNESS. J. Reyes*, D. Lewis* and M.C. Diamond (SPON: L. Stark). Department of Physiology-Anatomy, University of California, Berkeley CA 94720.

Investigators have demonstrated that some regions of the brain increase in size and cellular content when opioid receptors are blocked (Zagon, I.S. Life Sciences., 35:2057, 1984). However, the effects of opioid receptor blockade on the frontal and occipital cortex have not been investigated. This study examines the relationship between opioid receptor blockade, cortical thickness, and laterality in 9 regions of the rat cortex. Male and female Long-Evans rats were injected subcutaneously with either saline, 1, 10, or 50mg/kg of Naltrexone, an opioid receptor antagonist, for the first 21 days of postnatal life. On day 21, animals were sacrificed and morphometric analysis of 2 frontal, 3 somatosensory and 4 occipital cortical regions were made on transverse, frozen, 40 mu sections stained with a modified Thionin stain. Results indicated significant dose effects in the frontal and occipital cortical regions. The 50mg/kg group showed increases in cortical thickness, while 1mg/kg animals decreased in cortical thickness in comparison with saline injected controls. The frontal and occipital association cortices were most affected. In somatosensory cortex, no significant effects were found with any dose, thus, not offering support to the findings of previous investigators. Lateralization of the frontal, somatosensory and occipital cortices were affected in a dose and sex dependent manner. As the concentration of Naltrexone increased, male lateralization shifted from right to left; in female animals, the opposite occurred. In conclusion, our results support previous findings that opioid receptor blockade increases growth in neural tissue. However, we do not confirm previous work indicating a dose dependent growth in the somatosensory cortex. In addition, our results reveal a reversal in cortical lateralization in both male and female rats as a consequence of receptor blockade.

309.1 GANGLIOSIDES STIMULATE THE BREAKDOWN OF POLYPHOSPHOINOSITIDES IN CNS NEURONS IN VITRO. S.D. Skaper, M. Favaron*, L. Facci* and A. Leon. Fidia Neurobiological Research Laboratories, Abano Terme, Italy.

GM1 ganglioside has been shown to ameliorate the deficits following injury to the adult mammalian CNS. In addition, cultured neurons, both from the CNS and PNS respond to gangliosides with morphological changes characteristic of cell differentiation, the latter in the presence of defined neuronotrophic proteins. An understanding of ganglioside effects on neuronal performances will first require elucidation of the underlying molecular mechanism(s) operating in vitro.

Hydrolysis of membrane polyphosphoinositides is now a widely accepted transmembrane signalling pathway for a variety of external stimuli. These extracellular signals rapidly induce the breakdown of phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol. Very strong evidence supports the view that these two inositol lipid intermediates serve as second messengers, one by inducing a transient rise in $(Ca^{2+})_i$, the other by activating protein kinase C.

With this in mind, the following experiments were carried out. Neuronal cultures from embryonic avian and rodent CNS were prelabeled with ^{3}H-inositol for 24 hr, presented with ganglioside and the formation of inositol phosphates evaluated in the presence of Li^+. The different inositol phosphates were separated using ion exchange chromatography. GM1 elicited, in a dose-dependent manner a several-fold increase in IP_3 in neurons from E8 chicken cortex, as well as in inositol 1,4-bisphosphate (IP_2). Similar results were obtained using neurons from E8 chicken neural retina, E18 rat hippocampus, and E14 mouse mesencephalon. Elevations of IP_1 were also detected, but depended upon the tissue and species used. AsialoGM1 and oligosaccharideGM1 were without effect. Thus, gangliosides appear able to activate polyphosphoinositide breakdown. These results provide the first evidence for a direct action of gangliosides in transmembrane signalling in neurons. A possible relationship between this effect of gangliosides and neuronotrophic factor action is currently being explored.

309.2 MORPHOLOGICAL AND IMMUNOCYTOCHEMICAL CHARACTERIZATION OF HUMAN FETAL BRAIN CULTURES. F. Gremo, S. Torelli*, V. Sogos*, A. Riva *, C. Marcello * and U. Lecca* Dept. of Cytomorphology and Inst.of Clinical Obstetrics and Gynecology, School of Medicine, 09100 Cagliari, (Italy)

Tissue cultures provide a very useful tool to study the effects of endogenous and exogenous substances on neuronal differentiation and maturation. Primary cultures from brain contain different types of cells and cell precursors which need to be characterized.

Human brains, obtained from either spontaneous or medically induced abortions, were freshly dissected, when possible divided into regions and incubated with sterile trypsin. Cells were mechanically dissociated, diluted with medium (DMEM) + 10% fetal calf serum and plated on polylysine-treated dishes. At different intervals, cells were fixed and processed for Scanning Electron Microscopy (SEM) and for immunocytochemistry. Monoclonal antibodies against acetylcholine-esterase (AChE), glial fibrillar acidic protein (GFA-P), vimentin (VT), neurofilament subunits (NF68K, NF160K, NF200K), proliferating cells (PC) and peanut lectin (a-PNA) were used. Also fluorescein-conjugated peanut lectin (PNA) was used.

Results showed that cultures were enriched in neurones, which survived in vitro for several weeks. Cell passages produced a decrease in neuron content and an increase in non-neuronal cells. Microglia-like cells were also observed. Double staining showed the presence of both VT and NF in immature neurones. Non neuronal cells were positive for both VT and GFA-P in early cultures. PNA stained non-neuronal cells, but never well differentiated neurones. Among the PNA-positive cells, some were also GFA-P positive. Interestingly, GFA-P positive cells were negative for a-PNA. On the contrary many cells positive for a-PNA were also NF200 positive. a-PNA staining was both intracytoplasmatic and on the surface of the cells. These results suggest that during development neuronal and non-neuronal migration might be regulated by the production of lectin-like proteins and lectin-receptors from different cells. PC never stained well differentiated neurons, but only flat cells on the bottom of neuronal aggregates. Whether PC-positive cells are non-neuronal or neuronal precursors is under investigation.

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309.3 MORPHOMETRIC ANALYSIS OF PYRAMIDAL CELLS IN ADULT AND IMMATURE HAMSTERS FOLLOWING PYRAMIDOTOMY. T.E. Durica. Department of Anatomy, Rush Medical College, Chicago, IL 60612.

Pyramidotomy in the adult and immature (15 day old) hamster results in a differential morphological response of the pyramidal cells that is age dependent. A morphometric analysis of normal and injured pyramidal cells was performed to determine if the cytological changes are accompanied by changes in neuronal size.

Unilateral pyramidal tract lesions were made in the medulla, rostral to the pyramidal decussation, in adult and 15 day old hamsters. Animals were cardiac perfused with a formal saline-gum acacia solution at 4 or 8 days postoperative (dpo). The cerebral cortices were serially sectioned, every 10th section collected, mounted and stained with buffered Thionin. Point counting was used to measure the total cell, cytoplasmic, and nuclear areas.

In the adult, at 4 and 8 dpo, most of the pyramidal cells displayed a slight paling of their Nissl substance without any noticeable changes in other cellular components. Also, there were a few neurons that were rounded and pale with a mottled nucleolus. In the 15 day old, at 4 and 8 dpo, the pyramidal cells displayed a focal chromatolysis; at 8 dpo there were a few rounded and pale neurons with a mottled nucleolus as in the adult.

Measurements of injured neruons included both types of reacting cells. Normal adult pyramidal cell measurements, in square microns, for total cell, cytoplasmic, and nuclear areas were 194.85, 115.64, 79.21 respectively. After pyramidotomy there was a slight decrease in total cell and cytoplasmic areas at 4 dpo but measurements at 8 dpo were not significantly different from normal. Normal 15 day old pyramidal cells measurements were 192.48, 111.48, 80.99, which are the same size as in the adult. There were no changes in the area of any of these components at either postoperative times.

The pyramidal cells of the 15 day old hamster have characteristics of both immature and mature neurons. This may account for a morphological response that is typical of an immature neuron and the lack of change in the size of the measured areas which is similar to the adult.

309.4 ELECTROPHYSIOLOGICAL PROPERTIES OF CEREBELLAR PURKINJE CELLS AFTER DISSOCIATION FROM LATE EMBRYONIC AND EARLY POSTNATAL RATS. P.E.Hockberger, H.Y.Tseng*, and J.A.Connor, Dept. of Molecular Biophysics, AT&T Bell Laboratories, Murray Hill, NJ 07974

Cerebellar Purkinje cells (PCs) display several stages of morphological development during late embryonic and early postnatal periods. These stages correspond to a progressive differentiation from round neuroblasts to mature neurons with extensive dendritic arborization (J.Altman, *J. Comp. Neurol.* 165:31,1976). Electrophysiological correlates of this differentiation process have been restricted to extracellular recordings. A more cellular analysis is difficult *in vivo* because developing PCs are small and inaccessible with conventional intracellular recording techniques. We have chosen therefore to examine the electrophysiological properties of these cells at various stages of development following tissue dissociation. This approach yields isolated cells some of which can be identified and examined immediately after dissociation (<1 hr.), while offering the prospect of long-term studies of development *in vitro* (>30 days).

Whole-cell patch recording (intracellular and voltage clamping) from isolated PCs was performed immediately (0.5-3 hrs.) following tissue dissociation between embryonic day 20 (E20) and postnatal day 9 (P9). PCs were identified by size, morphology, and immunocytochemical staining patterns. Between E20-E22 isolated PCs were 10μm spheres which stained moderately for neuron-specific enolase (NSE; 1:1000 dilution)) and G-kinase (GK; 1:2000). These cells were inexcitable and showed no membrane potential response to iontophoretically-applied glutamate. Under voltage clamp most of these cells exhibited small voltage-dependent conductances. Between P1 and P4 isolated Purkinje cells were 12μm in diameter with a prominent apical dendrite, and they stained moderately for NSE, GK, and Thy-1 (1:40). By this stage most of the Purkinje cells were excitable, depolarized by glutamate, and displayed several large voltage-dependent conductances. By P9 PCs were 15μm in diameter and stained more intensely for NSE and GK than at earlier stages, and these cells were also excitable. We found no evidence for spontaneous activity in isolated PCs at any of these stages.

Cultures were prepared from dissociated cerebella using methods described elsewhere (Ahmed et al., *J. Neurosci.* 3:2248, 1983) for studies of PC development *in vitro*. Purkinje cell survival was significantly better in embryonic cultures compared with those prepared from postnatal cerebella (by P9 PCs did not survive for more than a few hrs.). Embryonic PCs cultured for several weeks had larger somata (up to 25μm in diameter) and thicker apical dendrites than cultures of postnatal cells.. The most impressive dendritic arbors developed on PCs maintained in normal potassium growth media. However the development of excitability required growth media containing high potassium (25mM). Supplementing the growth media with glutamate (0.5 or 5μM) or aspartate (0.5 or 5μM) did not mimic the effects of high potassium in either embryonic or postnatal cultures. In fact these treatments were toxic to all nerve cells which rarely survived under these conditions for more than 2-3 weeks. on the other hand PCs from embryonic cultures grown in high potassium media survived as long as cultures were maintained (40-50 days). They developed electrical excitability after 1-3 weeks, displayed several large voltage-dependent conductances, and were depolarized by glutamate, kainate, NMDA, or quisqualate.

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309.5 **ONTOGENY OF SUBSTANCE P- AND SEROTONIN-CONTAINING NEURONS IN THE MOUSE.** Ni Li* and G. Miller Jonakait, (Spon: Joan Morrell) Dept. of Biological Sciences, Rutgers University, Newark, New Jersey 07102

Substance P (SP) is located in serotonin (5-HT)-containing neurons of the rodent medulla (Johansson *et al.* 1981). In order to investigate possible pharmacological interactions between these transmitters during mouse embryonic development, we have initiated an immunohistochemical study to compare the developmental profile of serotonin and its co-existing peptide partners.

Adjacent 15 um sections from mice of gestational day 11 (E11) through postnatal day 3 (P3) were prepared for detection of 5-HT (Newton, B.W., B.E. Maley & R.W. Hamill [1986] *Brain Res.* 376, 155-163) and SP (Kessler et al. [1984] *Devel. Biol.* 103, 71-79) using the peroxidase-antiperoxidase method with 4-Cl-naphthol as chromagen. To determine the percentage of co-localized cells, the 5-HT-antibody complex was removed and tissue was reacted for a second time with antibody to SP (Tramu *et al.* 1978).

SP and 5-HT initially appear on E12. 5-HT cells and fibers are found in the midline just caudal to the mesencephalic flexure as well as in bilateral columns on either side of the medullary midline. Early SP-positive cells are clustered at the pontine flexure and in a continuous bilateral column of cells extending from rostral levels of rhombencephalon throughout the entire length of the neural tube. These small, round SP-positive cells have neither dendrites nor fibers and do not contain 5-HT. Preadsorbed controls show no SP immunoreactivity.

Over the next 4 gestational days, SP-positive cells appear in areas of both diencephalon and telencephalon including the pyriform cortex, bed nuc. of the stria terminalis, striatum, amygdala, and in more caudal regions including the nuc. parabrachialis colliculi, central gray, n. tractus spinalis trigemini and vestibular nuclei. Immature cells seen in the medulla and neural tube at E12 are no longer evident by E14, but SP-positive cells are, at these later stages, included in the medullary raphe nuclei and in the neural tube lateral to the central canal. During this period, 5-HT-positive cells increase their number and attain a mature distribution into recognizeable B1-B9 subgroups.

SP and 5-HT are co-localized in cells of the medullary raphe nuclei (and only there) as early as E13. The percentage of 5-HT cells containing both neurotransmitters attains its highest value at E15 (34.9±.29%; n=3). Thereafter, the intensity of SP staining in the medulla diminishes. Concomitantly, the percentage of 5-HT cells with detectable SP falls to about 10% by postnatal day 2. (Supported by NS 23687. GMJ is a Johnson & Johnson Discovery Research Fellow.)

309.6 PRENATAL ONTOGENY OF THE CHOLINERGIC SYSTEM IN THE MOUSE FOREBRAIN: AN IMMUNOCYTOCHEMICAL STUDY. U.B. Schambra*, K.K. Sulik*, P. Petrusz*, and J.M. Lauder. (Spon: D.L. McIlwain). University of North Carolina, Chapel Hill, North Carolina.

Prenatal development of cholinergic neurons in the forebrains of C57Bl/6J mice has been studied with a monoclonal antibody to choline acetyltransferase (anti-ChAT, Boehringer) from gestational day 14 (GD14) to 18. Embryos or fetuses were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, sectioned at 80 μm using the Vibratome, and immunostained using the ABC peroxidase method. At GD14, immunoreactive (IR) neurons arising in the ventricular zone (VZ) of the olfactory ventricle were seen to form the olfactory tubercle and olfactory nuclei, while IR neurons developing in the anterodorsal VZ of the lateral ventricle (LV) migrated anteriorly to form part of the frontal cortex. More caudally, IR neurons for the pre-optic nuclei and the nucleus basalis magnocellularis developed in the VZ of the floor of the third ventricle, while other IR neurons were formed in the roof. The epiphysis stained intensely at this time. IR neurons were also formed in the dorsomedial VZ of the LV from which the hippocampus arises, and were later seen to mature within the hippocampus proper (GD17). At GD15 most of the neurons designated for the nucleus of the diagonal band of Broca, the nucleus accumbens and the septal nuclei developed from the mediventral VZ of the LV. The VZ of the ganglionic eminence produced IR neurons which migrated into the interior to form cholinergic neurons of the striatum. IR neurons designated for the cingulate cortex differentiated last with a peak of development at GD16. In the VZ of the LV and third ventricle distinct clusters of IR neurons were observed, some of which were located next to the lumen. Based on their position, some of these cells could be mitotic suggesting that ChAT may be present in dividing cells in certain regions of the developing brain. IR cells were also observed in the meninges and in regions where structures such as the frontal cortices and olfactory bulbs adjoined in the midline.
(Supported by NCARA grant #8601).

309.7 CARBOHYDRATES AND HISTOGENESIS IN CEREBELLAR DEVELOPMENT IN THE ABNORMAL (REELER) AND NORMAL MOUSE. G.P. Sinson*, M. Breen*, R.G. Higbee, W. Goossens*, D.G. McLone and P.A. Knepper. Division of Neurosurgery, The Children's Memorial Hospital and Northwestern University Medical School, Chicago, IL 60614; and V.A. Medical Center, North Chicago, IL 60064.

Neurohistogenesis requires the timely and orderly migration of cells away from the germinative zone toward often distant sites. Cell-surface and extracellular carbohydrates have been implicated in certain cell contact relationships, including migration and differentiation. To define the carbohydrates that accompany cerebellar differentiation: 1) serial plastic sections (1 um) of prenatal (E-14, 16, and 18) and postnatal (P-1, 4, 6, 8, 10, 14, and 28) day Reeler (a mouse mutant with cerebellar ataxia) and age-matched normal mice (C57BL/6J) were stained with fluorescent-labeled lectins; and 2) the results were correlated with polyacrylamide gel electrophoresis under reduced conditions and 3) Western blots using biotinylated Concanavalin A. The staining patterns of five lectins (Con A, recognizes mannose and glucose; WGA, N-acetylglucosamine and sialic acid; RCA, galactose; Ulex, fucose; and LPA, sialic acid) were determined by low-light intensity video image analysis and computer-automated densitometry.

The normal cerebellum was characterized by time-dependent expression of Con A-, RCA-, and WGA-positive material. The external granular cells were moderately stained by Con A, RCA, and WGA prior to migration (P-1, P-4, and P-6) and during migration (P-8 and P-10) through the Purkinje layer of cells. The molecular layer (i.e., parallel fibers and Purkinje dendritic projections) exhibited intense focal areas of transient FITC staining: WGA, days P-8 and P-10, and Con A, days P-10 and P-14. The normal Purkinje cells displayed RCA granular cytoplasmic staining on day E-18 and all subsequent time points, whereas the Reeler Purkinje cells were delayed by 7 days, and the granular cells were only moderately stained. Western blots of Con A-binding proteins of normal cerebellum showed: a) a time-dependent increase in two proteins (44 and 46 Kd) from P-8, which increased with age; and b) a transient increase in a 66-Kd protein in the time frame of P-8 to P-14. Studies are in progress to further characterize these differences by additional lectins.

These results indicate: 1) time-dependent points occur in the appearance of carbohydrates during cerebellar histogenesis; 2) the different patterns of lectin staining suggest the presence of a transient glycoprotein during granular cell migration and interction with the Purkinje cells; and 3) modifications of carbohyrates in the Reeler may relate to abnormal differentiation, cell positioning and/or maturation of parallel fibers.

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309.8 EXPRESSION OF NEURON-SPECIFIC ENOLASE IN THE RAT SENSORIMOTOR CORTEX: A MARKER FOR DIFFERENTIATION? K.M. Hamre, M.D. Cassell* and J.R. West. Dept. of Anatomy, Univ. of Iowa, College of Medicine, Iowa City, IA 52242.

During development, the expression of the enolase enzyme changes from the non-neuronal isoform to the neuron-specific isoform. It has been suggested that the timing of this change is correlated with the arrival of afferent inputs and/or cellular differentiation. This correlation has not been demonstrated in rat sensorimotor cortex where the cell layers and afferent inputs are arranged in the adult pattern by postnatal day 7. The purpose of this study was to examine the time course of laminar differentiation in the sensorimotor cortex of the rat, as measured by staining with an antibody against neuron-specific enolase (NSE). Male rats from timed pregnancies were perfusion-fixed on postnatal days 1 (gestational day 23), 3, 5, 8, 10, 12, 15 and 20. Frozen sections (10 μ) were stained with an anti-NSE antibody (Polysciences, Inc.) in a dilution of 1:1000 using the avidin-biotin immunocytochemical method. On day 1, the sensorimotor cortex consists of no adult pattern cell layers, but of a molecular layer and cortical plate. There is considerable staining in the upper, more densely packed area of the cortical plate. By day 3, layer V is definable and well stained, but there is only sparse staining in the cortical plate above it. Layer VI also exhibits staining with a conspicuous band of stained cells in its deepest layer, just superficial to the corpus callosum. On day 5, layer V is intensely stained and scattered cells in layers II and III are also stained. There is an increase in the number of scattered cells stained in layer VI. On day 8, the pattern of staining is similar except that more cells are stained in layers II and III. On day 10, layers II and III are intensely labelled, however the staining in layer VI is diminished and the deep band of stained cells has disappeared. The pattern is continued on days 12, 15 and 20 with an increasing number of scattered cells in layer VI being labelled on successive days. The adult pattern of staining shows labelling in layers II, III and V and in scattered cells in layer VI -- the majority of neurons in layer IV do not stain. The laminar expression of NSE in the sensorimotor cortex loosely follows the "inside-out" developmental age gradient but appears poorly correlated with arrival of afferents. Moreover, in layer VI, there is a decrease in the number of NSE positive cells from days 8 to 12. These observations, and the finding that cells in layer IV seldom express NSE, question the validity of NSE as a definitive marker for differentiation in the sensorimotor cortex. (Supported by NIAAA grant AA05523 to J.R.W.).

309.9 PROTOONCOGENE EXPRESSION IN EMBRYONIC MAMMALIAN FOREBRAIN: COLUMNAR ORGANIZATION OF THE LATE EMBRYONIC VENTRICULAR ZONE
J.Johnston* and D. van der Kooy (spon:J.A.Connolly) Department of Anatomy, Univeristy of Toronto, Toronto, Canada, M5S 1A8

Oncogenes are homologues of normal cellular genes called protooncogenes. Protooncogenes may help regulate the normal development of embryonic tissues. We have used monoclonal antibodies to four different oncogenic proteins to investigate the distribution of their corresponding protooncogene products proteins during the late embryonic development of the rat forebrain. Rat brain sections obtained at 18 days of gestation were first incubated in mouse monoclonal antibodies directed against synthetic MYC, RAS, SIS and SRC peptides, and then incubated in fluorescent secondary antibodies. The predominant staining pattern in coronal sections was one of interdigitating labeled and unlabeled columns in the ventricular zone, with the MYC, RAS and SRC antibodies. The labeling appeared to be in groups of cells (3-15 cells) which had cell bodies near the edge of the ventricle and long processes that extended through the ventricular zone and sometimes out into the region containing postmitotic cells. Morphologically, the stained elements appeared to be columns of radial glial cells interdigitating with unstained columns. This supposition was supported by the dense staining of the same oncogene antibody labeled columns in the ventricular zone at embryonic day 18 with a rabbit polyclonal antiserum against vimentin, an intermediate filament protein that is expressed in radial glial cells during late embryogenesis. The columns unlabeled by the oncogene sera showed relatively less vimentin staining. The staining of ventricular zone columns did not appear to be an artifact of fixation or cutting. Antibodies against Dl.1, a marker of proliferating ventricular zone cells (provided by J. Levine, Stony Brook), stained uniformly through the protions of the ventricular zone where columnar staining was seen with the other markers. Adsorption controls with the appropriate synthetic peptides abolished the ventricular zone columnar staining seen with the MYC, SRC and RAS antibodies. These results suggest that cellular protooncogenes may play a more important role in the early differentiation, rather than in the internal regulation of proliferation, of embryonic brain cells. The oncogene staining of ventricular zone columns at embryonic day 18 may be marking groups of more metabolically active radial glial cells in the ventricular zone. This is suggested by the denser vimentin staining of similar columns and by preliminary results showing a slightly higher rate of neuronal proliferation (as judged by 3H - thymidine labeling) in the region of these oncogene stained columns. Perhaps the activated radial glial cells are regulating the proliferation and/or migration of columns of ventricular zone neuroblasts.

309.10 SEPARATE BLOOD AND BRAIN ORIGINS FOR PROLIFERATING CELLS FOUND AT KAINIC ACID LESION SITES IN THE MOUSE STRIATUM. C. Morshead*, M. Bradford* and D. van der Kooy. (SPON: P.A. Stewart). Department of Anatomy, University of Toronto, Toronto, Canada, M5S 1A8.

Gliosis, the accumulation of small darkly stained cells at a site of damage is one of the responses of the brain to injury. We attempted to characterize the properties and origin(s) of the gliotic response in an adult mouse model using kainic acid (KA) to induce a local striatal lesion and tritiated thymidine (3H-Thy) to follow the proliferating cells. Animals were pulse labeled with 3H-Thy 4 hours before sacrifice. The proliferative response was limited to the ipsilateral forebrain and could be divided into two phases depending on time post-lesion and distribution of 3H-Thy labeled cells. First, an early proliferative response 3 days post-lesion was seen in the caudate surrounding the injection site and to a lesser extent in the cortex. Second, at day 6 post-lesion the 3H-Thy labeling of cortical cells disappears, the number of labeled cells in the caudate is similar to that seen at day 3 post-lesion, and most dramatically there is a large increase in the number of labeled cells in the immediate vicinity of the striatal injection site. To trace the arrival of the labeled cells found in the immediate vicinity of the injection site at day 6 we pulse labeled mice on day 3 after KA injection and sacrificed them on day 4, 5, or 6. Over days 4 to 6 post-lesion the number of labeled cells in the caudate remained unchanged, the number in the cortex decreased dramatically, the number of labeled cells in the immediate vicinity of the injection site continued to increase despite the fact the 3H-Thy was administered only once at day 3. We hypothesized that the source of the labeled cells appearing in the immediate vinicity of the injection site is exogenous to the brain. It seems unlikely that migration of cells within the brain can explain this phenomenon as the cells in the immediate vicinity of the injection site continue to increase after a single pulse labeling while the population of labeled cells in the caudate does not decrease. In order to test whether a peripheral (haemopoetic) source was the origin of the late population of 3H-Thy labeled cells at the injection site, mice were irradiated (900rad) to kill the proliferating bone marrow precursors. Three days after irradiation KA was injected into the striatum. The mice were sacrificed 6 days after the KA injection and injected with 3H-Thy 4 hours before sacrifice. Preliminary results indicate that after irradiation , the population of 3H-Thy labeled cells found in the immediate vicinity of the injection site is significantly reduced compared to the number of labeled cells found at the injection site of non-irradiated mice. Furthermore, the number of labeled cells seen in the striatum farther away from the injection site is not affected by the irradiation treatment.

These results suggest that two populations of cells make up the proliferative gliotic response at the site of injury in the brain. The large population of late appearing cells found in the immediate vicinity of the site of injury are of heamopoetic origin while further from the injection site, in the striatum and cortex, there appears to be an early proliferative response of a population of uncharacterized cells that is endogenous to the brain at the time of lesion.

309.11 GENETIC MAPPING AND MOLECULAR CHARACTERIZATION OF THE MOUSE GENE ENCODING THE ALZHEIMER'S DISEASE-RELATED CEREBROVASCULAR AND NEURITIC PLAQUE AMYLOID PEPTIDE. R. Reeves*, R. Morgan, M.L. Oster-Granite, J.T. Coyle, J.D. Gearhart* and N. Robakis*. Depts. of Neurosci. and Physiol., Johns Hopkins Univ. Sch. of Med., Balto. MD 21205 and NYS Inst. Basic Res., Staten Island, N.Y. 10314.

Individuals with trisomy 21 (Down Syndrome, DS) who survive past the third decade invariably develop the neuropathologic stigmata of Alzheimer's Disease (AD), including deposition of cerebrovascular and neuritic plaque amyloid peptide (CVAP). The gene encoding a precursor molecule, including the CVAP, has been molecularly cloned and mapped in humans to chromosome 21 (HSA 21), band q21. This larger protein is expressed throughout development in many tissues of normal individuals. To understand the normal function of this protein and the relationship of its overexpression to the pathogenesis of AD in DS individuals, we have undertaken a molecular and genetic analysis of this gene and its product in the mouse. Genes linked to the "DS region" of HSA 21 are also found linked on mouse chromosome 16 (MMU 16). Trisomy 16 (Ts16) mice develop many stigmata resembling those seen in DS, and thus serve as a genetic model system for studies relevant to DS.

Using a human cDNA as a molecular probe, we mapped the mouse *Cvap* gene to the portion of MMU 16 distal to the T(2H3-4;16C3)28H breakpoint. This region of MMU 16 also contains two HSA 21 genes encoding the purine synthesis enzyme phosphoribosylglycinamide synthetase (*Prgs*) and the protooncogene *Ets-2*. A third HSA 21 gene, *superoxide dismutase-1* (*Sod-1*), is present in the region just proximal to the T28H breakpoint. Using a backcross [(Czech II x BALB/c) x Czech II] for genetic localization, we found that *Cvap*, *Ets-2*, and *Sod-1* are linked tightly within a region of 3.2 cM near the distal end of MMU 16.

At autopsy, normal aging human brains occasionally display neuritic plaques, while the brains of aged mice do not. To determine whether sequence variations in the protein may contribute to a difference in abnormal processing, we have cloned a mouse cDNA which contains 1.4 kb of information, including the entire region which, in humans, encodes the 42 amino acid (aa) residue A4 amyloid, characteristic of the neuritic plaques. The predicted aa sequence is similar overall, but includes variations near the 5' boundary of the plaque amyloid peptide.

In Northern blots of brain using this probe, we can demonstrate the presence of two forms of mRNA, a predominant 3.5 kb message and a 5.0 kb message expressed at lower levels. With *in situ* hybridization using this mouse cDNA probe we can demonstrate elevated expression of *Cvap* in the brains of fetal trisomy 16 mice (See abstract by Bendotti *et al.*).

Thus, we have determined that the gene encoding CVAP is located on the distal tip of MMU 16, linked tightly to other genes located in the putative "DS region" of HSA 21. Using a mouse cDNA probe for CVAP, we have shown that the gene is expressed in normal adult mice and is overexpressed in fetal Ts16 mice. Whether aging Ts16 ↔ normal mouse chimeras develop plaques containing CVAP remains to be determined; normal aging mice do not. *Supported by Grants PO1 HD 19920, RO1 HD 19932, and the McKnight Foundation.*

309.12 **ANALYSIS OF CELL BIRTHDATES AND CELL CYCLE DURATION IN TRISOMY 16 FETAL MOUSE BRAIN.** **M.L. Oster-Granite, J.T. Coyle, S. Fisher*, C.F. Hohmann, T. Huang-Chong*, J.E. Sweeney, and R. Wetts. (SPON: E. Lotter). Depts. of Neuroscience and Environ. Health, The Johns Hopkins University Schools of Medicine and Public Health, Baltimore, MD 21205 and Develop. Biol. Center, UC Irvine, Irvine, CA 92717.**

Down Syndrome (DS) usually results from triplication of human chromosome 21 (HSA 21). Of the five genes localized to the distal portion of HSA 21, all have been located on the distal quarter of mouse chromosome 16 (MMU 16) (see abstracts by Reeves *et al.*; Bendotti *et al.*), thus making murine trisomy 16 a good candidate for an animal model of DS. In addition to genetic homology, Ts16 mice exhibit a number of neurochemical, neuropathologic and neuroanatomic features found in DS individuals. Reduction in the number of cholinergic neurons has been noted in DS patients; therefore, we examined cells in regions of high and low cholinergic activity in Ts16 mice, namely the nucleus basalis magnocellularis (nBM) and cerebellum, respectively. We examined whether the hypocellularity observed in these regions was the result of 1) decreased proliferation during neurogenesis, 2) altered cell birthdates, and/or 3) premature cell death.

Pregnant mice were injected with single or multiple doses of [^{3}H]thymidine on days 9-15 of gestation (E9-15). For cell cycle studies, mice were killed at hourly intervals on days 10 - 16 gestation. For cell birthdating experiments, mice were killed on E16. Liver tissue (for karyotype analysis) was taken from each fetus prior to transcardial perfusion with 4% paraformaldehyde in 0.1M phosphate buffer. The brains were divided at the mesencephalon and processed for frozen sections (anterior portion) or paraffin sections (posterior portion).

We analyzed birthdates and cell cycle duration of neurons of the rhomencephalon, especially in the cerebellum, from E10-15 on 5-8 μm paraffin sections counterstained with toluidine blue following development of the autoradiograph. In 50 μm frozen sections stained for acetylcholinesterase (AChE), we determined cell birthdates of nBM neurons from E11-15.

As early as E9, the rhombencephalon contained 30-40% fewer cells in Ts16 mice. When we analyzed the duration of the cell cycle in the rhombencephalon, we found no change in the Ts16 mice versus normal littermates sufficient to account for the disparity in cell numbers that already existed at E10.

Cell birthdates were similar to those of normal littermate controls for both the cerebellar Purkinje cells and the AChE-stained neurons of nBM, presumptive cholinergic neurons. Most neurons in these regions were generated on E11-13, but continued through E15. Qualitatively, fewer cells were generated, although in both regions the proportions of mitotic to non-mitotic cells were similar.

In conclusion, these results indicate that the reduction in cell number observed in the rhombencephalon and nBM region of Ts16 mice is not due to alterations in the duration of the cell cycle or to alterations in the birthdates of principal neurons. The events that result in reduced cell number apparently occur before significant neurogenesis in these structures has taken place in Ts16 mice.

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309.13 NEUROCHEMICAL CHARACTERIZATION OF EMBRYONIC BRAIN DEVELOPMENT IN MURINE TRISOMY 19 (TS19): IMPLICATIONS CONCERNING THE SPECIFIC CONTRIBUTION OF ANEUPLOIDY IN NEURAL DEVELOPMENT. M.D. Saltarelli, G.L. Forloni, M.L. Oster-Granite, J.D. Gearhart*, and J.T. Coyle. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Autosomal aneuploidy is invariably asssociated with mental retardation in human infants who survive birth. Unfortunately, little is known about the neurochemical and neuroanatomical substrates underlying these profound behavioral alterations. While spontaneous chromosomal aneuploidy is rare in mice, one can generate trisomy for each autosome with high frequency through the use of selective breeding schemes involving mice with Robertsonian translocations. In our previous studies of trisomy 16 (Ts16) mice, we observed neuronal hypoplasia and a specific reduction of choline acetyltransferase (ChAT) activity in the developing basal forebrain. Coupled with demonstrated genetic synteny between human chromosome 21 (HSA 21) and mouse chromosome 16 (MMU 16), these studies support the Ts16 mouse as a model system for studies of brain development in human trisomy 21 (Down Syndrome; DS). However, the specfic contribution of qualitative effects of trisomy of specific genes on MMU 16 to abnormal neurogenesis has not been separated from the more quantitative effects of generalized autosomal aneuploidy itself. We have therefore characterized the neurochemical development of murine trisomy 19 in an attempt to clarify the qualitative versus quantitative effects of aneuploidy in the mouse.

Male mice doubly heterozygous for Robertsonian translocations Rb(5.19)1Wh and Rb(9.19)163H were mated to normal C57BL/6J female mice. The Ts19 conceptuses and their littermates were collected by caesarian section on days 14 to 18 gestation (plug date = day 0), and distinguished phenotypically by their developmental growth retardation and cerebral petecchiae. Their brains were removed rapidly, dissected, and frozen at -70°C until karyotypic confirmation.

We observed reductions in the total protein and wet weights of both the diencephalon/brainstem and cerebral hemisphere sections in Ts19 mice. While we observed no significant alteration in ChAT activity in the diencephalon/brainstem, we did observe a significant reduction in hemispheric ChAT activity compared to controls, progressing from 1.7 ± 0.2 nmol/mg protein/hr (X ± S.E.M., N=6, p<.01, 14% reduction) at E15 to 2.2 ± 0.2 nmol/mg protein/hr (X ± S.E.M., N=4, p<.03, 31% reduction) at E18. In addition, we found that synaptic neurochemical markers for GABA-ergic systems were not significantly reduced in either brain region.

Therefore, a specific reduction in hemispheric ChAT activity in Ts19 mice occurs without a corresponding decrease in the developing basal forebrain, suggesting selective vulnerability of striatal and/or cortical intrinsic cholinergic neurons. Since the pattern and character of neurochemical alterations differs depending upon the particular trisomy, the qualitative effects of the triplication of specific genes on chromosomes, rather than the quantitative effect of aneuploidy in general, may be responsible for the maldevelopment observed in neurochemical and neuroanatomical markers. *Supported by grants PO1 HD 19920, postdoctoral fellowship #MH 15330 (M.D.S.), and by the McKnight Foundation.*

309.14 *IN SITU* HYBRIDIZATION STUDIES OF EXPRESSION OF THE GENE ENCODING CEREBROVASCULAR AMYLOID PEPTIDE (CVAP) IN THE BRAINS OF NORMAL AND TRISOMY 16 MICE. C. Bendotti, R. Reeves*, R. Morgan*, M.L. Oster-Granite, J.D. Gearhart*, and J.T. Coyle. Depts. of Neurosci. and Physiol., Johns Hopkins Univ. Sch. of Med., Balto., MD 21205.

Cerebrovascular amyloid peptide (CVAP) has been identified as a major extracellular component of senile neuritic plaques and cerebrovascular amyloid in Alzheimer's disease (AD) patients and in Down Syndrome (DS; trisomy 21) individuals with AD pathology. Recently, the gene encoding CVAP has been cloned and mapped to human chromosome 21 (HSA 21), suggesting a genetic relationship between AD and DS. In studies showing a genetic homology between HSA 21 and mouse chromosome 16 (MMU 16), it has been proposed that mice with trisomy 16 (Ts16) might serve as a genetic animal model for studies relevant to DS. The genetic homology between HSA 21 and MMU 16 has been extended recently to the gene encoding the A4 amyloid protein (see abstract by Reeves *et al.*). Thus, the Ts16 mouse may also provide an experimental model system to examine the developmental expression of CVAP, when present in three copies as it is in DS, and possibly in AD as well.

We used the *in situ* hybridization technique on 10 um frozen coronal sections of normal adult mouse brain to localize the CVAP mRNA. By using a [35S]cDNA clone homologous to the mouse CVAP gene (see abstract by Reeves *et al.*), we observed an uneven distribution of hybridization signal throughout the mouse brain with highest densities in the frontal and parietal cortices, the tuberculum olfactorium, the pyramidal layer of the hippocampus, the medial habenula, and the cerebellar cortex, with intermediate levels in the hypothalamus, pons-medulla, and ventrotegmental area and with low levels of expression in the striatum, thalamus, and mesencephalon. The density of hybridization overlying individual positive neurons exhibited substantial regional differences. The specificity of the DNA-RNA hybrid was confirmed by a loss of hybridization signal after pretreatment of the sections with RNAse.

The same *in situ* hybridization procedure was used to study the CVAP gene expression in fetal Ts16 mice and their normal littermates. Again, 10 um frozen coronal sections were prepared from the heads of day 15 gestation, karyotypically identified, normal and Ts16 fetuses. With the same [35S]cDNA probe, we observed a clear amplification of the hybridization signal in the brain tissue of the Ts16 mice compared to their normal littermates on Ultrofilm autoradiographic images. Quantitative analysis of these images with a computer assisted RAS analyzer (Amersham) confirmed a significant increase in hybridization signal in various regions of the Ts16 mouse.

These data confirm the recent finding of overexpression of the CVAP gene as measured by Northern blot analysis in the brain tissue of DS fetuses and provide further evidence for considering Ts16 mice as a genetic model system for studies of DS. Moreover, a comparison of the developmental profile of CVAP expression at postnatal ages in normal and Ts16 ↔ normal chimeric mice may provide evidence for early changes that may occur in the brains of AD patients before symptoms become apparent. *Supported by grants PO1 HD 19920, RO1 HD 19932, and by the McKnight Foundation.*

309.15 SOMATOSTATIN EXPRESSION IN DWARF (*dw/dw*) MICE: GENETIC, PHARMACOLOGIC, AND MOLECULAR STUDIES. B. O'Hara*, R. Reeves*, C. Bendotti, J.D. Gearhart*, M.L. Oster-Granite, and J.T. Coyle. Depts. of Neuroscience and Physiology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Mice homozygous for the gene *dwarf* (*dw*), located on mouse chromosome 16 (MMU 16), have elevated levels of somatostatin (SMST) in extra-hypothalamic brain regions (Fuhrmann *et al.*, Brain Res. 328:161, 1985). When the gene encoding preprosomatostatin *Smst* was localized to MMU 16 as well, strength was added to the hypothesis that excess SMST may be a primary molecular defect in these mice. To localize abnormalities in SMST regulation further, we analyzed SMST mRNA levels in the brains of homozygous *dw* mice and their normal littermates by *in situ* hybridization. Qualitatively, *dw* mice have higher levels of expression of SMST mRNA in cortical and hippocampal regions, but lower levels in the hypothalamus. Presumably, the low levels of SMST mRNA (and of SMST peptide) in the hypothalamus result from a lack of growth hormone feedback in the hypopituitary *dw* mouse.

To ascertain whether *dw* and *Smst* were alleles, a backcross using *dw* and the subspecies *Mus musculus molossinus* (MOLD/Rk) [(*dw/J* x MOLD/Rk) x *dw/J*] was used to generate a three point cross with the immunoglobulin light chain lambda (*Igl-1*) as the third gene. We isolated a genomic clone of mouse *Smst* and used it as a probe to detect restriction fragment length polymorphisms (RFLPs) between *dw/J* and MOLD/Rk. Coupled with RFLPs for *Igl-1* that distinguished these subspecies, we were able to identify the *Smst* and the *Igl-1* allele contributed by each parent. When we analyzed 72 backcross progeny, we found the following map: (centromere) *Igl-1* - 0.04 - *Smst* - 0.31 - *dw*, showing conclusively that *Smst* and *dw* are distinct genes.

It was still possible that the dwarfism associated with *dw* resulted from a *dw*-mediated increase in *Smst* expression, and that excess levels of SMST caused the death of somatotrophs in the late stages of pituitary cell differentiation. To test this hypothesis, we injected neonatal mice with a potent SMST agonist, cyclo (N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe) II. Their growth curves, while reduced, were quite distinct from those of *dw* mice. On histologic examination, the pituitaries of these treated mice were essentially normal, with little or no change in the number of somatotrophs. Once treatment with cyclo II ceased, treated mice experienced a period of catch-up growth. While it is still possible that excess SMST could affect *dw* mice prenatally, the levels of SMST observed may be a secondary effect in *dw* mice. Currently, we are using specific hormone therapies to test this hypothesis.

In conclusion, although *dwarf* mice do exhibit increased expression of the gene encoding somatostatin, and both genes are located on MMU 16, they are distinct genes. Additionally, excess levels of somatostatin during the early postnatal period in the mouse do not account for the loss of somatotrophs in the anterior pituitary, nor the decreased levels of somatostatin in the hypothalamus that is observed in *dwarf* mice. *Supported by grants PO1 HD 19920, RO1 HD 19932, and the the McKnight Foundation.*

310.1 EFFECT OF FFX LESIONS ON CONCURRENT OBJECT AND CONDITIONAL PLACE/OBJECT DISCRIMINATIONS. C.G. Wible, S. McGinn*, S. Graham*, and D.S. Olton. Department of Psychology, Johns Hopkins University, Baltimore, MD 21218.

Animal models of human medial temporal lobe amnesia address questions about the anatomical and functional locus of the amnesia. Animal models using monkeys have predominately used nonspatial tasks with objects. Animal models using rats have predominately used spatial tasks. In order to develop a rat model of amnesia using tasks more similar to the ones used to test monkeys and human amnesics, we have trained rats to perform object discriminations. The performance of normal control (C) rats and those with fimbria/fornix (FFX) lesions were compared on two tasks. Experiment I used a concurrent object discrimination with 2 pairs of objects, Experiment II used a conditional place/object discrimination in which the correct object was conditional upon the location of the pair of objects within a room. Different groups of rats were trained on the two tasks. Rats in Experiment I and II were trained preoperatively and were then given either FFX lesions or underwent surgical procedures without a lesion (C).

Two pairs of objects were used in the concurrent discrimination; the same object in each pair served as the S+ on every trial. Both the FFX and C groups learned the task; there was no difference in performance between the two groups.

In the conditional place/object discrimination, two testing areas were located in distinct locations in a room. In place 1, one of the two objects was correct; in place 2, the other object of the same pair was correct. Rats with FFX lesions failed to learn the conditional place/object discrimination, but C rats did learn the task.

The results of both the concurrent and conditional object/place tasks show that normal rats can learn object discriminations relatively easily. FFX lesions have an effect on conditional place/object tasks, but not on a concurrent discrimination using two pairs of objects. This opens avenues for further research in which more difficult object discriminations can be used to identify the brain mechanisms involved in memory.

310.2 AN ANATOMICAL AND BEHAVIORAL STUDY OF THE FOREBRAIN CHOLINERGIC SYSTEM IN THE MARMOSET (CALLITHRIX JACCHUS). A.C. Roberts*[1], T.W. Robbins*[1], B.J. Everitt*[2], T.E. Sirkia*[2] and G.H. Jones*[1]. (SPON: B.J. Sahakian). Depts. of Psychology[1] and Anatomy[2], Univ. of Cambridge, Cambridge, CB2 3EB, United Kingdom

This study investigates the anatomy and function of the forebrain cholinergic system, particularly that arising from the nucleus basalis of Meynert (nbM), and its projections to the cerebral cortex, in the Common marmoset.

The distribution of choline acetyltransferase immunoreactive (ChAT-IR) neurons was studied using immunohistochemistry. In the forebrain, ChAT-IR neurons were found in the medial septal nucleus, vertical and horizontal limb of the diagonal band and the nbM. Of particular interest to this study was the organisation of the projections from ChAT-IR neurons of the nbM to the overlying cortex. Combining the retrograde transport of HRP-WGA with ChAT immunohistochemistry revealed the distribution of neurons in the nbM projecting to the dorsolateral pre-frontal cortex. In addition, lesions at various loci within this nucleus resulted in differential patterns of ChAT loss in the cortex, suggesting some degree of topographical organisation of nbM projections.

The second phase of the study investigated the role of the anterior nbM, innervating mainly pre-frontal/anterior frontal areas, in visual discriminationand reversal performance. Computer generated visual stimuli were presented on a VDU screen which was fitted with a touch sensitive screen for monitoring the animals responses. All animals were first trained to discriminate between two shapes followed by training on a series of reversals, using banana-favoured milkshake as the reinforcer. Animals then received either a lesion of the anterior nbM using N-methyl-D-aspartic acid or a sham lesion (vehicle injection). Post-operative retention of the discrimination and, to some extent, reversal performance was impaired in the nbM-lesioned animals. In addition, these animals showed a significant and long-lasting perseveration of the consummatory response of licking the spout that provided the reinforcement, indicating impaired stimulus control.

These results will be discussed in terms of the possible "frontal" nature of the behavioral deficits.

310.3 ACQUISITION OF A COMPLEX PLACE TASK IN RATS WITH SELECTIVE IBOTENATE LESIONS OF HIPPOCAMPAL FORMATION: COMBINED LESIONS OF SUBICULUM AND ENTORHINAL CORTEX VS. HIPPOCAMPUS. J.P. Bouffard* and L.E. Jarrard (SPON: H.E. King). Dept. of Psychology, Washington and Lee Univ., Lexington, VA. 24450.

Examination of the brains of patients diagnosed as having Alzheimer's disease has revealed an extensive loss of cells in the deep layers of the entorhinal cortex and the subiculum (Hyman et al, 1984). This pattern of damage was interpreted as effectively isolating the hippocampus from the neocortex by interrupting the major inputs from entorhinal cortex and major outputs to the subiculum. One purpose of the present study was to determine the effects of combined ibotenate damage to subiculum and entorhinal cortex on the acquisition of a complex spatial task designed to vary temporal contextual information (massed vs. distributed practice trials). A second purpose was to compare the performance of rats with subiculum and entorhinal cortex lesions with performance of animals having the hippocampus removed and controls.

Rats were divided into 2 control (operated, unoperated) and 2 lesion groups. Multiple injections of IBO were made into the subiculum and entorhinal cortex in one group and the hippocampus in the other. Using a within-subjects design the rats underwent training on a series of problems (4 arms baited out of 8) in an 8-arm radial maze. Eight problems were presented for 11 trials each, with half the problems given under massed (45 sec) and the other half under distributed (10 min) practice trials.

Even though histology indicated that the cell loss in the subiculum and deep layers of entorhinal cortex was extensive, acquisition in these rats was like that of controls. In contrast, removing the cells in the hippocampus had a devastating effect on acquisition of the complex spatial task. Distributed practice trials (10 min) served to improve performance of hippocampals over massed practice (45 sec) more than was found in the other groups. These data show that removing the cells in the hippocampus with an axon-sparing neurotoxin impairs acquisition of a complex place task, but eliminating most cortical inputs to and outputs from hippocampus has no effect under the conditions studied.

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310.4 LESIONS OF THE ANTERIOR TEMPORAL STEM IMPAIR DELAYED MATCH TO SAMPLE PERFORMANCE IN MONKEYS. R.A. Cirillo[1]*, J.A. Horel[2]*, P.J. George[1]*, and S.G. Spence[2]*. 1. Dept. of Psychology, Syracuse University, Syracuse, NY, 13244. 2. Dept. of Anatomy and Cell Biology, SUNY Health Science Center at Syracuse, Syracuse, NY 13210.

Resection of the medial temporal lobe in humans produces a profound anterograde amnesia in which past memories are seemingly intact, but the ability to form new memories is severely compromised. Efforts to develop a model of this human amnesia using non-human primates have relied extensively on the delayed non-match-to-sample (DNMS) task, and its relative, the delayed match-to-sample (DMS) task. DNMS deficits have been found with combined damage to the amygdala and hippocampus, but not to the adjacent white matter (the temporal stem) that connects the temporal cortex to other brain areas (S. Zola-Morgan et al., Science, 218: 1337-1339, 1982). Previous work in our laboratory, however, has produced delayed match-to-sample (DMS) deficits with lesions to anteroventral temporal cortex (J.A. Horel et al., Soc Neurosci Abstr, 200.13, 1986), and lesions to orbitofrontal cortex (M.L. Voytko, Physiol. Psych., 13 (4): 219-229, 1985). The antroventral temporal cortex and the orbitofrontal cortex are interconnected through the temporal stem.

In the present study, we examined the hypothesis that the temporal stem lesion of Zola-Morgan et al., (1982) was not sufficiently rostral to include the fibers exiting from the anteroventral temporal cortex and the temporal pole. We transected the anterior extreme of the temporal stem via an incision through the superior temporal sulcus. Our cut begins deep to the temporal pole and continues posteriorly for 2 cm.

We have found that animals with this preparation exhibit a severe deficit on DMS, particularly at longer delays (30-45 seconds). In addition, these animals were impaired in relearning a preoperatively learned visual discrimination, and in the postoperative learning of a new visual discrimination. Therefore, it would appear that insofar as DMS is a measure of human amnesia, this syndrome can be produced by damage to the temporal stem. (This work was supported by NINCDS Grant NS 1829-05.)

310.5 EFFECT OF MEDIAL FRONTAL CORTEX LESIONS ON OBJECT RECOGNITION MEMORY IN THE RAT. L.A. Rothblat and L.F. Kromer, Dept. of Psychology, George Washington University, Washington, D.C., 20052 and Dept. of Anatomy and Cell Biology, Georgetown University School of Medicine, Washington, D.C., 20007.

Delayed nonmatching-to-sample with trial unique stimuli (DNMS) has become the currently accepted standard for demonstrating object recognition memory in monkeys (e.g. Mishkin and Delacour, J. Exp. Psych., 1:326, 1975), and, as such, is being widely used to identify critical brain regions which when damaged produce amnesia (e.g. Mishkin, Nature, 273:297, 1978; Zola-Morgan and Squire, Behav. Neuorsci., 100:155, 1986). Thus far, this work has been successful in demonstrating the involvement of a number of different neural structures, e.g. hippocampus, amygdala, medial thalamus and frontal cortex, but there is still disagreement as to whether these brain areas can function independently in recognition memory, or work in combination. To further explore this question, we began testing rats on DNMS utilizing procedures which are virtually identical to those used with primates (Rothblat and Hayes, Behav. Neurosci., In press). Previously, we showed that rats with septo-hippocampal damage were not impaired on DNMS, even though they displayed a marked deficit on a test of spatial memory (Rothblat, Hayes and Kromer, Soc. Neurosci. Abstr., 12:742, 1986). The present study was an attempt to further define the neural circuitry of memory by evaluating the role of rat frontal cortex in object recognition.

For testing on DNMS, we used a pool of 250 small "junk" stimuli. On each trial, 2 stimuli were selected from the object pool; one member of the pair was designated as the sample. On the first part of a trial, the sample run, the rat traversed an elevated runway and displaced the sample object for food reward. On the second part of a trial, the choice run, the rat had to choose between the previously presented sample and the second member of the pair. Reward on the choice run followed selection of the novel object. DNMS performance of previously trained rats was evaluated following bilateral aspiration lesions of medial frontal cortex. In addition, performance on a measure of spatial memory, discrete trial rewarded alternation, was assessed.

The results of the study showed that rats with lesions of medial frontal cortex, as is the case with hippocampally damaged animals, are not impaired on DNMS. Medial frontal animals were impaired on the spatial alternation task, though the magnitude of the deficit was less severe than that displayed by rats with damage to the septo-hippocampal system. Thus, although both brain regions seem to play a role in spatial memory, the critical structures for object recognition remain to be determined. (Supported by the March of Dimes and a NIH Biomedical Research Support Grant to G.W.U.).

310.6 FIMBRA-FORNIX LESION IMPAIRS LEARNING PERFORMANCE OF RATS IN A 14-UNIT T-MAZE. E. Bresnahan, H. Kametani*, E. Spangler*, M. Chachich*, P. Wiser*, and D. Ingram*. Essex Comm. College, Baltimore, MD 21237, Dept. Psychol., Towson State Univerity, Towson, MD 21204, and Gerontology Res. Ctr., NIA, NIH, Baltimore, MD 21224

Results from a previous study demonstrated that systemic injections of the muscarinic antagonist, scopolamine, impaired learning of young (3 mo) rats in a shock-motivated 14-unit T-maze (Spangler, E. et al., Pharmacol. Biochem. Behav., 25:673, 1986). This drug-induced dysfunction simulated the age-related impairment in learning of rodents observed in this task (Ingram, D., Annals NYAS, 444:312, 1985). The objective of the present study was to determine the involvement of the septo-hippocampal cholinergic pathway in memory systems required to learn this task. Young (3-mo) male F-344 rats received electrolytic lesions to the fimbra-fornix or served as sham-operated or unoperated controls. One week later, rats received one-way active avoidance training (US = 0.8 mA) in a straight runway (1 m long). The criterion of 8/10 successful avoidances on the last of three consecutive 10-trial daily sessions was met equivalently by all groups. Beginning 24 hours later, rats were provided two 10-trial daily sessions in the complex maze task. The rat was required to traverse each of five maze segments within 10 sec to avoid footshock (0.8 mA). Dependent measures of maze performance analyzed included errors (deviations from the correct path as detected by photocells), run time, number of shocks, duration of shock, and alternation errors (errors made if the rat were using an alternation strategy). According to t-test analyses, the sham-operated and unoperated controls significantly differed only in alternation errors ($p = 0.046$). For all other analyses the two control groups were combined and compared to the lesioned group on each variable by way of 2 (group) by 4 (block of 5 trials) ANOVAs. Control and lesioned groups demonstrated learning by significantly ($p < 0.01$) reduced error rate across trial blocks. However, the results also demonstrated that the fimbra-fornix lesion produced significant ($p's < 0.01$) impairment relative to controls in all performance measures. Further analysis of subgroups among lesioned animals indicated that significantly ($p < 0.05$) greater impairment was observed when transection of the fimbra-fornix appeared complete across several thionin-stained serial sections. This dysfunction in maze performance was similar to that observed following high doses of scopolamine (3 mg/kg). Performance with partial transections simulated that observed in young rats with lower doses of scopolamine (0.5 - 1.0 mg/kg) or that of aged rats (22-24 mo). Thus, the integrity of the septo-hippocampal pathway affects the proficiency of learning performance in this task and is implicated as a system involved in the age-related impairment observed.

310.7 THE EFFECTS OF NUCLEUS BASALIS OR FIMBRIA-FORNIX LESIONS ON A SPATIAL T MAZE ALTERNATION OR MATCHING TASK. R. Etherington* and S.B. Dunnett. Dept. of Experimental Psychology, University of Cambridge, Downing Street, Cambridge, CB2 3EB, England.

Recent research has suggested that there is a dissociation between the effects of lesions of the cortical and septo-hippocampal cholinergic pathways. A reference memory deficit is seen on a radial arm maze task after lesions of the nucleus basalis magnocellularis (Murray and Fibiger, Neuroscience, 1985,14:1025-1032) while in contrast there are impairments in working memory functions after lesions of the fimbria-fornix (Olton et al., Behav and Brain Sciences, 1979,2:313-365) . In the present experiment different groups of rats were trained on either an open T maze matching or alternation task. This procedure required the rats to make a forced choice entry into one arm then, after a 10 second delay, a recognition choice entry into the relevant arm depending on the type of task. Rats in each condition were given either quisqualic acid lesions of the NBM, or aspiration lesions of the fimbria-fornix, and both post op. acquisition and retention tests were carried out. The performance of the fimbria-fornix rats was impaired on both matching and alternation tasks and these animals showed a bias to respond to a particular arm of the maze. NBM lesions, on the other hand, produced a more variable effect. On the alternation task the rats showed no deficit during either acquisition or retention performance at the 10 second delay, although a small but significant deficit was apparent at longer delays (30-120 seconds). NBM rats in the matching task appeared to learn the task faster than controls in the early trials but did not reach criterion levels of performance, and this lesion x trials effect was highly significant. The pattern of performance on the two tasks suggests that the nature of the deficit induced by fimbria-fornix lesions was not mnemonic, but rather was of the classic perseverative type. In contrast, the NBM animals showed more clear cut deficits on the matching than the alternation task, which could be attributable to the former task requiring more "effortful" levels of cognitive processing than the latter, where spontaneous alternation strategies are reinforced.

310.8 HANDLING STIMULATION IN INFANCY AND GENDER: EFFECTS UPON MAZE LEARNING AND BEHAVIORAL ASYMMETRY. S. H. Freter*, A. Mikolowski* R. H. Fitch*, A. S. Berrebi, D. A. Yutzey and V. H. Denenberg. Biobehavioral Sciences Graduate Degree Program. Univ. of Conn., Storrs, CT 06268.

Extra handling stimulation in infancy enhances left-right behavioral asymmetry (Sherman et al., Brain Res., 1980, 192:61; Camp et al., Physiol. Beh., 1984, 33:433); and females have a different asymmetry pattern than males (Sherman et al., Life Sci., 1983, 33:189). Better left-right spatial discrimination is associated with cerebral laterality (Zimmerberg et al., Brain Research, 1976, 140:194), suggesting that animals with greater behavioral asymmetry should learn a complex maze more easily. We tested this hypothesis.

Newborn Purdue-Wistar rat pups were not disturbed or were handled daily for 3 min for the first 21 days of life, resulting in four groups: handled males (N = 13), handled females (N = 13), nonhandled males (N = 10), and nonhandled females (N = 9). Starting on day 70 the rats were given one trial a day for 12 days on a water version of the Lashley III maze. Two kinds of errors were recorded: T-Choice errors (making the wrong choice at a T point) and Perseveration errors (continuing straight ahead into a blind alley rather than making a turn).

On the initial trial the rats do not know the location of the goal. At T-choices the animals were more likely to go left than right ($p<.01$), a bias which is indicative of endogenous asymmetry. This is consistent with prior findings from this laboratory that our rat is behaviorally asymmetrical with a left preference.

Thereafter, females made more errors than males while learning ($p<.01$). Handling in infancy interacted with Sex, Kind of error, and Trials ($p<.02$). Analysis of this interaction revealed that three general rules, or strategies, were used to learn the maze: (1) at choice points go left rather than right; (2) use extramaze cues as guides toward the goal box; and (3) learn the internal structure of the maze. Nonhandled females only used the first two strategies; whereas the other three groups used all three rules.

Animals that only use spatial information will get through the maze but will make errors. To eliminate errors, it is necessary that intramaze cues be employed for associative learning. These results indicate that males make better use of associative learning than females, possibly indicating gender differences in use of strategies to solve this task.

310.9 IBOTENATE LESIONS OF THE RAT NUCLEUS BASALIS CAUSE ENDURING IMPAIRED PERFORMANCE OF A NON-MATCH-TO-SAMPLE DISCRIMINATION. B. E. Lerer, D. Hepler, K. Zeller*, J. Bradd* and R. Pierdomenico*. DuPont Pharmaceuticals, Medical Products Dept., Wilmington, DE 19898.

CNS cholinergic neurons mediate critical learning and memory processes; in fact, the cognitive deficits observed in Alzheimer's Disease have been correlated with the extent of loss of neocortical cholinergic integrity. In the rat, neurotoxic lesions of the nucleus basalis magnocellularis (NBM) produce significant decreases in markers of cholinergic neurons in frontal and parietal cortex and also behavioral deficits suggestive of learning and memory dysfunction.

Male Sprague-Dawley rats were trained to respond for food on a fixed-ratio 5 (FR5) schedule with a food reward and retractable lever located beneath a cuelamp. The location of the lever on each trial varied randomly from the right to the left side of the experimental chamber. After training, rats were randomly assigned to either a NBM or SHAM surgery group. Bilateral NBM lesions were induced with 40 nmol injections of ibotenic acid. The SHAM group had a clean cannula inserted into the NBM without injection. Three weeks after surgery, all rats were tested in the barpressing procedure and, when they were responding without error, discrimination training began. The non-match-to-sample (NMTS) test consisted of a SAMPLE trial followed by a CHOICE trial. On the SAMPLE trial, rats were rewarded for FR5 responding on the lever extended below a lit cuelamp. The SAMPLE varied randomly from the right to the left lever on each trial. On the CHOICE trial, two levers were extended and a FR5 to the uncued lever was rewarded.

NBM rats were severly impaired on this NMTS discrimination (although they are not impaired in a match-to-sample discrimination) and after 18 sessions they made about 65% correct responses while the SHAM group made over 80% correct responses. Testing was suspended about 4 months and resumed for an additional 15-21 sessions. Although it had been almost 6 months since surgery, the NBM rats were still significantly impaired relative to SHAM performance. After 4 sessions, the NBM group reached an asymptotic level of about 65% as compared to 87% correct responses in the SHAM group. At 27 weeks post-surgery, several animals from each group were sacrificed and cortical samples were assessed for acetylcholine release and choline acetyltransferase activity. In the NBM group, both markers were significantly reduced by about 35% as compared to the SHAM group.

Our results indicate that bilateral NBM lesions produce profound and sustained behavioral and biochemical deficits that appear stable and non-recovering for at least 6 months.

310.10 **DISRUPTION IN MAZE PERFORMANCE AFTER CORTICAL LESIONS IN TURTLES (Chrysemys picta).** **M. A. Petrillo* and A. S. Powers. Department of Psychology, St. John's University, Jamaica, NY 11439.**

The dorsal cortex (cd) of turtles, a three-layered structure on the surface of the hemisphere, has been implicated in learning (Grisham & Powers, 1986, Society for Neuroscience Abstracts, 12). Like the neocortex of mammals, the cd receives input from cholinergic cells in the basal forebrain (Mufson et al., 1982, Brain Research, 323, 103-108). The present study was undertaken to investigate the role of the cd in retention of a maze habit.

Postoperative maze performance was assessed in turtles after bilateral ablation of the cd. The turtles were required to learn a four arm maze for water reward. Each arm ended with a well, one of which was filled with water (the goal well). The turtles were placed in another well, the start well, and after a sixty second adjustment period, allowed to make an unlimited number of choices until they reached the goal well or until five minutes had elapsed. From the start well, the turtles had to make a left turn in the center to reach the goal well. A ratio of number of correct choices to total number of choices for a day was computed for each turtle. The turtles were trained until they reached a criterion of 67% correct choices per day for two successive days. Once criterion was met, the turtles were anesthetized with Equithesin (ip; dose .20 ml per 100 g body weight). Lesions of the cd (n=4) were made by aspiration. Control lesions (n=4) consisted of merely opening the skull. After a five to seven day recovery period, the turtles were reintroduced to the maze. The turtles were run postoperatively until they reached criterion or until they amassed three times as many days postoperatively as preoperatively. Postoperative days to criterion were computed for each turtle. Turtles with cd lesions were significantly impaired relative to shams ($U \leq 1$, p=.029). These data suggest that spatial memory, as measured by maze performance, involves the cd. Previous research on the Morris milk maze has yielded similar results in rats after lesions of the nucleus basalis (Dokla et al., 1985, Society for Neuroscience Abstracts, 11, 332).

310.11 **THE IMMUNOHISTOCHEMISTRY AND CYTOARCHITECTURE OF THE AVIAN HIPPOCAMPUS.** J.R. Krebs*, J.T. Erichsen and V.P. Bingman. Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794

The avian dorsomedial forebrain (Hp-APH) was considered by early comparative anatomists (Ariens-Kappers et al., 1936) as homologous with the mammalian hippocampal complex (Hp). Recently, behavioral and anatomical studies have provided further support for this suggested homology. First, like the mammalian Hp (e.g. O'Keefe & Nadel, 1978; Olton et al., 1979), the avian Hp-APH has been shown to play an important role in spatially guided behavior and memory (Bingman et al., 1987; Sherry & Vaccarino, in press). Second, a pathway tracing study has found similarities in the connectivity of avian Hp-APH and mammalian Hp (Casini et al., 1986). In the present study, we have employed immunohistochemical techniques to investigate the distribution of a variety of neuropeptides and neurotransmitter-related enzymes in the homing pigeon, a bird known to suffer spatial behavior deficits following damage to Hp-APH. Our goals were to: (1) characterize the subdivisions of Hp-APH, and (2) explore further the hypothesis of homology with mammalian Hp by comparing the distributions of different kinds of immunoreactivity.

Antibodies to five neuropeptides (somatostatin [SS], avian pancreatic polypeptide [APP], cholecystokinin [CCK], leu-enkephalin [ENK], and substance P [SP]), an indoleamine (serotonin [5-HT]) and three transmitter-related enzymes (choline acetyltransferase [ChAT], glutamic acid decarboxylase [GAD] and tyrosine hydroxylase [TOH]) were used in the immunohistochemical analysis of Hp-APH. A variety of SS-positive cell types, including pyramidal-like cells, were located in medial Hp-APH. APP-positive cells were also found medially and were characterized by the regular orientation of their dendrites. A sparse population of small cells, lying ventrally and along the medial wall, contained CCK. Immunoreactivity for these three peptides, as well as for SP and ENK, was also found in dense terminal fields in restricted regions. A population of 5-HT-positive fibers appeared to terminate in a dense dorsomedial field. This serotonergic projection probably arises from the medial raphe nucleus (Casini et al., 1986). ChAT-positive fibers were observed medially with terminals sparsely distributed dorsomedially. This projection may originate from the ventral septum where a few ChAT-positive cell bodies were observed (Casini et al., 1986). GAD- and TOH-positive terminal fields were also situated dorsomedially.

The distribution of immunoreactive staining for these peptides and transmitter-related enzymes revealed the existence of several distinct subdivisions of Hp-APH. Based on these results, we will offer a tentative cytoarchitectural characterization of Hp-APH and a comparative analysis of the avian and mammalian hippocampus.

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310.12 EXPLORATORY BEHAVIOR OF LONG-EVANS HOODED RATS ON THE MAIER 3-TABLE MAZE FOLLOWING NEUROTOXIN-INDUCED LESIONS OF THE HIPPOCAMPUS. C. Wages. Dept. of Psychology, Georgia State Univ., Atlanta, GA

O'Keefe and Nadel (1978) stressed the necessity of locomotion through an environment if the animal is to acquire information concerning the distance and direction between stimuli in the environ-ment (i.e., cognitive map). Specifically, they argued that as the dentate cells can only fire at one phase of the theta cycle (Ranck, 1975), they could gate stimulus input; so that stimuli detected at different locations in the environment would be encoded in different areas of the CA3 field.

O'Keefe and Nadel further contrasted exploratory activity with general activity suggesting that exploratory activity would decrease across time as a neural representation of the environment was formed; whereas general activity would not decrease.

While impairment on certain spatial tasks may indicate that some cognitive map process has been impaired, it is usually difficult to determine whether the the impairment reflects an inability to access information encoded in the map, or a disruption of the neural systems that had encoded the distance and direction information.

To the extent that locomotor activity decreases when a cognitve map is encoded, one can identify if an impaired animal has a cognitive map by observing its locomotor activity. Animals with damage to the septum, medial frontal cortex, or hippocampus are deficient on many spatial tasks. In contrast to normal animals, animals with septal damage failed to decrease activity during a daily 15 minute opportunity to explore a test environment (Ellen and Weston, 1983; Herrmann, Poucet, and Ellen, 1985) indicating that they were not able to encode spatial information into a cognitive map. Animals with medial frontal cortex damage failed to habituate locomotor activity during early exploration of the test environment, but with repeated exposure the activity within an exploration session decreased (Herrmann et al, 1985) indicating that the failure on the task indicated an inability to use the encoded spatial information.

In this experiment, the exploratory behavior of animals with dentate damage, or massive or minor CA damage was analyzed on a spatial problem that could only be solved if the animals were able to use spatial information that was acquired on separate days.

310.13 SPATIAL BEHAVIOR AFTER HIPPOCAMPAL LESIONS IN PIGEONS V. P. Bingman and W. Hodos. Dept. of Psychology, Univ. of Maryland, College Park, MD 20742.

The avian hippocampal (Hp) and parahippocampal (APH) areas have been suggested as the homolog of the mammalian hippocampal complex. Indeed, a recent pathway-tracing study revealed considerable similarity in the extrinsic connections of the avian Hp-APH and the mammalian hippocampus (Casini, et al., J. comp. Neurol., 245, 454-470, 1986). Given the important role of the hippocampus in the neural organization of spatial behavior, as revealed by laboratory studies in rodents, the role of the Hp-APH complex in naturally-occurring spatial behavior in birds was examined.

Homing behavior of pigeons with Hp-APH lesions was compared to homing in pigeons with control lesions in the anterior telencephalon. The Hp-APH-ablated pigeons showed a persistent impairment in the amount of time required to return home, which suggests a spatial deficit. The basis of the deficit is obscure at present, although anecdotal evidence suggests that Hp-APH-ablated pigeons are impaired in their ability to rely on landmarks to locate their loft. This hypothesis, if verified, would be consistent with data from mammals that indicate a mapping function for the hippocampus.

Psychophysical techniques were used to test the possibility that the deficits in homing behavior might be due to sensory losses. Hp-APH ablation was found to have no effect on either visual acuity or size-difference thresholds. Thus, an explanation based on visual impairment would seem unlikely as a cause of the homing deficit.

Currently, a "spatial relations" test is being used to examine the ability of pigeons to make use of the location of landmarks (colored squares on a video monitor) as cues to which pecking responses would lead to a food reward. Preliminary results indicate that the post-operative performance of Hp-APH ablated pigeons is impaired.

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310.14 ACCELERATED RATE OF FORGETTING OF SPATIAL INFORMATION WITH AGING AND LONG TERM ETHANOL CONSUMPTION IN MICE: EVIDENCE FOR TWO FORMS OF AMNESIA. R. Jaffard, D.J. Béracochéa and A.N. Tako*. Lab. Psychophysiologie, Univ. Bordeaux I, 33405 Talence, France.

Studies of amnesic patients have consistently produced evidence for two dissociable forms of amnesia (Squire, Ann. Rev.Neurosc., 5:241, 1982). The purpose of the present experiment was twofold: (i) to compare the rates of forgetting of aged and alcohol-treated mice; (ii) to determine whether the observed deficits correspond to two different types of amnesia.

At 2 months of age, mice of the alcohol groups were given a solution of ethanol (12% v/v) as their only source of fluid for periods ranging from 2 to 12 months; they were compared to pair-fed and free-feeding animals after ethanol had been omitted from the diet for at least 4 weeks. Spontaneous alternation behavior (S.A.) in a T maze was used as a learning paradigm consisting of two forced trials (acquisition) followed, at varying retention intervals (R.Is) by a free test trial (retention). Thus, performance was measured both as a function of age (4,9,12 and 17 months) and alcohol consumption duration (respectively 2,6,9 and 12 months). No between-groups differences were observed for S.A. rates at the shorter (5 mn) R.I. (between 78.1 and 82.9 %). However, as compared to younger subjects (4-12 months) which still significantly alternated at the 24 hrs R.I. (mean: 70.2 %), older mice (17 months) responded at chance level at the 6 hrs interval (53.2%). The same accelerated rate of decay of S.A. was observed in young (9 months) alcohol-treated mice which exhibited chance level performance at 6 hrs (51.3 vs 75.0 % for controls).

The second part of the experiment was aimed at testing whether a change of intra-maze context occuring on the retention test trial would improve performance of aged and alcohol-treated mice (see Béracochéa and Jaffard , Behav. Neurosc., 101:101, 1987 for a similar procedure which was found to reverse the spatial memory deficits of mammillary body-lesioned mice). Results showed that this change of context totally alleviated the deficit of alcohol-treated mice but had no effect in aged animals. Moreover, this procedure was totally ineffective in improving performance of untreated young subjects whatever the R.I.(from 6 to 72 hrs).

These results suggest that both alcohol consumption and aging result in an accelerated rate of forgetting of spatial information but that the alcohol-induced deficit would stem from a basic failure in retrieval while the age-related one would result from a faster decay of the memory trace.

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310.15 DIFFERENTIAL EFFECTS OF ELECTROLYTIC AND CYTOTOXIC CINGULATE CORTEX LESIONS ON ACQUISITION RETENTION AND REVERSAL OF A SPATIAL DISCRIMINATION TASK IN MICE .M.Meunier*,R. Jaffard, C. Messier and C.Destrade. Lab. Psychophysiologie, Univ. Bordeaux 1, Avenue des Facultés, 33405 TALENCE Cedex FRANCE.

We have shown that restricted electrolytic lesions of the posterior but not anterior cingulate cortex produced a transitory facilitation of learning in Balb/c mice (Meunier et al., Physiol. Behav. 37,903,1986). In the present experiments we compare the effects of bilateral electrolytic (HF) or ibotenic acid (IBO) lesions of the anterior (ANT) or the posterior (POST) cingulate cortex on acquisition,retention and reversal of a spatial discrimination task in a T-maze.

HF Lesions were produced by passing anodal high frequency current (500 KHz; 0.35 mA) for 30 sec through the tip of an stainless-steel electrode. The IBO injections were made through a glass micropipette.(0.24 µl at a rate of 0.02 µl/min). One week after surgery the lesioned mice and the sham operated controls were food deprived and tested over 5 consecutive days. The baited T-maze arm was reversed each test day. The daily test ended when 5 consecutive correct responses were made. An additional session was carried on 10 days later. Three single-trial retention tests were given 5min, 6h and 24h after the end of each session.

POST-HF lesioned animals reached the criterion significantly faster on the first 2 days of testing (acquisition and 1st reversal). This effect disappeared on the 3 following days, and was reversed (impairment) ten days later. However,the POST-HF group was significantly impaired in the 24h-delay retention test. In contrast, ANT-HF lesioned mice reached the criterion significantly later on the 3rd day of testing (2nd reversal) but did not differ from controls in any of the other 5 sessions.Retention was slightly but not significantly impaired for the 3 retention intervals (5min, 6h and 24h).

The behavioral effects observed in POST-HF lesioned group were not found in POST-IBO lesioned animals. However certain POST-IBO mice were impaired on the first two days of testing when compared to controls. In contrast to POST-HF mice, POST-IBO animals were not impaired in the 24h-delay retention test. No significant effects were observed in ANT-IBO lesioned animals.

These results indicate an involvement of the posterior cingulate cortex, and in particular the cingulum bundle,in both the initial acquisition and long-term memory processes.

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310.16 ISOLATION OF NEURAL REGIONS MEDIATING OLFACTORY LEARNING IN RATS. David Kucharski and W.G. Hall. Department of Psychology, Duke University, Durham, NC 27706.

Memory for conditioned olfactory preference can be functionally confined to one side of the brain in young rat pups by restricting odor input to one naris and corresponding olfactory bulb during training pairings of an odor with milk reward. While the memory is stored on the trained side of the brain, information can be accessed contralaterally from the untrained side of the brain. Twelve-day-old pups show a conditioned increase in preference for the odor when tested with either the trained or untrained naris stimulated, but the capacity for responding by the untrained naris depends on mature and intact components of the anterior commissure. These commissural fibers appear to provide contralateral access and "read-out" of unilaterally stored memories. The ability to probe olfactory memory from the untrained side provides the opportunity to further identify structures and substrates involved in conditioning and memory retrieval by anatomically isolating specific projections of the anterior commissure. Here, we trained 12-day-old rat pups to associate cedar odor with sucrose reward while olfactory input was restricted to one naris by a soft rubber plug in the opposite naris. We then made knife cut transections of commissural fibers near their projection site in anterior olfactory nucleus or piriform cortex, or transected the entire olfactory bulb on the trained side of the brain. If memory for the odor preference is indeed stored in one or more of these locations, then the animal should demonstrate a retrieval deficit when tested with the untrained naris open. Selectively disrupting the commissural projection to the AON resulted in a disruption of retention for animals tested with the untrained naris open. Neither presence of the trained bulb nor projections to the olfactory bulb or piriform cortex appear necessary for accessing olfactory memories. Thus, the anterior olfactory nucleus is implicated as a structure that once innervated, provides critical sites for, or pathways to, olfactory preference memories.

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310.17 THE ACQUISITION OF ASSOCIATIVE HABITS BY MONKEYS WITH MEDIAL THALAMIC LESIONS. E.C. Gower, S. Jacobsen, N. Butters & D.L. Kasdon*. VA Medical Center, Boston, MA 02130.

Damage to the primate medial thalamus impairs the acquisition and performance of a task which requires the retention of information over temporal epochs measured in seconds and minutes. Such monkeys further exhibit postoperative retention deficits for visual discriminations learned preoperatively, a result implying that medial thalamic damage may also accelerate forgetting over longer intervals, and for repeatedly experienced events (Gower, E.C., et al., Soc. Neurosci. Abstr. 11:831, 1985). The present experiment characterizes associative learning in monkeys with medial thalamic lesions, and compares postoperative with preoperative rates of acquisition exhibited by the same subjects for the same type of visual discrimination problem. Three monkeys (M. fascicularis) with radiofrequency lesions of the medial thalamus instituted under visual guidance comprised the experimental group. Two monkeys in the control group of 5 sustained the transcallosal approach surgery.

Pattern Set I (learned preoperatively) consisted of 3 pairs of stimuli, and Set II (learned postoperatively) of 4 pairs. The discriminanda were geometric figures or letters mounted on plaques. Each pattern pair was learned individually to a criterion of no more than 3 errors in 3 consecutive 10-trial blocks. The discriminanda and the procedure encountered in the two training periods were thus similar. Moreover, exposure to Sets I and II was separated by nearly 2 years and a lengthy series of other tasks based on the non-matching principle.

The number of trials required for acquisition of Set I preoperatively did not differentiate the two groups (t_{20} = .92, $p > .10$). Normal monkeys learned the second problem set more rapidly than the first (t_{31} = 2.15, $p < .025$). However for the experimental subjects, the mean acquisition score for Set II did not differ from Set I (t_{18} = 1.06, $p > .10$). In addition, they were significantly impaired in the acquisition of Set II when compared with normal monkeys learning Set II (t_{29} = 3.46, $p < .005$).

The results provide further evidence that the impairment sustained by monkeys with medial thalamic lesions is not restricted to the time course exemplified by the recognition memory task, but also extends into the domain of associative learning. Whether the deficits revealed by these two task types are dissociable, or have a common underlying cause is a question that requires further analysis. (Supported by VA Medical Research funds).

310.18 THIAMINE DEFICIENCY IN THE RAT PRODUCES BILATERALLY SYMMETRICAL LESIONS OF MEDIAL THALAMUS AND IMPAIRS ACQUISITION OF AVERSIVELY CONDITIONED BEHAVIOR. R.L. Knoth, T.A. Otto, T.P. Goodness*, R.G. Mair. Psychology Department, University of New Hampshire, Durham, NH. P.J. Langlais. VA Medical Center, Brockton, MA.

A subacute bout of thiamine deficiency in the rat (pyrithiamine [0.5 mg/kg/day] + thiamine deficient diet) produces consistent medial thalamic lesions in the region of the intralaminar nuclei (examined in one hemisphere only) and significant alterations in the concentrations of brain monoamine and aminoacid neurotransmitters (BRAIN RES, in press; P.J. Langlais, et al., this meeting). Behavioral tests of these animals revealed a long lasting impairment in some appetively motivated tasks (spatial delayed alternation, spatial delayed non-matching-to-sample) but no impairment in others (light/dark discrimination) (BRAIN RES 360, 273-284; NEUROSCI ABST 12, 745). The purpose of the present experiments was to extend the neuropathological findings in PTD animals and to assess the behavioral deficits in tasks employing aversive motivation.

In this study, 8 post-thiamine deficient (PTD) rats were compared with 8 controls in a variety of aversively conditioned tasks: one way escape, one way active avoidance, spatial non-match-to-sample escape, and in a series of habit learning tasks (see below). Although PTD rats exhibited escape latencies comparable to controls after three training sessions, they exhibited a consistent and significant impairment of avoidance responses throughout four training sessions. In the non-match-to-sample escape task, controls improved from 54% correct in the first 24 trials to errorless performance after 120 trials. The PTD group performed significantly worse, improving from 54% to only 77% correct after 120 trials and showed no further improvement over 72 additional training trials. PTD rats were also impaired on a left-right discrimination escape task but were equivalent to controls on all other habit learning measures (spatial reversal, light/dark discrimnation; 24 hr. savings on spatial, spatial reversal, and light/dark tasks). These results are consistent with those obtained using appetitive motivation. Histopathological analyses of behaviorally impaired animals showed consistent bilaterally symmetrical lesions in medial thalamus involving ventral portions of the mediodorsal, as well as the entire extent of central median, paracentral, and central lateral nuclei.

These results, together with those cited above, suggest that PTD animals are unimpaired on tasks requiring only "procedural" types of memory but show consistent deficits on both appetitively and aversively conditioned tasks requiring "declarative" types of memory. The results also demonstrate the bilateral nature of the thalamic lesions associated with post thiamine deficiency.

310.19 APPETITIVELY-MOTIVATED BRIGHTNESS AND SPATIAL DISCRIMINATION LEARNING ARE IMPAIRED FOLLOWING LESIONS OF THE N.BASALIS IN RATS. A.C. Santucci*, V. Haroutunian, and K.L. Davis (SPON:V. Guanowsky). The Mount Sinai Sch. of Med., New York, N.Y. 10029.

Lesions of nucleus basalis of Meynert (nbM) in rats produce learning and memory deficits on aversively and appetitively motivated tasks. In general, lesion-induced learning and memory deficits have not been assessed on different tasks in identically-lesioned animals, nor have lesioned rats been challenged by reversal paradigms. Accordingly, the present study examined whether lesions of the nbM would impair original and reversal learning of a brightness (Expt. 1) or complex spatial (Expt. 2) discrimination.

Both experiments employed male adult rats which received either bilateral ibotenic acid (5 ug in 1 ul/side) lesions of the nbM or sham operations. In Expt.1, animals were required to approach the "dim" arm of a Y-maze in order to receive water reinforcement. Entrance into the incorrect arm, stem, or correct arm without drinking was scored as an error. Training (1 trial/day) continued until a criterion of 4 out of 5 consecutive errorless days was reached. After reaching acquisition criterion, reversal procedures were implemented by reinforcing "bright" arm choices (criterion=4/5 errorless days). In Expt. 2, subjects were trained on a complex spatial discrimination identical to the one employed by Murray & Fibiger (Behav. Neurosci., 100, 23, 1986) for six consecutive days (1 trial/day). Animals were required to find a receptacle containing saccharine-flavored water located among a 6x5 array of receptacles. An error was recorded when an animal searched an unbaited receptacle. For some animals, reversal training followed.

Lesioned animals were severely impaired on the acquisition ($p<.01$) and reversal ($p<.02$) of the brightness discrimination task, often failing to reach reversal criterion. Lesioned rats were similarly impaired on the complex spatial task. Although both lesioned and sham operated groups made an equivalent number of errors during the first two days of training ($ps>.10$), controls continued to improve on subsequent days, whereas lesioned subjects did not ($ps<.05$). These data extend the generality of nbM-lesion learning deficits and show that lesion-induced learning deficits persist despite extensive training and prolong post-lesion survival times.

310.20 RADIAL MAZE PERFORMACE IN AN ANIMAL MODEL OF STATUS EPILEPTICUS. R.M. Sapolsky, B.T. Volpe and H.P. Davis (Spon: F.McDowell). Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305, Cornell Univ. Med.Coll., and Univ. Colorado.

Severe epilepsy in both humans and animal models can eventually injure neurons. The hippocampus which plays a critical role in learning and memory is among the most vulnerable of brain regions to seizure induced neurotoxicity. Our recent work has uncovered endocrine and metabolic manipulations which substantially decrease hippocampal damage in a rat model of epilepsy. Naturally, it is of interest to determine whether such interventions reduce functional as well as neuropathological consequences of epilepsy. Thus, we have developed an animal model of the effects of status epilepticus upon hippocampal dependent behavior. Male Sprague Dawley rats were injected every third day with kainic acid (10mg/kg ip) for 15 days. Kainic acid, a glutamate analogue, produces status epilepticus seizures which share a number of traits with human temporal lobe epilepsy including paroxysmal discharge originating in the hippocampus and preferential damage to hippocampal CA3 neurons. In order to characterize the behavioral impairment in these animals after seizure, we used a 12-arm radial maze to examine their performance for memory of trial specific information (working memory) and trial independent information (reference memory). After three weeks of recovery, animals with seizure treatment (N=12) and control animals (N=32) were adapted to the maze for three days, placed on a food deprivation schedule that maintained them at 80% of ad lib weight, and then given a daily training trial for 32 days. Seven of the 12 arms were baited with food pellets and the baited arms remained constant relative to room cues throughout the experiment. Entry into a baited arm was considered a correct response, entry into a nonbaited arm a reference error, and reentry of an arm a working error. Both control and seizure rats improved on reference and working performance over trials ($p,<.001$), and there was no significant interaction between condition and trials ($p>.25$). However, seizure animals were significantly impaired compared to control animals on reference ($p<.001$) and working performance ($p<.001$). Thus, the behavioral impairment of seizure animals for both reference and working performance remained constant relative to control rats throughout behavioral testing. This preliminary study extends the assessment of this animal model of status epilepticus to behavior. Additionally, this initial behavioral characterization provides a paradigm for assessing whether pharmacological interventions which have been shown to reduce the neuropatholotical consequences of seizures will also reduce the behavioral sequelae of seizures.

311.1 LOCALIZATION OF SEROTONIN 5-HT_{1B} BINDING SITES: EFFECTS OF NEURONAL LESIONS. E. Edwards, P.M. Whitaker-Azmitia and E.C. Azmitia. Dept. Psychiatry, SUNY at Stony Brook, NY 11794 and Dept. Biology, New York University, NY 10003.

We have recently demonstrated by binding and competition studies that ^{125}Iodo-cyanopindolol ((±) ^{125}I-CYP) labels a 5-HT_{1B} site in the rat hippocampus (Neuropharmacology 26(1), 1987). The present experiments were devised to gain some information about the cellular localization of this site by examining the effect of various types of neuronal lesions on the binding of (± ^{125}I-CYP) to rat hippocampal membranes.

The selective degeneration of serotonergic neurons two weeks after an injection of 5,7 dihydroxytryptamine in the fornix-fimbria region (5 μg in 400 nl of .025% ascorbic saline; 15° angle from the vertical line, coordinates in mm: P 6.7, L 1.0, V 4.5 to bregma) was associated with a significant decrease in ^{3}H-serotonin uptake (-70%) and a loss of 5-HT_{1B} binding sites to the hippocampus (-37%).

The possible localization of 5-HT_{1B} sites on cholinergic projections in the hippocampus was examined by assessing the effect after nine days of unilateral kainic acid lesions in the septum and substantia innominata region (1 μg kainic acid in 0.9% saline; coordinates in mm: A 6.5, L 2.3, V 2.0 to lambda). Choline acetyl transferase (ChAT) activity was decreased by 76% in the hippocampus but no significant decrease in ^{125}I-CYP binding was observed.

We have also examined the effect of a kainic acid injection in the dorsal hippocampus (0.5 μl, 4 mg/ml in 0.9% saline; 90° angle to skull, coordinates in mm: A 4.5, L 1.5, V 3.7 to lambda). Nine days after lesions, the destruction of hippocampal intrinsic neurons was evident with a significant loss of 5-HT_{1B} binding sites in the hippocampus (-73%).

We conclude from these experiments that the 5-HT_{1B} sites in hippocampus, labeled by ^{125}I-CYP, occur both pre- and post-synaptically. Approximately one-third of these sites occur on serotonergic terminals and the remainder on neurons or astroglia intrinsic to the hippocampus.

311.2 ANALYSIS OF SEROTONERGIC RECEPTOR SUBTYPES IN AUTONOMIC REGIONS OF THE RAT MEDULLA USING QUANTITATIVE AUTORADIOGRAPHIC TECHNIQUES. K.B. Thor, A. Blitz*, and C.J. Helke. Dept. Pharm., Uniformed Services Univ., Bethesda, MD 20814

The nucleus tractus solitarius (NTS), as well as parasympathetic efferent nuclei in the medulla, contain high levels of serotonin (5HT). Furthermore, injections of 5HT drugs into these regions can change autonomic function. The present study was conducted to determine the distribution of 5HT receptor subtypes associated with these regions.

Adult male rats were killed by decapitation. Frozen sections (20μm) were mounted on slides and incubated in buffered solutions of ^{3}H-5HT (5 nM) to label 5HT_1 sites, ^{3}H-8OH-dipropyl-aminotetraline (^{3}H-8OHDPAT, 2 nM) to label 5HT_{1A} sites, ^{125}I-cyanopindolol (^{125}ICYP, 50 pM) with 30 μM isoprenaline (ISOP) to label 5HT_{1B} sites, and ^{125}I-lysergic acid diethylamide (^{125}I-LSD, 1 nM) with 1 μM domperidone (DOMP) to label 5HT_2 sites. Nonspecific binding of 5HT_1 ligands was assessed by adding 1 μM 5HT to incubation buffers, while nonspecific binding of ^{125}I-LSD was assessed by adding 10 μM ketanserin to incubation buffers. Blocking experiments were conducted by adding 1 μM cold 8OHDPAT, 10 μM propranolol (PROP), or 10 μM chlorimipramine (CMI) to incubation buffers. Slides were apposed to ^{3}H-sensitive Ultrafilm for 2-5 days (^{125}I compounds) or 3-6 months (^{3}H compounds). Sections were scraped from slides and radioactivity measured directly with scintillation or gamma counters.

^{3}H-5HT binding in medullary sections was saturable, and >85% of the total binding at a concentration of 5 nM was specific. Since 20-35% of the specific ^{3}H-5HT binding was CMI sensitive, it was included in incubation buffers. Blocking experiments with 8OHDPAT and PROP indicated specific ^{3}H-5HT binding was to the 5HT_{1A} (30%) and 5HT_{1B} (60%) receptors. ^{3}H-5HT binding was high in NTS, dorsal motor nucleus of the vagus nerve (DVN), and hypoglossal nucleus (HN). Nucleus ambiguous (NA) exhibitted an abscence of binding. Because CMI reduced specific ^{3}H-8OHDPAT binding 40-50%, it was included in the incubation buffer. 80% of the remaining ^{3}H-8OHDPAT binding was specific for 5HT_{1A} binding sites. Autoradiographs of ^{3}H-8OHDPAT binding will be presented. 66% of the ^{125}I-CYP binding was specific for 5HT_{1B} sites. Specific ^{125}I-CYP binding was high in the NTS, DVN, and HN. NA showed an abscence of binding. Nonspecific binding was high in the HN, and the 7th motor nucleus. Only 33% of the ^{125}I-LSD binding was specific for 5HT_2 sites and was primarily associated with the inferior olives.

These results indicate that 5HT_1 binding sites are present in the NTS and DVN, while few are in NA. Furtheremore, 5HT_{1A} and 5HT_{1B} sites appear in a ratio 1:2. 5HT_2 sites are few in all autonomic portions of the medulla. Support: NS20991 and MD. AHA

311.3 DIFFERENCES BETWEEN THE IN VIVO DISTRIBUTION OF ^{3}H-IMIPRAMINE IN BRAIN WITH THE TOPOGRAPHY OF ^{3}H-IMIPRAMINE BINDING TO BRAIN SECTIONS IN VITRO. G.E. Duncan*, I.A. Paul,* G.R. Breese, and W.E. Stumpf. Dept. of Anatomy and Biol. Sci. Res. Center, Univ. of North Carolina, Chapel Hill, NC 27514.

We have applied high resolution autoradiographic techniques to examine ^{3}H-imipramine binding in vivo and in vitro. For the in vivo study, rats were implanted with chronic jugular catheters. Three to 5 days after surgery, the rats were injected via the catheter with ^{3}H-imipramine (2 μCi/g body weight) and killed 1 hr later. Four micron cryostat sections were thaw-mounted on Kodak NTB-3 nuclear emulsion coated slides to produce autoradiograms. For in vitro binding, 4 micron cryostat sections of brain were thaw-mounted on coverslips, incubated in 5 or 10 nM ^{3}H-imipramine, and apposed to NTB-3 nuclear emulsion coated slides. The neuroanatomical topography of ^{3}H-imipramine binding in vitro was identical to that reported by others. Striking differences were observed in the patterns of ^{3}H-imipramine binding in vitro, compared to in vivo. Some brain regions that exhibited very high binding of ^{3}H-imipramine in vitro displayed low binding in vivo. Among such regions are the molecular layer of the dentate gyrus, central grey, and caudate putamen. In other regions, such as the striatum radiatum of the hippocampus and basolateral nucleus of the amygdala, binding of ^{3}H-imipramine was high both in vivo and in vitro. Low delivery of ^{3}H-imipramine in vivo does not explain the differences observed compared to in vitro binding, since high levels of ^{3}H-imipramine occur in all brain regions 5 min after i.v. injection of the compound. It is unlikely that metabolism of ^{3}H-imipramine in vivo accounts for the observed differences. Relatively higher binding in vivo, compared to in vitro would be expected if the radioactivity in brain in vivo represents a metabolite of ^{3}H-imipramine. Endogenous compounds have been extracted from brain that compete for ^{3}H-imipramine binding in brain homogenates. The highest concentrations of these compounds were found in regions (Barbaccia et al., Eur. J. Pharmacol. 123:45), where we find large differences between the in vivo and in vitro conditions . We are currently examining the possibility that the low binding of ^{3}H-imipramine in certain brain regions in vivo, relative to in vitro, is due to endogenous compounds competing for ^{3}H-imipramine binding sites in vivo. Supported by USPHS Grants MH-36294, HD-03110 and NS-09914.

311.4 DIFFERENT CATECHOLAMINE TURNOVER RATES IN STRIATAL COMPLEX IN THE PRESENCE OF REMOXIPRIDE. L.D. Loopuijt* and J.B. Sebens* (SPON:ENA) Dept. Biological Psychiatry, Psychiatric Clinic, Oostersingel 59, 9713 EZ Groningen, the Netherlands.

The striatal complex can be considered as an entity, but is a heterogeneous structure, in which the dopaminergic afferents from the substantia nigra and ventral tegmental area are fitting into the striatal mosaic. In addition, different types of dopaminergic fibers have been described on the ground of morphological criteria (Fuxe et al., Acta Pharm. Suppl. 1983, pp 60-79; Gerfen, Neurosci. Abstr.10,1984,9). Recently, the dopamine D2 receptor antagonist remoxipride has been shown to decrease the turnover of dopamine in a subpopulation of dopaminergic fibers (Fuxe et al. In: Recent Developments in Parkinsons Disease, Raven Press 1986, pp 17-32). Thi prompted us to study the location of the dopaminergic fibers with slow dopamine turnover in the presence of remoxipride. As a marker for the anatomical heterogeneity of the striatal complex was chose acetylcholinesterase (ACHE) histochemistry.

17 days old rats or adult male rats were injected with the tyrosine hydroxylase inhibitor alpha-methyl-DL-p-tyrosine /aMPT) or remoxipride plus aMPT (Astra, Sweden) and sacrificed 2 h later by decapitation, after freezing the brain, sections were cut and subsequently subjected to a procedure for catecholamine fluorescence (de la Torre, J. Neurosci. Meth.3(1980)1-5 or, alternatively, ACHE histochemistry (Geneser-Jensen and Blackstad, Z.Zellforsch. 114 (1971)460-481).

In 17 days old rats, 2 h after injection of remoxipride plus aMPT, fluorescence intensity is high in the mediodorsal part of the nucleus accumbens, at the dorsal end of the major island of Calleja and at the medial rim of the caudate, adjacent to the lateral ventricle. The rest of the caudate-putamen or nucleus accumbens does not show fluorescence above background level. In adult rats, in the nucleus accumbens, its mediodorsal part plus a small area dorsal to the major island of Calleja shows fluorescence. In the entire caudate-putamen fluorescence is above background level, but the intensity is non-homogeneous, being highest in the medial rim of the caudate and in discrete areas in the medial caudate. Comparison of the patterns of fluorescence with ACHE staining show that intense fluorescence coincides with low ACHE staining; in adult animals this includes striosomes.

So, under the conditions used, two populations of catecholaminergic fibers can be shown, which differ in catecholamine turnover rate. Fibers with slow turnover rates are located in areas that do not receive afferents from the motor cortex, but from the prelimbic cortex (striosomes; Donoghue and Herkenham, Brain Res.365 (1986)397-403) or hippocampus (dorsomedial nucleus accumbens; Kelley and Domesick, Neurosci. 7(1982) 2321-2335).

311.5 DIVERGENT PROJECTIONS OF NORADRENERGIC CELLS OF THE A7 GROUP TO THE MOTOR NUCLEUS OF THE TRIGEMINAL NERVE AND THE SPINAL CORD. W.E. Lyons* and R. Grzanna. Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Cells of the A7 group are the principal source of the noradrenergic (NA) innervation of the motor nucleus of the trigeminal nerve (MoV). We have previously shown that approximately 40% of the cells in the A7 group project to MoV and that more half project to the spinal cord. The present study was conducted to determine whether NA projections to MoV and the spinal cord originate from the same or separate populations of A7 cells. Rhodamine (Rh) labeled latex beads and True Blue (TB) were used as retrograde tracer in combination with immunohistochemistry of NA neurons. Rh-labeled beads were injected into MoV and TB was injected into low thoracic segments of the spinal cord of rats. Following a two week survival period, brainstem sections were processed for immunohistochemistry using antibodies to dopamine-beta-hydroxylase (DBH), a marker protein of NA neurons. NA neurons were visualized by the indirect immunofluorescence method using fluoresceinisothiocyanate (FITC) as label. Sections were viewed in the fluorescence microscope using different filter combinations for TB, Rh and FITC. NA cells and retrogradely labeled NA cells were mapped using a computer assisted fluorescence microscope. The position of each Rh and TB labeled NA cell in the A7 group was recorded and the percentage of NA cells containing both retrograde tracers was determined. Since the NA projection to MoV is strictly unilateral, only A7 cells ipsilateral to the injection site were mapped. Following tracer injections into MoV and spinal cord, 47% of the ipsilateral A7 cells contained Rh and 60% contained TB; 26% of the A7 cells did not contain any tracer. The most striking finding of this study was that 71% of Rh-labeled A7 cells also contained TB and that 55% of TB filled A7 neurons contained Rh.

The results of this retrograde transport study demonstrate that divergent projections to brainstem and spinal cord are a prominent anatomic feature of A7 cells. Although the exact site of termination of descending A7 cell axons remains to be determined, one might predict that collaterals of A7 cell axons which innervate motoneurons in MoV terminate on motoneurons in the spinal cord. We propose that A7 cells have widespread projections to spatially separate but functionally similar populations of neurons at different levels of the neuraxis. (Support: NIH grants MH 41977 and NS 16654).

311.6 QUANTIFICATION OF REGIONAL AND LAMINAR DENSITIES OF THE NORADRENALINE INNERVATION IN ADULT RAT CEREBRAL CORTEX. M.A. Audet, G. Doucet, S. Oleskevich and L. Descarries (SPON: J. de Champlain). Centre de recherche en sciences neurologiques (Dép. physiologie), Université de Montréal, Montréal, (Québec), Canada H3C 3J7.

To quantify noradrenaline (NA) innervation density in adult rat cerebral cortex, we have applied an improved method for the radioautographic visualization of these axonal varicosities in semi-thin sections of whole brain hemisphere (Doucet, G . and Descarries, L., 1984, Soc. Neurosci. Abstr. 10: 422). Brain slices (200 µm-thick) were incubated with 1 µM [^{3}H]NA in the presence of a monoamine oxidase inhibitor (10 µM pargyline) and of an inhibitor of catecholamine uptake by dopamine neurons (10 µM benztropine (BZ) or 5 µM GBR-12909 (GBR)). Control incubations were carried out with no uptake inhibitor and in the presence of the NA uptake blocker, desipramine (DMI, 5 µM), with or without BZ or GBR. The slices were fixed with glutaraldehyde and osmium, dehydrated, embedded in Epon and radioautographed as 4 µm-thick sections. NA axonal varicosities, detected as small silver grain aggregates, were numbered with the aid of a computer-based image analysis system (Dapple ImagePlus+). Comparison with control conditions indicated that, in the presence of BZ or GBR, NA varicosities were specifically labeled, and that neither BZ nor GBR reduced their number (paired t-test). In order to estimate the total number of NA varicosities per mm^3 of cortical tissue, correcting factors for incomplete detection at the chosen section thickness and exposure times were determined experimentally, and the average diameter of the labeled profiles was measured by electron microscope radioautography. Counts were then obtained across cortical areas Cg3 and AID at the transverse level A-11.7 mm (interaural), and Cg2, Pr1, Par1, AID and Pir at level A-9.7 mm (Zilles stereotaxic atlas, 1985). The mean regional density of cortical NA innervation (± s.d.) amounted to 1.2 ± 0.2 x 10^6 varicosities per mm^3 without significant difference between these cortical regions (one-way ANOVA). The laminar distribution also showed only minimal variations between regions. Laminar density was always the highest in layer I, with a mean of 1.46 ± 0.2 x 10^6 per mm^3, and showed a slight decreasing gradient towards deeper layers, with 0.96 ± 0.1 x 10^6 per mm^3 in the intermediate layers and 0.53 ± 0.1 x 10^6 per mm^3 in the deepest portion of layer VI. These data indicate that the NA innervation of cerebral cortex is significantly denser than was previously estimated (Lapierre, Y. et al., 1973, Brain Res. 63: 175), and substantiate the rather uniform regional and laminar distributions of these nerve terminals. (Supported by FRSQ and MRC grant MT-3544).

311.7 EPINEPHRINE IN RAT CORPUS STRIATUM: IN VIVO MICRODIALYSIS AND HPLC ANALYSIS. J.L. Baird*, S.D. Glick, J.N. Carlson and K.L. Drew (SPON: L.B. Hough). Department of Pharmacology and Toxicology, Albany Medical College, Albany, New York 12208.

While a high density of B adrenergic receptors has been demonstrated in the rat corpus striata, the presence of epinephrine in this brain region has not been reported. We now report, for the first time, the presence of epinephrine in rat corpus striatum.

In vivo microdialysis has become a powerful tool in the neurochemical analysis of discrete brain regions. In this study bilateral probes were inserted into the corpus striatum using the following coordinates (Pellergrino et al., 1979): anterior, +2.2 (from bregma) and lateral, ±3.0. The probes were lowered to a dorsoventral position of -4.0 (from dura) and the dialysate was recovered from -4.0 to -6.0. Samples were analyzed by reverse phase HPLC on the ESA Coulechem with a model 5011 detector cell. Baseline epinephrine concentrations (N=20) were approximately 50% of baseline dopamine concentrations.

Epinephrine was also assayed in striatal homogenates. The samples were analyzed by reverse phase HPLC on the BAS LC 48 electrochemical detector. Once again peaks corresponding to those of epinephrine were identified; epinephrine concentrations were 36.1±2.9 nmol/gram tissue weight (mean ± SE; N=8), or about 0.3% of dopamine concentrations. These whole tissue homogenates were then spiked with an epinephrine standard and the peak previously identified as epinephrine was increased in peak height and area corresponding to the concentration of epinephrine added. We are currently pursuing the functional role of epinephrine in this brain region. The relatively greater levels of epinephrine in dialysates, compared to homogenates, suggest that epinephrine may be released from a highly active neuronal system. (Supported by NIDA grant DA03817.)

311.8 LOCALIZATION OF ALPHA-2 ADRENORECEPTORS IN THE RAT CNS WITH THE SELECTIVE ANTAGONISTS, [3H]-YOHIMBINE AND [3H]-IDAZOXAN. M. Madar*, D. Bylund, J. P. Yezuita*, J. K. Wamsley (SPON: A. S. Unis) Depts. of Pharm. and Tox. and Psych., Univ. of Utah, Sch. of Med., SLC, UT 84132, and Dept. of Pharm., Univ. of Missouri Columbia, MO 65212.

Alpha adrenoreceptors have been subclassified according to their pharmacological selectivity for agonists and antagonists irrespective of their anatomical localization. There is established evidence for the existence of alpha-1 and alpha-2 adrenergic receptor sites. Distribution of alpha-2 adrenoreceptors has been defined using only the alpha-2 agonist, para-aminoclonidine. Since tritiated agonists appear to preferentially label a high affinity guanine nucleotide sensitive alpha-2 receptor site, use of these ligands may provide only a partial assessment of the entire alpha-2 receptor population. Anatomic localization of these receptors using [3H]-Yohimbine and [3H]-Idazoxan, two selective alpha-2 antagonists, will define both the high and low affinity alpha-2 adrenoreceptors.

The binding characteristics of the selective alpha-2 antagonists have previously been established under less than optimal conditions. After extensive trial and error we determined what appear to be the optimal binding parameters for both [3H]-Yohimbine and [3H]-Idazoxan. Use of a glycylglycine buffer (25mM, pH 7.6) and a 2nM concentration of each drug provided a specific to nonspecific (signal to noise) ratio of 75-90%, with specific binding defined as that inhibited in the presence of 300uM phentolamine. Results of the experiments for both ligands indicate that a 25mM glycylglycine buffer (pH 7.6), devoid of any additional ionic compounds, optimizes the binding of both ligands. From our experiments it was clearly demonstrated that divalent and monovalent cations have a pronounced effect on the specific binding of these radioligands. Equally profound alterations of specific binding were also seen with slight pH changes.

It is now possible to localize the alpha-2 adrenoreceptors using the two antagonists by autoradiographic techniques. The distribution of alpha-2 receptors, as reported by Unnerstall, using [3H]-Para-aminoclonidine, were found in areas innervated by norepinephrine and epinephrine containing neurons as well as in discrete areas of the brain where alpha-2 agonists have been shown to elicit an effect. Qualitatively, the initial experiments correlate well with the regional distribution of alpha-2 adrenergic receptors as defined by [3H]-Para-aminoclonidine. Quantitatively, however, subtle differences were noted in the density and distribution of alpha-2 sites, presumably due to labeling of the low affinity sites.

311.9 **QUANTITATIVE AUTORADIOGRAPHY OF SUBTYPES OF β-ADRENERGIC RECEPTORS FOLLOWING ADMINISTRATION OF DESMETHYLIMIPRAMINE.** E. W. Johnson, B. B. Wolfe and P. B. Molinoff. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6084.

Antidepressant drugs are thought to exert their effects through modulation of the noradrenergic innervation of the CNS. Adult male rats were treated with desmethylimipramine (DMI; 10mg/kg twice daily), a tricyclic antidepressant that blocks the neuronal reuptake of norepinephrine. Animals were sacrificed after 14 days and the brains sectioned for autoradiography to measure the densities of β_1- and β_2-adrenergic receptors. Receptors were labeled with ^{125}I-iodopindolol (^{125}I-IPIN). Specific binding to total β-adrenergic receptors was the binding of ^{125}I-IPIN minus nonspecific binding defined in the presence of 100 μM l-isoproterenol. Binding to β_1-adrenergic receptors was defined as binding of ^{125}I-IPIN in the presence of 11 nM ICI 118,551, a β_2-selective antagonist, minus nonspecific binding. Binding to β_2-adrenergic receptors was defined as binding of ^{125}I-IPIN in the presence of 12 nM ICI 89,406, a β_1-selective antagonist, minus nonspecific binding. Decreases in the density of β-adrenergic receptors were observed in a number of cortical regions. The density of β_1-adrenergic receptors was decreased in the motor and somatosensory cortices while the density of β_2-adrenergic receptors did not change in those regions. The densities of both β_1- and β_2-adrenergic receptors decreased in the lateral and medial septal nuclei. There was no change in the density of either subtype in the dorsolateral caudate putamen following chronic treatment with DMI. These findings support the idea that the subtypes of the β-adrenergic receptor are independently regulated in the rat CNS. The observation that administration of DMI results in changes in the density of β_2-adrenergic receptors in some areas of the rat CNS suggests that these receptors receive a functional neuronal input. (Supported by USPHS Grant GM 34781.)

311.10 CHARACTERIZATION OF A NORADRENERGIC PATHWAY FROM THE DORSOMEDIAL MEDULLA (A2) TO THE HYPOTHALAMIC SUPRAOPTIC NUCLEUS (SON) IN THE RAT. Wilfrid Noel Raby and Leo P. Renaud, Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Quebec, H3G 1A4.

The SON contains both vasopressin (VP) and oxytocin (OXY) synthesizing neurons. This nucleus receives a catecholaminergic input from the A1 noradrenergic cells in the ventrolateral medulla (Sawchenko and Swanson, Brain Res Rev, 4:275, 1982), which selectively excites VP cells (Day and Renaud, Brain Res.,303: 233, 1984). Anatomical tracer studies (e.g. Tribollet et al., Neuroscience 15: 135, 1985) have reported that the A2 neurons in the dorsomedial medulla also project to SON. We now confirm that discrete SON injections of rhodamine labelled latex beads are transported retrogradely to approximately 10% of ipsilateral A2 catecholamine fluorescent cells, and a smaller number of contralateral A2 neurons.

The electrophysiology of this pathway was examined in pentobarbital anesthetized male Sprague-Dawley or Long-Evans rats. A transpharyngeal approach to the hypothalamus and brain stem allowed placement of a glass coated tungsten monopolar electrode in the dorsomedial medulla to activate A2 neurons (0.2 msec, < 150 microamps). A bipolar electrode in the neurohypophysis served to antidromically activate neurosecretory neurons. During recordings, VP cells were characterized by their phasic or continuous firing pattern, and a depression in activity when blood pressure was elevated by i.v. alpha-agonists; OXY cells fired in a continuous fashion, and remained unaffected by blood pressure elevation. Results indicate that 70% of both VP cells (n=40) and OXY cells (n=20) demonstrate a brief (30-50 msecs) increase in firing probability, (latency range: 50-75 msecs) following ipsilateral A2 stimulation. Similar responses were also noted after contralateral A2 stimulation.

These findings support the anatomical tracer studies and identify an excitatory A2 noradrenergic innervation of SON, distributed to both VP and OXY neurons. The neural information conveyed by this pathway may relate to cardiovascular, visceral, or osmotic homeostasis. (Supported by the MRC)

311.11 AN ULTRASTRUCTURAL CHARACTERIZATION OF A GABA-MEDIATED INPUT TO RAT SUPRAOPTIC NUCLEUS (SON) FROM THE DIAGONAL BAND OF BROCA (DB). J.H. Jhamandas, J. Rogers*, R.M. Buijs* and L.P. Renaud. Neurosciences Unit, The Montreal General Hospital and McGill Univ. Montreal, Quebec, Canada, H3G 1A4

Recent electrophysiological evidence suggests that γ-aminobutyric acid (GABA) may serve as an inhibitory neurotransmitter in a pathway from the DB that selectively depresses the excitability of the vasopressin (VP)-secreting neurons of the hypothalamic SON. The present study was conducted to ascertain the anatomical configuration of this input at the ultrastructural level using a combined horseradish peroxidase (HRP) and immunocytochemical approach.

Animals received an injection of 30% HRP in the DB. After survival (18-24 hrs), animals were perfused and fixed brains processed for HRP enzymatic activity using diaminobenzidine (DAB). Injection sites were verified at the light microscopic level and the distribution of the anterogradely labelled terminals in the region of the SON evaluated at the ultrastructural level. GABA-synthesizing terminals were identified by a post-embedding colloidal gold technique. Simultaneous localization of VP and GABA on DAB stained sections was obtained by a combined pre- and post-embedding immunocytochemical approach. HRP- and GABA-positive terminals were counted both within and outside the SON.

The greatest accumulation of HRP-labelled terminals was found in a region immediately dorsal to the SON (18,000 per mm^2). Substantially lower values (1200 per mm^2) were recorded within the SON. Terminals displaying GABA immunoreactivity were preferentially distributed within the SON and constituted two thirds of terminals contacting VP-immunopositive elements. GABA was seldom co-localized within HRP-labeled terminals either in the SON or the perinuclear area.

These observations indicate that the axons of DB coursing towards the SON terminate preferentially in the perinuclear zone. The lack of GABA immunoreactivity within these perinuclear terminals, but its presence in the SON suggests that a GABAergic inhibitory interneuron, located in the perinuclear area, is interposed in the pathway from DB to SON VP-secreting neurons. (Supported by MRC).

311.12 IN VIVO BINDING OF [^{3}H]HALOPERIDOL AND [^{3}H]d-SKF 10,047 IN MOUSE BRAIN. A.D. Weissman, E.P. Broussolle* and E.D. London. Neuropharm. Lab., NIDA Addiction Res. Ctr., Baltimore, MD 21224.

Quantitative autoradiographic studies of slide-mounted sections of guinea pig brain in other laboratories and ours have demonstrated specific labelling of sigma binding sites using [^{3}H]d-SKF 10,047 (SKF) and [^{3}H]haloperidol (HAL) as ligands (Largent, B.L. et al., J. Pharm Exp. Ther. 238:739 1986). As an extension of these experiments, we examined the regional in vivo specific binding of the same radioligands used in vitro.

Male ICR mice received an injection of tracer amounts of HAL or SKF via the tail vein (3 μCi/mouse). Total and nonspecific binding were determined in different mice which received s.c. injections of either saline or a combination of sigma and phencyclidine (PCP) receptor agonists or antagonists, respectively. HAL binding to sigma sites was assayed with spiperone to block binding of the ligand to D2 dopamine receptors and haloperidol or d-pentazocine to displace specific binding. Specific SKF binding to sigma sites was defined by displacement with N-(1-[2-thienyl]cyclohexyl)3,4-piperidine (TCP) and haloperidol. SKF binding to PCP sites was identified by displacement with TCP alone. The doses and the times of injection of these cold displacer drugs were selected from information about their behavioral effects, brain entry, metabolism and toxicity. The mice were killed by decapitation 30 min after injection of the radioligands. Brains were rapidly removed, and several regions dissected out, dissolved in hyamine hydroxide and counted by liquid scintillation spectroscopy.

Specific HAL binding to sigma sites was highest in the cerebellum, medulla-pons and midbrain. Specific binding in the thalamus, striatum and cortex was approximately half of that in the former regions, and was even lower in the hippocampus. A similiar distribution of in vivo sigma binding was obtained with SKF. In these experiments, the medulla-pons had the highest specific binding, but all other brain regions showed the same relative level as that seen with HAL. Specific binding of SKF to PCP receptors appeared to have a more uniform regional distribution in the brain in contrast to the results obtained with this ligand in vitro.

The results of this study demonstrate a similar pattern of regional sigma receptor binding in the mouse brain using either HAL or SKF, in agreement with in vitro data in several rodent species. In vivo specific binding of SKF to PCP receptors, however, does not show the regional distribution seen with in vitro studies, indicating that SKF may not be a satisfactory ligand to visualize PCP receptors in vivo.

311.13 CORTICAL LESIONS DECREASE BASAL AND AMPHETAMINE-INDUCED RELEASE OF ASCORBATE IN THE NEOSTRIATUM. Allison Basse-Tomusk and George V. Rebec. Dept. Psychol., Indiana Univ., Bloomington, IN 47405

The neostriatum contains very high levels of extracellular ascorbate (AA) that show marked circadian (O'Neill et al. (Neurosci. Lett, 42:105, 1983) and drug-induced changes (Kamata et al. Brain Res. 362:331, 1986). However, the source or sources of neostriatal AA release are unknown. O'Neill et al. (1983) have suggested that basal extracellular AA levels in the neostriatum are regulated by the corticostriate pathway since cortical lesions decrease extracellular AA levels by 80%. In the present experiment, we sought to determine whether the corticostriate pathway also is involved in amphetamine-induced AA release.

Adult, male rats received bilateral-suction lesions of the dorsal aspect of the neocortex from 1mm posterior to bregma to the frontal pole. Another group of animals received sham lesions in which only the dura was removed. Following an 8 to 10 day recovery period, basal and amphetamine-induced (2.0 mg/kg, i.v.) AA release were assessed with in vivo voltammetry using electrochemically-modified carbon fiber electrodes. These electrodes provide a voltammetric wave for AA that is resolved easily from that for catechols and all other electroactive species in the mammalian brain. Voltammetric scans, obtained at 2-min intervals, were displayed in a differential form.

Consistent with previous reports, cortical lesions decreased basal levels of AA by 80%. Furthermore, amphetamine-induced AA release also was reduced dramatically. These results suggest that the corticostriatal pathway plays a crucial role in the neuromodulatory actions of neostriatal AA.

Supported by NSF Grant BNS 84-16303 (GVR).

311.14 HALLUCINOGENS BIND TO COMMON RECEPTORS IN THE RAT FOREBRAIN: A COMPARATIVE STUDY USING ^{125}I-LSD AND ^{125}I-DOI, A NEW PSYCHOTOMIMETIC RADIOLIGAND. D. J. McKenna*[1], C. A. Mathis*[2], A. T. Shulgin*[2], & J. M. Saavedra[1] (SPON: N. Buckholtz). 1. Section on Clinical Pharmacology, Laboratory of Clinical Science, NIMH, Bethesda, MD 20892 2. Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

Autoradiographic methods were applied to the characterization of hallucinogen-specific receptors using two $5HT_2$ specific ligands, ^{125}I-LSD, and a new psychotomimetic radioligand, [^{125}I]-4-iodo-2,5-dimethoxyphenylisopropylamine (^{125}I-DOI). The R(-) and S(+) enantiomers of ^{125}I-DOI were synthesized to radiochemical purity at a specific activity of 1700 Ci/mmol and 1200 Ci/mmol, respectively. In rat cortical homogenates R(-)-[^{125}I]-DOI showed saturable, specific binding (Kd: 1.41 nM, Bmax: .112 pmol/mg protein, one site model). Sixteen micron rat forebrain sections incubated in 200 pM concentrations of each enantiomer showed high densities of specific binding in the cortex (layer IV), claustrum, lateral olfactory tracts, nucleus acumbens, and diagonal band. Both enantiomers were completely displaced from all sites by unlabelled DOI and unlabelled LSD (1 uM). Sections incubated with ^{125}I-LSD under identical conditions showed a similar pattern of regional specific binding. ^{125}I-LSD also showed specific binding in the caudate-putamen, while ^{125}I-DOI showed virtually no specific localization in this region. Incubation of 200 pM ^{125}I-LSD or the ^{125}I-DOI enantiomers in the presence of various hallucinogens and hallucinogen analogs (500 nM) showed selective, regional displacement. ^{125}I-LSD was specifically displaced from the claustrum and cortex, but not from the caudate, nucleus acumbens, or olfactory tracts, by both enantiomers of DOI and DOB, but not by the inactive analog alpha-ethyl DOM. The tryptamine hallucinogens DMT and 5-MeO-DMT at 500 nM partially displaced ^{125}I-LSD. ^{125}I-DOI showed a similar displacement pattern in the presence of these unlabelled ligands. The displacement of two psychotomimetic radioligands from binding sites in the claustrum and cortex by members of each structural class of hallucinogens provides evidence that indole, ergoline, and phenylisopropylamine hallucinogens act at common receptors located in these brain regions.

311.15 COLOCALIZATION OF TRANSMITTER BINDING SITES ON THE LEVEL OF HIPPOCAMPAL LAYERS IN THE RAT AND HUMAN BRAIN. K. Zilles*, A.Schleicher, E.Horvath, D.Spencer. Anatomical Institute, University of Köln, 5000 Köln 41 and Troponwerke, 5000 Köln 80, FRG.

Information processing in the hippocampus depends on the action of many different transmitters. As a first step, to analyze possible interactions, the correlation in regional distribution of receptors for the classical transmitters acetylcholine (ACh), glutamate (Glu), GABA and serotonin (5-HT) was studied in all regions and layers of the rat and human hippocampus on frozen sections (20µm) with quantitative receptor autoradiography. The muscarinic ACh receptors were labelled with (3H)N-methyl-scopolamine(0.2nM), the M1 subtype with (3H)pirenzepine (5-10nM), The M2 subtype with (3H)oxotremorine-M, the Glu receptors with (3H)L-glutamate (100nM) both in presence or absence of Ca^{++}/Cl^{-}, the GABA receptors with (3H)muscimol (5-10nM), the 5-HT1 receptors with (3H)5-HT (2.5nM), and the 5-HT1A subtype with (3H)ipsapirone (5nM). Specific binding was measured in presence of the respective unlabelled compounds with an image analyzer (Zilles,K. et al., J.Neurosci.Meth., 18:207,1986).

The comparison of the quantitative distribution pattern of the actual binding sites with the immunohistochemically identified axonal terminals shows a clear mismatch in all cases, which suggests a different regulation of transmitters and their receptors. The comparison between the distribution in rat and man shows a high similarity in the Glu receptors, but no concordance in 5-HT receptors, which indicates caution in generalizing the rat model. The most important result is the colocalization of some, but not all of these receptors on the level of hippocampal layers. High correlations between the distributional pattern of 5-HT1A receptors on one side and M1 or Glu receptors on the other side, as well as between M1 and Glu receptors have been found. This can be an argument for interactions of different receptors in the same layer, which has already been demonstrated for Glu and α_1-adrenoreceptors (Nicoletti,F. et al., Proc. Natl.Acad.Sci.USA, 83:1931,1986).

311.16 NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA OBLONGATA CONTAIN MULTIPLE MESSENGERS. D.E. Millhorn, T. Hökfelt and K. Seroogy. Dept. of Physiology, University of North Carolina, Chapel Hill, N.C. 27514 and Dept. of Histology, Karolinska Institute, Stockholm, Sweden.

Although it is generally accepted that neuronal networks in the ventrolateral aspect of the medulla oblongata play an essential role in control of the respiratory and cardiovascular systems, relatively little is known about the chemical nature of cells in this region of the brainstem. The present study was devoted to identifying coexistence patterns of neurotransmitters and peptides in a region of the ventrolateral medulla that corresponds anatomically to nucleus paragigantocellularis (PGL).

Adult rats were pretreated with colchicine 24-48 hr prior to transcardial perfusion with a mixture of picric acid and formalin. Sections were cut on a cryostat (14µm) and processed for an indirect immunofluorescence technique that allowed simultaneous identification of multiple chemical messengers in individual cells. In some instances Fluoro-Gold (FG), a retrograde transported dye, was injected into either the nucleus tractus solitarii (NTS) or spinal cord 7 days prior to colchicine pretreatment.

Three types of coexistence (transmitter-transmitter, transmitter-peptide and peptide-peptide) were found. For example, a substantial number of individual cells in PGL stained positive for the classical transmitters serotonin (5-HT) and GABA. Moreover, a number of 5-HT/GABA cells were labelled with FG that had been injected into the thoracic spinal cord. We also found that 5-HT in the region of PGL coexists with several peptides including enkephalin (ENK) and cholecystokinin (CCK). The type of coexistence most often encountered involved the peptides somatostatin (SOM) and ENK. Numerous perikarya that showed positive immunostaining for both SOM and ENK were found at all rostral-caudal levels of PGL. Furthermore, a substantial proportion of the SOM/ENK cells were labelled with FG that had been injected into either NTS or the spinal cord. Finally, we found that essentially all 5-HT somata in PGL and caudal raphe complex as well as the epinephrine-containing cells in the C1 area of the ventrolateral medulla showed positive immunostaining for acetylcholinesterase. (Supported by Swedish MRC grant 04X-2887 and NIH grant HL 33831).

311.17 DISTRIBUTION OF MULTIPLE SECOND-MESSENGER SYSTEMS IN THE TEMPORAL CORTEX AND HIPPOCAMPUS OF THE RHESUS MONKEY. D.L. Price†, M.V. Wagster†, P.F. Worley*§, L.C. Walker† and J.M. Baraban*§. †Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med, Baltimore, MD 21205; §Dept. of Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Some neurotransmitters act through second-messenger systems to modulate synaptic interactions. To help define the role of second-messenger systems in the brain, the distribution of key components of these systems has been mapped in rat brain using in vitro receptor autoradiography [Worley et al., Ann. Neurol. 21:217-229, 1987]; discrete, heterogeneous labeling patterns were demonstrated with [^{3}H] forskolin, [^{3}H] phorbol 12,13-dibutyrate (PDBu), and [^{3}H] inositol 1,4,5-trisphosphate (IP3), which label the Gs-adenylate cyclase complex, protein kinase C, and IP3 receptor binding sites, respectively. Because receptor distributions may vary among species, the present autoradiographic study mapped these receptor sites in the temporal neocortices and hippocampi of two-year old rhesus monkeys (Macaca mulatta) (n = 5). In neocortex, [^{3}H] forskolin autoradiography showed a significant difference (ANOVA, $p < 0.0001$) in binding among six cortical layers, with the highest concentrations occurring in layers II and III and the lowest levels occurring in layer VI. In hippocampus, [^{3}H] forskolin binding was highest in CA4 and the dentate gyrus; the molecular layer showed higher binding than the granule cell layer. In neocortex, [^{3}H] PDBu binding varied significantly (ANOVA, $p < 0.0001$) among the six cortical layers, with the highest binding occurring in layer II and the lowest in layer IV. In hippocampus, [^{3}H] PDBu binding was highest in CA1. In the dentate gyrus, a slightly higher binding concentration was seen in the molecular layer than in the granule cell layer. As in the rat brain, the pattern of [^{3}H] IP3 binding was similar to that observed with [^{3}H] PDBu. In general, binding of the three ligands in temporal cortex exhibited distinct laminar distributions, with binding concentrations higher in the superficial than in deeper neocortical layers. However, in hippocampus the distribution of receptor binding for the adenylate cyclase system was markedly different than that of markers for the phosphatidylinositol system. In areas examined in monkeys, binding patterns of distribution of each of these three ligands were similar to those demonstrated in rat, indicating that the distribution of second-messenger systems may be conserved in mammalian brain.

Tissues were obtained from the Regional Primate Research Center at the University of Washington, supported by NIH grant RR 00166.

MODULATORS II

312.1 INTERACTIONS OF ETHANOL WITH GLUTAMATE AND GABA EVOKED RESPONSES OF CEREBELLAR PURKINJE NEURONS. R.-S. Lee, N. Shimizu, and D.J. Woodward. *Dept. of Cell Biology and Anatomy, The University of Texas Health Science Center at Dallas, TX 75235.*

Acute ethanol intoxication is known to produce substantial impairment of motor function and to result in certain cerebellar symptoms, such as ataxia. Previous studies from this laboratory demonstrated that ethanol may enhance the inhibitory action of GABA on cerebellar Purkinje cell (P-cell) discharge. To further examine the effect, it is of interested to study the action of ethanol on other putative neurotransmitter within cerebellum. The goal of the present study was to determine (1) the effect of acute ethanol intoxication on the response of individual P-cells to glutamate (GLUT), a putative neurotransmitter in cerebellum and (2) whether imidazobenzodiazepine Ro15-4513 (ethyl-8-azido-5,6- dihydro-5-methyl-6-oxo-4H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate), a putative antagonist of ethanol-GABA interaction (P. D. Suzdak et al., Science, 234: 1243-1247, 1986), antagonizes the enhancement of GABA-mediated inhibition by ethanol in P-cells of the cerebellum.

Long-Evans rats were anesthetized with halothane (2.0 % or 0.7 % in oxygen during surgery and electrophysiology, respectively). An intraperitoneal catheter used for administration of ethanol (1.5 to 2.0 gm/kg, 10-15% in saline, w/v) and Ro15-4513 (4-6 mg/kg as a 4% Tween 80 in saline suspension) was implanted before the recording session. Extracellular activity of single cerebellar P-cells was recorded with five-barrel micropipettes. GABA (0.2 M, pH=4.3) and GLUT (monosodium salt, 0.5 M, pH=8.2) were administered by microiontophoretic pulses (10 sec every 40 sec). Spontaneous discharge, and influences to GABA and GLUT responses in P-cells were monitored before and 1 to 1.5 hours after systemic administration of ethanol. A transient inhibition of spontaneous neuronal activity was observed usually within 4-8 minutes of ethanol injection. This effect lasted 2-4 minutes in 5 of the 8 rats tested. After the recovery of spontaneous discharge to control levels, we found: (1) GLUT-mediated excitatory responses were increased (10-35%) (n=5) relative to pre-ethanol values between 15 and 90 minutes post-injection, (2) imidazobenzodiazepine Ro15-4513 (4-6 mg/kg) failed to antagonize the enhancement of GABA-mediated inhibition by ethanol (n=3). These results indicate that ethanol may enhance both the inhibitory action of GABA and the excitatory actions of GLUT on spontaneous discharge of P-cells. The slow onset reaction of P-cell to both GABA and GLUT suggested that a possible indirect metabolic action was elicited by ethanol via systemic administration. Our view is that the modulatory actions of ethanol on synaptic physiology observed in the present study may reflect mechanisms of the CNS effects of acute ethanol intoxication. *Supported by grants AA-3901, DA-02338 to DJW and the Biological Humanics Foundation.*

312.2 PERIPHERAL ADMINISTRATION OF ASCORBATE MODULATES UNIT ACTIVITY IN THE NUCLEUS ACCUMBENS. L.K. White, A. Basse-Tomusk, and G.V. Rebec. Dept. of Psychology, Indiana Univ., Bloomington, IN 47405.

Ascorbate (AA) is present in high concentrations in the mammalian brain, and appears to play a neuromodulatory role in several brain areas. In the neostriatum, AA accelerates the firing rate of neurons when administered intraperitoneally (Ewing et al., Brain Res., 261:101, 1983), or when applied iontophoretically in conjunction with glutamate (Gardiner et al., Brain Res., 344:181, 1985). These results have important behavioral implications because AA appears to modulate certain drug-induced responses that appear to involve neostriatal neurons. AA, for example, potentiates the catalepsy produced by haloperidol and attenuates the behavioral response to amphetamine (Rebec et al., Science, 227:438, 1985). Like the neostriatum, the nucleus accumbens, which also has been implicated in these drug-induced behaviors, contains a high level of extracellular AA. It is conceivable, therefore, that AA modulates neuronal activity in this brain region in much the same way that it does in the neostriatum. As a first step in our investigation of this hypothesis, we monitored the response of nucleus accumbens neurons to systemic injections of AA.

Adult, male rats, anesthetized with urethane, received 500, 1000, or 1500 mg/kg AA via an indwelling intraperitoneal catheter. Single-unit activity in the nucleus accumbens was monitored for at least 1 hour following AA administration. At each dose approximately 80% of all neurons sampled showed a clear change in firing rate that typically began within 15 min. after AA administration and that persisted throughout the recording session. Both excitations and inhibitions were recorded with equal frequency. These results suggest that, as in the neostriatum, AA plays an important neuromodulatory role in the nucleus accumbens.

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312.3 **THE MAJOR 27 kDa GAP JUNCTION PROTEIN IS PHOSPHORYLATED BY cAMP DEPENDENT AND Ca^{2+} DEPENDENT PROTEIN KINASES.** J.C. Saez, A.C. Nairn*, D.C. Spray, E.L. Hertzberg+, P. Greengard*, M.V.L. Bennett. Albert Einstein Coll. Med., Bronx, NY *Rockefeller University, New York, +Baylor Coll. Med. Houston, TX.

In a number of cell types intercellular coupling mediated by gap junctions can be modulated by second messengers, for example cAMP and Ca^{2+}, which can have opposite effects. In hepatocytes from rat elevation of intracellular cAMP increases junctional conductance between pairs of cells and stimulates phosphorylation of the major gap junction protein (PNAS 83: 2473, 1986). The present study shows that both Ca^{2+}/calmodulin dependent protein kinase (Ca^{2+}/CaM kinase II) and protein kinase C (both purified from rat brain) phosphorylate the 27 kDa protein in gap junction membranes from either rat or mouse liver. The 21 kDa protein which is particularly abundant in mouse was not phosphorylated by either of these kinases or by cAMP-dPK. The maximal stoichiometry for Ca^{2+}/CaM kinase and for kinase C was 0.3 mol/mol and 0.1 mol/mol respectively. Phosphorylation by kinase C required triton X-100. Moreover, optimal phosphorylation was obtained following addition of phosphotidylserine and diacylglycerol, which presumably at least partially reconstituted the lipid environment for the reaction.

cAMP-dPK, and protein kinase C phosphorylated only serine residues. Ca^{2+}/CaM kinase II phosphorylated mainly serine but also threonine to a minor extent. Two-dimensional tryptic mapping showed that Ca^{2+}/CaM kinase II phosphorylated a different serine from cAMP-dPk. Protein kinase C phosphorylated mainly the site phosphorylated by cAMP-dPK; however additional sites were phosphorylated to a smaller degree. There was no difference between rat and mouse 27 kDa protein in respect to sites and extents of phosphorylation.

A polyclonal antibody that recognizes the 27 kDa protein was used to immunoprecipitate the protein from intact hepatocytes. Cells dissociated into small groups were labeled with ^{32}P (PO_4^{3-}) for 1 hr. Cells were stimulated with forskolin (10 uM), Ca^{2+} ionophore A23187 (40 uM in 1 mM Ca^{2+}) or phorbol ester (PDBu 10-100 nM). Incorporation of ^{32}P into the 27 kDa protein rapidly (5 min) increased during all three treatments. Phosphoamino acid analysis of the immunoprecipitated protein showed only phosphoserine, and tryptic fingerprints were similar to those obtained from the 27 kDa protein phosphorylated in purified gap junctions by either cAMP-dPk or protein kinase C. Although it has been reported that in several cell types phorbol esters cause uncoupling, in this system PDBu or PMA (10-100nM) had no effect on dye transfer (Lucifer Yellow). Measurements of electrical coupling after phorbol ester or A23187 stimulation will help to clarify the role of the different kinases in modulating intercellular communication.

312.4 **HYDROLYSIS OF PI, PIP, AND PIP_2 IN ROD OUTER SEGMENTS OF DARK-ADAPTED BOVINE RETINA IS REGULATED BY DIFFERENT GTP-BINDING PROTEINS.** C.L. Jelsema* and A.D. Ma* (SPON: C.R. Merril). Laboratory of Cell Biology, NIMH, Bethesda, MD 20892.

We have previously demonstrated that light stimulates phospholipase A_2 activity in dark-adapted rod outer segments (DROS) of bovine retina by a transducin-dependent mechanism (Jelsema, JBC,262:163,1987) involving the $\beta\gamma$ subunits of this GTP-binding protein (G protein) (Jelsema and Axelrod, PNAS, in press). We now report that phospholipase C (PLase C) activity in DROS is also stimulated by light and is modulated by pertussis toxin (PT)- and cholera toxin (CT)-sensitive G proteins. PLase C activity of DROS membranes was measured by in vitro assay using either ^3H-inositol-labeled phosphatidylinositol (PI), PI-4-phosphate (PIP), or PI-4,5-bisphosphate (PIP_2) as the phosphoinositide substrate. While PIP was the major PLase C substrate in these membranes, its hydrolysis was unaffected by exposure to light and/or addition of GTPγS. In contrast, hydrolysis of both PI and PIP_2 was increased upon light exposure. While addition of GTPγS had no effect on either PI or PIP_2 hydrolysis in DROS, it markedly enhanced light-induced PIP_2 hydrolysis while inhibiting light-induced PI hydrolysis. This differential sensitivity suggests that the hydrolysis of these phosphoinositides may be regulated by different G proteins. This hypothesis was further supported by the different effects of CT and/or PT pretreatment of DROS on phosphoinositide hydrolysis. PT pretreatment (10 ng/ml) enhanced both basal and light-induced hydrolysis of PI and PIP_2 . In contrast, CT pretreatment (50 ng/ml) had no significant effect on the light-induced hydrolysis of either phosphoinositide, but inhibited basal PI hydrolysis while stimulating basal PIP_2 hydrolysis. The basal and light-induced hydrolysis of both phosphoinositides thus appeared to be regulated by (a) pertussis toxin-sensitive G protein(s), whereas basal hydrolysis was additionally controlled by (a) CT-sensitive G protein(s) able to stimulate PIP_2 hydrolysis and inhibit PI hydrolysis. PIP hydrolysis, which was unaffected by light and/or GTPγS, was inhibited by CT in the presence or absence of light, while PT proved inhibitory only in the absence of light or in the presence of GTPγS. PIP hydrolysis, therefore, also appears to modulated by (a) PT- and CT-senstivite G protein(s), but its regulation is distinct from the regulation of either PI or PIP_2 hydrolysis.

These results demonstrate that multiple G proteins regulate PLase C activity in bovine ROS. More importantly, it appears that hydrolysis of each phosphoinositide is regulated either by different G proteins or by different G protein-dependent mechanisms. A role for transducin in the light-induced hydrolysis of PI and PIP_2 is suggested by the capacity of exogenous transducin to partially restore the light-induced PLase C activity of dark-adapted transducin-poor ROS.

This work was supported by a National Research Science Award (IF32MH09423-01) to CLJ and by a Howard Hughes Medical Institute Research Scholarship to ADM.

312.5 **PHOSPHORYLATION OF THE α SUBUNITS OF Gi, Go, Gs AND TRANSDUCIN BY cAMP-DEPENDENT PROTEIN KINASE AND THREE ISOZYMES OF PROTEIN KINASE C: SUBSTRATE SPECIFICITIES AND FUNCTIONAL CONSEQUENCES.** A.D. Ma*, S. Jaken*, and C.L. Jelsema* (SPON: B.B. Stanfield). Laboratory of Cell Biology, NIMH, Bethesda MD 20892 and the W. Alton Jones Cell Science Center, Lake Placid NY 12946.

The α but not the $\beta\gamma$ subunits of the GTP-binding proteins (G proteins) Gi, Go, Gs and transducin (T) serve as substrates for protein kinase C and cAMP-dependent protein kinase (A kinase) when assayed in vitro using isolated G protein subunits together with isolated kinases. Three distinct forms of protein kinase C (types 1, 2 and 3) were employed in this study along with the catalytic subunit of protein kinase A. The C kinases, isolated from rabbit brain, have been previously characterized using histone III-S as the substrate (Jaken and Kiley, PNAS, in press). For each of the C kinases, Giα was the preferred G protein substrate. The phosphorylation of Giα observed with C kinase type 2 was 2.5 times that obtained with either C kinase type 1 or 3. In contrast, phosphorylation of Goα was greatest with C kinase type 3, where it approached the level of Giα phosphorylation obtained with this kinase. The phosphorylation of Goα induced by C kinases type 1 and 2 was 30 and 60%, respectively, that obtained with the type 3 C kinase. For both Giα and Goα, the A kinase-induced phosphorylation was less than 5% that observed with the type 3 C kinase. While Giα and Goα were clearly better substrates for the C kinases than for A kinase, the opposite was true for Gsα and Tα. The 52K form of Gsα was phosphorylated equally by kinase A and the C kinases, and its phosphorylation by A kinase was twice that observed with Giα and Goα. The 45K form of Gsα, however, showed a marked preference for the A kinase. The levels of phosphorylation observed with A kinase were at least 4 times that observed with any of the C kinases, and there was again little difference in the phosphorylating capacity of the C kinases. Tα proved to be the least favored substrate for the C kinases, with the type 3 C kinase resulting in phosphorylation levels less than 10% that obtained with Giα or Goα. While the A kinase-induced phosphorylation of Tα was only a third that obtained with the 45K form of Gsα, it was more than double the level of A kinase-induced phosphorylation of Giα and Goα. These results demonstrate that the G protein α subunits are preferentially phosphorylated by different kinases. In addition, the binding of GTP or GDP (or their thiol analogs) as well as the state of association or disassociation of the G proteins (induced either by addition of GTP or GDP or by addition of the $\beta\gamma$ subunits) differentially altered their capacity for phosphorylation by specific kinases. Furthermore, phosphorylation of these α subunits, in addition to previously reported effects on adenylate cyclase activity, was found to alter the role of these G proteins in the modulation of phospholipases A_2 and C. This work was supported by a Howard Hughes Medical Institute Research Scholarship to ADM and a National Research Science Award (1F32MH09423-01) to CLJ.

312.6 GTP-BINDING POLYPEPTIDE PATTERNS OF RAT BRAIN REGIONS AND SUBCELLULAR FRACTIONS. S. P. Mahadik, J. Satav*, and M. J. Modak*. Div. of Neurosci., N. Y. State Psychiatric Institute and Dept. of Biochem. & Molecular Biophys., Coll. of Physicians & Surgeons of Columbia Univ. NY 10032 and Dept. of Biochem. Univ. of UMDNJ, Newark, NJ 07103.

In brain, GTP-binding proteins (G-Ps) are assumed to play functional role in a variety of cellular processes (neurotransmitter and hormonal receptor-mediated signal transduction, ionic homeostasis and protein synthesis). They are considered to exist as a large family of proteins to carry out unique cellular functions. Since each region of the brain consists of several types of neuronal and glial cells it is difficult to identify G-protein associated with a specific cell type or with a specific cellular process. We report the regional and subcellular distribution of G-proteins in adult rat brain. The proteins were labeled by photoaffinity labeling with α-P32-GTP and labeled polypeptides were analysed by SDS-polyacrylamide slab gel electrophoresis followed by autoradiography (Basu & Modak, J. Biol. Chem.262:2369, 1987). The labeling was dependent on the presence of divalent metal ions. Distinct labeling patterns were seen in the presence of Mn, Mg and Ca. In the presence of EDTA three polypeptides (80 kDa, 35 kDa & 24 kDa), which were labeled faintly in presence of Mn, were labeled intensely. Following studies were carried out in the presence of Mn. A total of 18 labeled polypeptides (ranging in molecular wts. 20 to 200 kDa) were identified in cortex, hippocampus, striatum and cerebellum. All but one (73 kDa) polypeptides required UV exposure for stable labeling. The labeling was eliminated by pretreatment with heat or protease or GTP. Labeling was reduced by pretreatment with GDP>ATP and no changes were found after pretreatment with c-GMP and c-AMP. The region-specific differences in the amount of individual G-P were consistently noted. G-Ps of subcellular fractions (nuclei, microsome, mitochondria, myeline and synaptic plasma membranes, SPM) of cerebral cortex were also analysed. Each fraction showed a characteristic pattern. There was an enrichment in a distict set of G-Ps in each fraction. The pre- and postsynaptic neurotransmitter receptor containing SPM fraction showed four major G-Ps (110 kDa, 56 kDa, 48 kDa & 21 kDa) and the concentrations of two of them (56kDa & 21 kDa) were highest compared with other fractions. It is not possible yet to identify these G-Ps with any particular receptor. Observations described here are the first report on the G-binding protein patterns in the various brain regions. These studies should permit determination of the quantitative and qualitative changes in individual GTP-binding proteins as a function of physiological change or as a result of pathological condition.

312.7 IDENTIFICATION, PARTIAL PURIFICATION AND IMMUNOHISTOCHEMICAL LOCALIZATION OF A 94 kD PHOSPHOPROTEIN PRESENT IN ASTROCYTE-LIKE CELLS IN THE BRAIN. A-M. O'Carroll*, J. Patel* and D.M. Jacobowitz, Laboratory of Clinical Science, NIMH, Bethesda, MD 20892.

A cAMP-stimulated phosphoprotein with an apparent molecular weight of 94 kD on SDS-PAGE was identified in the rat neostriatum. Subcellular fractionation of the neostriatum revealed that the 94 kD protein was predominantly localized to the cytosolic fraction. This protein has been partially purified from this source. Purification was monitored by autoradiograpy of ^{32}P-phosphorylated samples separated by SDS-PAGE. The isoelectric point of the phosphorylated protein was revealed to be 4.7 by two-dimensional gel electrophoresis and phosphorylation was found to occur exclusively on serine. A rabbit antibody was prepared to this protein by the injection of the purified protein band cut from a SDS-gel.

Immunohistochemical localization of the 94 kD protein in formalin-fixed vibratome sections revealed astrocyte-like cells with processes in the gray and white matter of the rat and monkey cortex, striatum and cerebellum. Processes were also observed to make connections with blood vessels. In the cerebellum Bergmann fibers were observed in addition to processes which enveloped purkinje cells. In conclusion, preliminary results reveal that we have isolated a 94 kD cytosolic phosphoprotein that appears to be specifically localized to a population of astroglial cells.

312.8 POTENTIATION OF VOLTAGE-ACTIVATED CURRENTS BY SEROTONIN IN XENOPUS OOCYTES INJECTED WITH RAT CAUDATE mRNA. B. Gillo*, S. Sealfon*, T.M. Moriarty*, J.L. Roberts and E.M. Landau. Dept. of Psychiatry and Dept. of Neurobiology, Mt. Sinai School of Medicine and Bronx V.A. Hospital, New York, N.Y.

Oocytes of the African frog Xenopus laevis have the capacity to translate injected foreign messenger RNA (mRNA) and express functional receptors and voltage-activated channels. We used the oocyte electrophysiological preparation to study the interaction of brain serotonin (5-HT) receptors with brain voltage-activated currents.

Total cytosolic RNA was extracted from 15 day old rat caudate nucleus by the urea/lithium chloride method. Total RNA was microinjected into defolliculated Xenopus oocytes (100-450 ng/oocyte). Cells were tested for functional expression of mRNA at least two days after microinjection. Single cells were two-electrode voltage clamped in a standard superfusion apparatus. Voltage step protocols were controlled by micro-computer.

The oocyte was voltage clamped near resting potential. The membrane was hyperpolarized to -100 mV for five seconds followed by a series of depolarizing voltage steps. Voltage jumps from -100 mV to 0 mV evoked two peaks of hyperpolarizing current of 500-3000 nA (T_{out}). Voltage jumps from -100 mV to +40 mV evoked a hyperpolarizing current of 100-1000 nA (H_{out}). H_{out} could be elicited in calcium free medium. These evoked responses were attenuated on repetition of the voltage step protocol.

Bath application of 20 nM 5-HT induced a rapid transient depolarizing current of 500-2000 nA followed by a sustained and slowly decaying 50-200 nA depolarizing current. When the voltage step protocol was run during the sustained phase of the 5-HT response, H_{out} was markedly potentiated (2-5 fold). The second peak of T_{out} was also markedly potentiated. This effect was blocked by 1 uM mianserin.

We show here that the voltage-activated channels expressed in Xenopus oocytes can be modulated by neurotransmitter acting on expressed foreign receptors.

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312.9 MODULATION BY SEROTONIN OF A LOCAL INHIBITORY CIRCUIT IN THE HIPPOCAMPAL SLICE. S. A. Springfield. Dept. of Biology, City College of the City University of New York, New York, N.Y. 10031.

Recent evidence from electrophysiological studies has suggested a neuromodulatory role for serotonin (5-HT) in the CNS. I report here a reduction in local inhibition in a hippocampal slice preparation treated with 5-HT.

The experiments were performed on 300-400 um thick transverse slices prepared from rats weighing 150-300 g. The slices were constantly superfused with a bicarbonate-buffered balanced salt solution equilibrated with 95% O_2/5% CO_2 and maintained at 32^{o} C. Evoked population spikes, elicited by electrical stimulation of afferent fibers in the stratum radiatum, were recorded with a single barreled glass microelectrode from the somal layer of CA1 pyramidal neurons. Inhibitory recurrent interneurons were activated by positioning a second stimulating electrode in the alveus of the hippocampus. This placement antidromically activates the axons of CA1 pyramidal cells, the collaterals of which contact the inhibitory interneurons. When the antidromic stimulation followed the afferent stimulation by short intervals (10-150 msec) it produced a reduction in the amplitude of the second spike; whereas longer interstimulus intervals produced facilitation of the second spike. These events have been attributed to the action of GABA-mediated inhibition superimposed upon orthodromic excitation.

Serotonin (10 uM) added to the superfusate elicits a decrease in the amplitude of the population spike evoked from a single stimulus. For paired stimulation, 5-HT administration resulted in an increase in the amplitude of the second spike, evidence of a reduction in local inhibition. This reduction was observed 25-30 min after 5-HT application and persisted for 80 min. Recovery occurred 60 min after 5-HT was eliminated from the superfusate.

These preliminary experiments demonstrate a diminution in the efficacy of GABA-mediated inhibition by serotonin and is consistent with the idea that 5-HT has a modulatory role in the CNS. (Supported by NSF grant BNS 86-06419 and a PSC-CUNY award.)

312.10 ADENOSINE AND (-)-BACLOFEN HAVE A NEUROMODULATORY ROLE IN THE RAT SPINAL DORSAL HORN. I. Kangrga*, M. Randic and S. Jeftinija, (SPON: T. Sowa). Iowa State University, Dept. of Vet. Physiology and Pharmacology and Dept. of Anatomy, Ames, Iowa, 50011, U.S.A.

Adenosine and (-)-baclofen can depress the spontaneous and evoked activity of neurons in the central nervous system, including the spinal dorsal horn. Adenosine deaminase and 5-nucleotidase, the enzymes involved in adenosine metabolism, and a high level of adenosine receptors are present in the spinal dorsal horn. Behavioral studies have also shown that adenosine agonists increase and the antagonists decrease nociceptive thresholds in animals. (-)-Baclofen, a β-chlorophenyl derivative of gamma-aminobutyric acid (GABA), is a selective agonist for $GABA_B$-receptors. To gain more insight into the depressant effects of adenosine and (-)-baclofen, we have investigated the membrane actions of these agents on dorsal horn neurons using the rat spinal cord slice preparation.

Rats 14-26 days old were used. Conventional intracellular recording from dorsal horn neurons using 3M K-acetate-filled microelectrodes was employed. Fast and slow excitatory synaptic potentials were evoked by electrical stimulation of lumbar dorsal roots. All compounds were applied by bath perfusion.

Adenosine (10^{-6} to $5x10^{-5}$M) or 5'(N-ethyl) carboxamide adenosine (NECA, 10^{-6} to 10^{-5}M) or (-)-baclofen (10^{-7} to 10^{-6}M) elicited a dose-dependent hyperpolarization of dorsal horn neurons accompanied by a decrease in membrane input resistance. Spontaneous and evoked fast and slow postsynaptic potentials were inhibited by all three compounds. (-)-Baclofen, in addition, depressed after-hyperpolarization that follows action potential. Concentrations of bicuculline that blocked responses of dorsal horn neurons to GABA had no effect on the depressions of e.p.s.p.s. or the hyperpolarizing effect produced by (-)-baclofen. Spike frequency adaptation is significantly increased following administration of adenosine or (-)-baclofen. In the presence of TTX and TEA, adenosine and (-)-baclofen produced a shortening of duration of the high threshold calcium spike of dorsal horn neurons. Perfusion of slices with caffeine ($5x10^{-6}$ to 10^{-3}M) resulted in a reversible and concentration-dependent antagonism of the adenosine-induced effects on membrane potential and synaptic activities. Caffeine, and theophylline (5×10^{-5} to 10^{-4}M), per se, produced a decrease in resting membrane potential, increased synaptic activity and action potential firing. Caffeine induced also rhythmic oscillations of membrane potential.

It is suggested that adenosine and (-)-baclofen may modulate sensory transmission in the rat dorsal horn neurons through presynaptic sites possibly by decreasing transmitter release and they also may directly regulate the endogenous neuronal excitability through an activation of the postsynaptic recognition sites. Supported by NSF and USDA.

312.11 NEUROTRANSMITTER REGULATION OF LIPID METABOLISM IN CULTURED RAT SENSORY NEURONS, T.M. Perney* and R.J. Miller (SPON: P.J. Kontur). Dept. of Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Recently, several lines of evidence have suggested that the activation of protein kinase C (PKC) is involved in the receptor mediated inhibition of Ca^{+2} currents in sensory neurons. Activation of PKC is often associated with substances that produce their effects by stimulating the breakdown of phosphatidyl inositol-4,5,- bisphosphate (PIP_2). Degradation of PIP_2 catalyzed by the enzyme phospholipase C may then lead to the production of both inositol trsiphosphate (IP_3) and diacylglycerol (DAG). We therefore, examined the ability of norepinephrine (NE) and neuropeptide Y (NPY) to stimulate the hydrolysis of PIP_2 in cultured dorsal root ganglion (DRG) neurons.

We observed that both 1 μM NE and 10 nM NPY stimulated the synthesis of [^{3}H]DAG in DRG neurons prelabeled with [^{3}H]arachidonic acid. In both cases, two peaks of DAG production were observed. The first of these was transient, lasting approximately 30 sec, while the second was more sustained, peaking at 3 min and lasting at least 5 min. Both peaks of NE stimulated DAG formation could be inhibited by 10 μM yohimbine suggesting that this effect was mediated through activation of α_2 receptors. In addition, preliminary studies indicated that synthesis of the first peak of DAG production was blocked by pertussis toxin whereas the second was not.

Interestingly, neither NE or NPY were able to stimulate much release of [^{3}H]IP_3 from [^{3}H] inositol prelabeled DRG neurons. These agents were only able to produce a two fold increase which occured during the first 30 sec of exposure. This result indicates that much of the DAG produced by NE and NPY may not be derived from PIP_2, but possibly from phosphatidylinositol (PI), PI-glycan or even another phospholipid such as phosphatidylcholine. Neither NE or NPY were able to stimulate the release of arachidonic acid (AA) from DRG neurons.

These results for NE and NPY are in contrast to those found for the nonapeptide, bradykinin (BK). BK induced DAG synthesis was similar in magnitude to that produced by NPY and NE. However, IP_3 production was much greater (approx. 10-15 fold stimulation). In addition, BK was a potent stimulator of AA release.

We also examined the ability of NE to produce translocation of PKC in DRG cells. Translocation of PKC from the cytosol to the cell membrane has been related to the activation of the enzyme. We found that 10 μM NE caused a 50% decrease in the amount of PKC in the DRG cytoplasm suggesting movement to another cellular compartment. However, we have not yet been able to demonstrate an increased association of PKC with the cell membrane. This may be due to rapid dissociation of the enzyme during membrane preparation.

These biochemical results are consistent with activation of PKC in DRG neurons by inhibitory neurotransmitters such as NE and NPY, and a role for PKC in the control of DRG Ca^{+2} currents.

312.12 FORSKOLIN AND PHORBOL ESTERS REDUCE THE LARGE TRANSIENT CALCIUM CURRENT IN MOUSE SENSORY NEURONS. R.A.Gross and R.L.Macdonald, Department of Neurology, University of Michigan, Ann Arbor, MI 48104, U.S.A.

Cyclic AMP analogs and forskolin (FOR), an activator of adenylate cyclase, have been used to study the cyclic AMP/A kinase system. These compounds increase or decrease K conductances in various neurons, and enhance Ca conductance in invertebrate neurons and vertebrate cardiac cells. Analogs of diacylglycerol and the phorbol esters activate protein kinase C directly, and have been used to study the phosphatidylinositol-linked second messenger system. These compounds also reduce K conductances but also reduce Ca conductance in vertebrate neurons. We studied the effects of the adenylate cyclase activator FOR and the C kinase activators phorbol 12,13-dibutyrate (PDBu) and oleoylacetylglycerol (OAG) on the three calcium currents in mouse DRG neurons.

Intracellular recordings were obtained with micropipettes filled with 3M CsCl (20-30 MΩ) from mouse DRG neurons in culture. Recording medium (pH=7.3-7.4) suppressed Na and K currents, and contained (in mM): Tris 13.0; choline-Cl 67; KCl 5; TEA 100; $CaCl_2$ 2.0; glucose 5.6; $MgCl_2$ 0.8. A single electrode voltage clamp was used to record calcium currents with a switching frequency of 6-8 kHz and a 70-30 duty cycle. Voltage steps were generated and digitized data stored (2-6 kHz sampling) using a microcomputer. Drugs were applied by pressure and/or diffusion from blunt-tipped (5-20 μM) micropipettes. Three calcium currents were recorded. A small transient current was recorded at -60 to -50 mV when evoked from negative holding potentials (-100 to -90 mV) (T-type). A moderate very slowly-inactivating current was recorded at potentials positive to -20 mV when evoked from holding potentials of -50 to -40 mV (L-type). A large transient current (N-type) was recorded with the L-type current at potentials positive to -40 mV when evoked from holding potentials of -80 mV.

FOR (100 μM) had no effect on the T-type current. Within 2 min of application, FOR reduced the peak current of combined N- and L-type currents without an effect on the late ($\geq$300 msec) current, suggesting a selective effect on the N-type current. Both OAG (50 μM) and PDBu (500 nM) had similar effects, with similar latencies. Effects of all compounds lasted $\geq$ 5 min. The inactive phorbol, 4-α-phorbol, had no effect. The effects of OAG and PDBu were voltage-dependent, being negligible on currents evoked from holding potentials negative to -80 mV, and maximal when evoked from holding potentials of -70 to -50 mV. Although these compounds may have effects other than activation of their respective kinases, these data suggest that activation of A and C kinases may selectively modulate calcium conductances.

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312.13 DEPOLARIZATION OR PHORBOL ESTER TREATMENT STIMULATE EFFLUX OF ARACHIDONIC ACID AND ITS METABOLITES FROM MOLLUSCAN NERVOUS SYSTEM. R.O. Carlson* and I.B. Levitan. (SPON: P. Robinson). Grad. Dept. of Biochemistry, Brandeis University, Waltham, MA 02254

The effects of depolarizing agents and phorbol esters on efflux of eicosanoids (arachidonic acid and its metabolites) were studied with ganglia from the molluscs *Aplysia californica* and *Helix aspersa*. To follow eicosanoid efflux, ganglia were preincubated in appropriate saline supplemented with up to 50 nM [^{3}H]arachidonic acid (specific activity 240 Ci/mmol). [^{3}H]arachidonic acid taken up into the ganglia was found to be stored almost exclusively in neutral lipids and phospholipids. After washing to remove excess [^{3}H]arachidonate not taken up, the efflux of eicosanoids was monitored by measuring accumulation of radioactivity in the medium (containing 5 mg/ml fatty acid free BSA). Exposure of ganglia to elevated KCl or 100 μM veratridine was used to induce depolarization. Eicosanoid efflux increased about 2 fold in response to depolarizing conditions in both *Helix* and *Aplysia*. The phorbol ester 12-O-tetradecanoyl-13-acetate (TPA) also stimulated eicosanoid efflux from both *Helix* and *Aplysia* ganglia, with EC_{50} values of 140 nM and 15 nM respectively. Saturating concentrations of TPA were 500 nM for *Helix* and 200 nM for *Aplysia*, stimulating efflux three times and five times the basal rate, respectively. The inactive phorbol ester, 4-α-phorbol, did not stimulate eicosanoid efflux at a concentration of 1 μM in either molluscan nervous system. Removal of calcium chloride from the bathing medium and addition of 20 mM cobalt chloride completely blocked the effect of depolarizing agents or saturating concentrations of TPA. The effect of varying extracellular $CaCl_2$ concentration was studied more systematically with *Aplysia*. TPA-stimulated eicosanoid release was maximally inhibited when the extracellular $CaCl_2$ concentration was lowered to 100 μM. The results of cobalt substitution and lowering extracellular $CaCl_2$ suggest that the stimulation of eicosanoid release by depolarization or TPA may involve a common mechanism that is dependent on extracellular calcium. Furthermore, the lack of effect of 4-α-phorbol and the TPA dose-response range for *Aplysia* are consistent with a role for C kinase in stimulation of eicosanoid efflux.

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312.14 PHORBOL ESTER BINDING SITES IN MAMMALIAN BRAIN. M. Dalpé* and R. Quirion. Douglas Hospital Research Centre and Dept. of Psychiatry, Faculty of Medicine, McGill University, 6875 LaSalle Blvd, Verdun, Quebec, Canada H4H 1R3.

It is well known that neurotransmitter receptors are coupled to second messenger systems. Among them, adenylate cyclase and the phosphoinositide (PI) cycle have been extensively studied. Interestingly, products derived from the PI cycle appear to be associated with protein kinase C, a major calcium and phospholipid-stimulated phosphorylating enzyme. Recently, evidences have shown that phorbol esters bind to protein kinase C. Thus, we have used [^{3}H]phorbol 12, 13 dibutyrate ([^{3}H]PDBu) as a way to study the presence and distribution of protein kinase C in mammalian brain. Brains obtained from male Sprague-Dawley rats, male albino guinea pigs and human dying from non-neurological diseases were used in all experiments. Membranes were prepared as follows: Tissues were homogenized in 50 mM Tris.HCl buffer, pH 7.7 at 4°C containing 100 mM NaCl and 1 mM CaCl, and then centrifuged for 15 min at 49,000g. Pellets were resuspended in the same buffer at 25°C. For the assays, 200 μl (1.0-1.5 mg protein) of this preparation was incubated for 45 min in 50 mM Tris.HCl buffer, pH 7.7, containing 100 mM NaCl, 1 mM $CaCl_2$ and various concentrations of [^{3}H]PDBu ranging between 0.1-100 nM (15.8 Ci/mmol; NEN). Incubations were terminated by rapid filtration (3 x 5 ml washes) using a Brandel Cell Harvester. For in vitro receptor autoradiography, brain sections were prepared as described before (Quirion, Eur. J. Pharmacol. 117, 139, 1985) and then incubated with 2.5 nM [^{3}H]PDBu for 60 min as described above. At the end of the incubation, slides were rinsed for 4 min in cold incubation buffer and dipped in distilled water to remove ions. Incubated sections were then apposed to LKB-Ultrofilm for 8-10 weeks. Specific binding was determined as the difference in ligand bound in the presence and absence of 10 μM phorbol 12, 13 - dibutyrate (Sigma). Under these conditions, [^{3}H]PDBu bound with high affinity and specificity (85-90%) to its sites in mammalian brain. Autoradiographic data clearly demonstrated the discrete distribution of [^{3}H]PDBu binding sites in mammalian brain. In rat and guinea pig brain, high densities of sites were especially found in the hippocampal formation, various cortical areas and cerebellum, lower densities of [^{3}H]PDBu binding were present in striatum and hypothalamus. Most brainstem area contained only low densities of sites. In human brain, high densities of [^{3}H]PDBu sites were found in cortical and hippocampal areas. Thus, it appears that the distribution of protein kinase C/[^{3}H]PDBu sites is unique in mammalian brain. We are currently investigating possible alterations in [^{3}H]PDBu/protein kinase C binding characteristics in neurological diseases.

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313.1 CLONING OF A NOVEL Mr 60K CALMODULIN-BINDING POLYPEPTIDE WITH PROTEIN KINASE ACTIVITY FROM ADULT RAT BRAIN. C-A Ohmstede*, H. LeVine*, J. Gray*, P.M. Huff*, and N.E. Sahyoun* (Spon: Elizabeth B. Hollingsworth) Department of Molecular Biology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Screening a rat brain cDNA library in lambda gt11 with a monoclonal antibody directed near the calmodulin binding site of the brain-specific Ca^{2+}/calmodulin-dependent protein kinase II(PK-CAMII) produced several clones which also bound labeled calmodulin. Restriction enzyme analysis of one of these clones (C36) demonstrated a 1.2 kb cDNA insert. The recombinant β-galactosidase fusion protein from the C36 lambda lysogen was purified by affinity chromatography on calmodulin Sepharose and was injected into rabbits for the production of antibodies. Western blot analysis of soluble brain extracts demonstrated binding of the C36 antibody to one polypeptide with Mr value of 60K. The antibody, however, did not react with the α or β subunits of PK-CAMII from soluble or post-synaptic density preparations. Moreover, Western blot analysis of fractions from gel filtration columns indicated that the C36 native protein could be resolved from PK-CAMII. Screening many rat tissues by Western blots indicated that the C36 protein was localized to the brain. Developmental analysis indicated that the protein was absent in the newborn rat but was synthesized during the first postnatal week. Immunoprecipitation of rat cytosol with the C36 antibody removed a Ca^{2+}/calmodulin-dependent kinase activity against histones which was not removed with non-immune rabbit serum. The calmodulin-dependent kinase activity was recovered by adsorbing the specific immune complex on Protein A-Sepharose.

The DNA sequence of C36 was determined. The clone contained 1231 bp of sequence including a poly A+ tail. The 5'-end of the clone contained an open reading frame of 480 bp which would code for a peptide of about 18000 molecular weight. Comparison of the sequence with that from a mouse cDNA clone isolated by calmodulin binding demonstrated about 90 % homology in the amino acid coding sequence (Sikela, J.M. and Hahn, W.E., in press). Northern blot analysis with total poly A+ RNA demonstrated hybridization of C36 with 4 RNA species; >9.5 kb, 7.5 kb, 3.3 kb and 2.1 kb.

313.2 CEREBELLAR GRANULAR LAYER. PERSISTENT RETARDED MIGRATION SUBSEQUENT TO DIAZEPAM ADMINISTERED DURING GESTATION IN THE MOUSE. Ma. C. Márquez-Orozco*, A. Márquez-Orozco* and A. Escobar* (SPON: R. Salceda). Embryol. Dept. Sch. of Med., Natl. Univ. of México, P.O.B. 70250, México, D.F., México 04510, Inst. Invest. Biomed., UNAM and National Inst. Neurol. Neurosurg., México 14410.

Diazepam (valium) is a drug amply used as a therapeutic agent during pregnancy and in complications of labor including pre-eclamptic and eclamptic states. The drug is capable of traversing the placental barrier in several animal species including humans. In the fetal nervous tissue it accumulates in most nuclei of the brainstem and in the cerebral and cerebellar cortices. The fetal nervous tissues concentrations of diazepam are eliminated slowly. Previous work has shown retarded neuroblast differentiation in the mesencephalon. The cerebellar cortex is a structure that shows evident immaturity at birth in aetricial animals. Hence, it seemed of interest to study the effect of diazepam upon the maturation process in the cerebellar cortex of the mouse prenatally exposed to diazepam. Diazepam was administered intraperitoneally in a single dose of 2.7 mg/kg b.wt. from the 6th through the 17th day of gestation. Gestation term remained unchanged. The litter were carefully manipulated to help their breathing since all of them showed signs of respiratory insufficiency, hypothermia, and hypotonia. The survivors were mixed with the control litter and transferred to a foster mother for nursing. Feeding was left ad libitum with Purine Chow. After i.p. anesthesia with pentobarbital (35 mg/kg b.wt.) the animals were sacrificed at 120 and 180 days of age by cardiac perfusion with isotonic saline and then with 10% formalin in isotonic solution. The brains were obtained 12 hours later to avoid manipulation artifacts. Fixation by immersion in 10% formalin was continued for a period of 2 weeks. Sagittal sections of each hemisphere were embedded in paraffin, and stained for myelin, nerve cells, and axons identification; a few selected blocks were impregnated with the Golgi technique.

Light microscopy demonstrated in both experimental groups the presence of abundant neuroblasts in the external granular layer, decrease in neuron density of the internal granular layer, paucity of parallel fibers in the molecular layer, as compared with the control group. Quantitative assesment of the neuroblast population in the external granular layer was significant.

Behavioral analysis of the experimental group (swimming, spontaneous motor activity) and some clinical findings correlated well with the histological changes. The results are discussed on the basis of previous experiments involving the mesencephalon and the corpus striatum.

313.3 **Inhibitory Effects of Leu-enkephalin on ^{3}H-5HT Uptake in Dissociated Mesencephalic Raphe Neurons.** **Davila-Garcia M.I., Hou, X.P., Murphy, R. and Azmitia, E.C. New York University. Biology Dept. New York, N.Y. 10003.**

Neuropeptides have previously been demonstrated to have an effect on various neuronal systems during development. We have shown that ACTH peptides are able to stimulate the maturation of serotonergic neurons when these are separated from their target. These trophic influences of neuropeptides are further explored. Our results suggest that Opioid peptides have an inhibitory effect on the maturation of serotonergic neurons grown in culture.

Serotonergic neurons were obtained from slices of mesencephalic raphe of 14 day gestation (DG) rat fetuses. The target cells were obtained from the hippocampus of fetuses 18-19 DG. Neurons were dissociated by gently repipetting with fired polished tips of decreasing diameter. Neurons were plated on poly-L-lysine substrate (15ug/ml) on 96 well culture plates (Limbro). The cells were grown for 2-4 days at 37°C in a CO_2 incubator. The maturation of the serotonergic neurons was assayed by measuring the uptake of ^{3}H-5HT (5×10^{-8}) from the media during a 20 min incubation. Various concentrations of Leu-enkephalin were prepared (1,000-0.01 ng/ml) and administered at the time of plating to cultures of raphe cells alone or to co-cultures with their target tissue. To asses the specificity of leu-enkephalin effects, naloxone (10mM-1000mM) was administered to some of the plates. Morphometric studies of serotonergic cells were done in a bioquant system using a specific 5-HT antibody on 8 well slides (Lab-Tek) prepared in identical manner as the plates and incubated for 24 hrs.

Leu-enkephalin has an inhibitory effect on ^{3}H-5HT uptake by serotonergic cells grown in culture at all concentrations studied and this effect is reversed by naloxone. Neurite cell length is significantly decreased by leu-enkephalin, while cell body area is unaffected. An interesting finding is that naloxone itself significantly stimulates maturation of serotonergic neurons as measured by uptake and it increases cell body area but has no effect on neurite cell length. Parallel studies were performed with CCK-8 and de-sulfated CCK-8 (dsCCK-8). Results showed that de-CCK-8 was equally potent as enkehalin in inhibiting ^{3}H-5HT uptake by cultures of mesencephalic raphe neurons. CCK-8 produced a biphasic response with high concentrations being inhibitory and low concentrations being excitatory. These effects were also shown to be dependent on initial cell densities and reversed by naloxone

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313.4 CREATINE KINASE AS A MOLECULAR MARKER IN THE ESTABLISHMENT OF INTERNEURONAL CONNECTIONS IN VITRO. O. Ramírez, V. Alemán, E. Jiménez* and B. Osorio*. Departamentos de Bioquímica and Neurociencias. Centro de Investigación y de Estudios Avanzados del IPN, 07000 México, D.F.

It has been shown that a fast brain neuronal multiplication, from the first to the third day of chick embryo incubation, is accompanied by a 270% increase in the cytosolic creatine kinase (CK) specific activity, in only a 10 hour period at the end of the second day of chick embryo development. Coinciding with the initiation of neuromuscular activity of the chick embryo in vivo, another increase of about 150% brain CK specific activity was observed from the 17th day of incubation until hatching. On the other hand, brain mitochondrial CK activity increased 375% during the same period (J. Neurochem. 28: 411, 1977). In the present work chick brain neurons from stage 30 (day 7) of incubation were cultured on polylysine coated Petri dishes. Cultures were fed on Dulbecco medium during 11 days. At two-day intervals, representative samples were utilized for both phase contrast observation and cytosolic CK assays. It was found that an increase in the number of long axonic and dendritic processes between nerve cell clusters was followed by a 123% increase in cytosolic CK specific activity in only 48 hours from the fifth to the seventh day of culture (Table I). Electrophoretograms of chick embryo brain BB-CK isoenzyme, obtained from in vivo or in vitro neurons, showed an average migration of 3.4 cm as compared to the rat brain BB-CK isoenzyme which migrates 6.5 cm from the origin (Table II).

Table I

Age of neurons in culture (days)	CK specific activity ± SD (n moles/CP/min/mg prot)	No. of bundles between neuronal clusters
1	51 ± 3	Non-observed
3	58 ± 3	Non-observed
5	61 ± 4	17.4 ± 1.6
7	131 ± 11	18.8 ± 0.8
9	124 ± 7	Non-determined
11	124 ± 14	14.9 ± 2.1

Table II

Age of neurons in culture (days)	BB-CK migration from the origin (cm)
1	3.5
3	3.7
5	3.7
7	3.2
9	3.3
11	3.2

313.5 SIMULTANEOUS ANALYSIS OF 5α-REDUCTASE, AROMATASE AND ANDROGEN RECEPTOR LEVELS IN FETAL MONKEY BRAIN. S.A. Sholl and K.L. Kim* (SPON: G. Davis). Wisconsin Regional Primate Research Center, Univ. of Wisconsin, Madison, WI 53715

Although the importance of androgens and estrogens in the development of sexually dimorphic brain activity has been established in rodents, little is known about the prenatal effects of steroids in primates. The availability of tissues has also restricted such efforts. With this in mind, we have begun to investigate steroid enzyme and receptor levels in various regions of the fetal rhesus brain including the medial basal hypothalamus (MBH), amygdala (AMG), corpus callosum (CCAL), cerebellum (CB) and cerebral cortex. To date, 3 fetuses (2 females, 1 male) have been studied at 160 days of gestation and 2 fetuses have been studied at 100 days (1 male, 1 female). Assays were developed to simultaneously assess aromatase, 5α-reductase and cystolic androgen receptor (AR) levels in neural tissue samples from these animals. Tissues were homogenized in a buffer consisting of 10 mM Tris-HCl, 1.5 mM EDTA, 1 mM mercaptoethanol, 25 mM Na_2MO_4 and 10% glycerol. This buffer system was chosen so as to optimize receptor detection as previously reported *(Sholl and Pomerantz. Endocrinol. 117:1625, 1986)*. Enzyme activities were measured in a 50 μl portion of the crude homogenate (~ .1-.6 mg protein) in 2α-^{3}H-testosterone, 1β-^{3}H-testosterone (1.2 μM, T), NADH and NADPH (.4 mM) were added as substrates. ^{14}C-Dihydrotestosterone (DHT) was also included to account for procedural and metabolic loss of the ^{3}H-DHT which resulted from 5α-reductase activity. Aromatase activity was estimated on the basis of ^3H_2O synthesized from 1β-^{3}H-T, while 5α-reductase activity was evaluated from the amount of ^{3}H-DHT formed from 1α,2α- and 1β-^{3}H-T. Samples were incubated for 3 hr, 37°C and the reaction products purified by column and thin layer chromatography. Receptor levels were estimated in 108,000 g tissue supernatants using ^{3}H-DHT (4 nM) as ligand and radioinert DHT (1 μM) as competitor. Free and bound ligand were separated on DEAE-cellulose columns. Both enzymes exhibited linear behavior with time, and the amount of ^3H_2O formed was stochiometrically equivalent to the amount of ^{3}H-estradiol produced. For 5α-reductase activity, K_m = 5.0 μM and V_{max} = 7.9 pmoles/mg protein.hr, while for aromatase activity, K_m = 0.43 μM and V_{max} = 0.83 pmoles/mg protein.hr. Both enzymes were detectable at 100 and 160 days of development. MBH, CB and AMG exhibited the highest levels of reductase activity ($p < 0.001$), while aromatase activity was greatest in MBH, AMG and CCAL ($p < 0.001$). Overall, enzyme rates were higher at the earlier stage of development. AR levels were detectable in all tissues at both stages; however, due to the limited number of samples, results were not statistically significant. These findings indicate that neural tissues, including those from females, have the potential for transforming androgens to products which could have greater or lesser activity. Receptors are also present through which these products could effect a genomic response. This capacity may be important in terms of normal sexual differentiation of neural activities as well as for potential teratogenic changes under abnormal metabolic or physiological conditions. (Supported by NIH grant HD-18865.)

313.6 DISCRIMINATION BETWEEN MU AND DELTA OPIOID RECEPTOR AGONISTS IN CONTROL OF FETAL SHEEP CARDIOVASCULAR FUNCTION. C.E. Dunlap III and R.K. Zoltoski*. Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

Early studies in our laboratory showed the opiate antagonists, naloxone, naltrexone, and levallorphan caused dose-dependent increase in ovine fetal and maternal blood pressure (BP) and heart rate (HR) (Dunlap and Rose, Fed Proc 44:513,1985). Logically, opiate agonists were expected to decrease these cardiovascular responses. However, morphine was found to be pharmacologically inert, producing no effects on either BP or HR when injected intravenously or intraarterially into the fetal sheep ranging from 124 to 135 days gestation (term = 145 days). In some instances the highest doses of morphine used (10 to 20 mg) mimicked the effects seen with opiate antagonists.

When leucine-enkephalin (LEU-ENK) was injected intraarterially over a dose range from 0.2 to 2 mg, a decrease in both BP and HR was consistently observed. The average drop in mean arterial pressure produced by 1 mg of LEU-ENK was -17.11±1.93 mmHg (n=5). Maximal response was extremely rapid, occurring about 22 seconds after the peptide was injected into the tibial artery, and duration of action was short, with mean arterial pressure returning to baseline 2 to 4 minutes after injection. Decreases in BP and HR produced by LEU-ENK were completely blocked by 3 mg of the opiate antagonist, levallorphan, while its pharmacologically inert stereoisomer, dextrallorphan, had no effect on LEU-ENK responses. Levallorphan was found to be 2 to 3 times more potent than naloxone in blocking LEU-ENK effects on fetal BP and HR.

Epinephrine (EPI) (0.04 mg) produced a sustained increase in fetal BP of between 15 and 20 mmHg above baseline (baseline mean arterial pressure = 45 mmHg) and decrease in HR, lasting up to 10 minutes in some experiments. LEU-ENK injected 2 minutes following EPI administration attenuated the EPI increase in BP, primarily through a further reduction in HR. LEU-ENK antagonism of EPI response lasted only 2 or 3 minutes, following which BP again increased in response to the remaining circulating EPI. A less consistent observation was the effect of levallorphan on EPI responses. In several but not all experiments, the area under the response-time curve for a 0.04 mg dose of EPI was significantly increased following levallorphan administration.

These observations lead us to hypothesize that intrinsic regulation of fetal ovine cardiovascular function may in part be mediated by delta, but not mu-type opioid receptors due to the absolute discrimination of these responses for the delta receptor agonist, LEU-ENK, but not the mu receptor agonist, morphine.
This research was supported by NIH grant HL34460.

313.7 DRUG BINDING SITES IN HUMAN FETAL BRAIN. M. Schlumpf and W. Lichtensteiger. Institute of Pharmacology, University of Zürich, Zürich, Switzerland

Using in vitro autoradiography, drug binding sites are found to be present in human fetal brain towards the end of the first trimester of pregnancy. At this early developmental stage tritiated (N)-methylscopolamine (NMS), flunitrazepam and the benzodiazepine antagonist Ro-15-1788 are labeling regions in lower and upper medulla oblongata rather uniformly. In the course of further development labeling becomes more concentrated in certain nuclear areas. During the first part of the second trimenon, at around the 18th week, muscarinic cholinergic binding sites thus appear in higher amounts in the spinal trigeminal and in the hypoglossal nucleus while benzodiazepine binding sites are seen more heavily concentrated in the area of medial and lateral lemniscus and also in the nucleus of the XII. cranial nerve. The development of a distinctly differentiated pattern might be seen in connection with onset of function. Of especial interest is the coincident labeling by 3H-NMS and 3H-flunitrazepam of the hypoglossal nucleus that is innervating intrinsic and extrinsic musculature of the tongue. Sucking difficulties have been described in infants born to mothers treated with benzodiazepines during pregnancy (Bavoux). In the striatum of 18 to 23 weeks of gestation, muscarinic cholinergic, benzodiazepine and dopamine D2 (sulpiride) sites exhibit an irregular dotted pattern which is similar to the one described for acetylcholinesterase in humen fetal striatum of comparable age (Graybiel). It is expected that in vitro autoradiography will provide information on drug-sensitive periods of defined areas in human fetal brain.

F. Bavoux, C. Olivier and G. Pons: Journées Parisiennes de Pediatrie, 123, 1982
A.M. Graybiel and C.W. Ragsdale: Proc. Natl. Acad. Sci. 77, 1214

313.8 POSTNATAL EXPRESSION OF ENKEPHALIN AND TACHYKININ GENES IN THE STRIATUM OF THE RAT: MODULATION BY NIGROSTRIATAL DOPAMINE Sivam, S.P., Li, S., Hudson, P., Lee, P., Hong, J-S, and Breese, G.R. Dept. Pharmacol. & Toxicol., Indiana Univ. Sch. Med., Northwest Ctr. Med. Ed., Gary, IN., Lab Behav. Neurol. Toxicol., NIEHS, NIH Research Triangle Park, NC., BSRC, Univ. North Carolina at Chapel Hill, NC.

Previous studies have shown that the nigrostriatal dopaminergic pathway exerts a strong influence to regulate the activity of striatal enkephalinergic and striatonigral tachykiniergic neurons. The purpose of the present study is to elucidate the postnatal development of enkephalin and tachykinin neuropeptide systems of the striatum and their plasticity following neonatal destruction of striatonigral dopaminergic pathway with 6-hydroxydopamine (6-OHDA) in Sprague-Dawley rats. Dopaminergic lesions were made by intracisternal administration of 6-OHDA during an early postnatal period (day 3). The animals were sacrificed at various times (0-35 days) and their striata removed for the biochemical determinations. A combination of methods, namely, radioimmunoassay, molecular hybridization and HPLC were used for the determination of peptides, specific mRNAs coding the precursors and for monoamines and their metabolites, respectively. The rate of postnatal development of proenkephalin-A derived peptide Met -enkephalin (ME) was faster than the development of protachykinin derived peptides substance P (SP) and neurokinin A (NKA). Administration of 6-OHDA during the early postnatal period led to marked depletion of dopamine and its metabolites whereas an increase in 5-hydroxytryptamine and its metabolite was observed. The dopaminergic lesion induced a minor initial decrease followed by a late increase in ME level and a persistent decrease in SP and NKA levels. Preliminary evidence indicates that the development of proenkephalin and protachykinin mRNA closely paralled the development of their respective peptide products. The 6-OHDA lesions did not affect the development of peptides (SP, NKA, ME and cholecystokinin) in the frontal cortex, indicating regional selectivity. These results indicate a differential plasticity of enkephalin and tachykinin systems (that is, an enhanced expression and a retarded expression) as a result of damage to the dopaminergic system. These studies provide evidence for the close interrelationship and interdependence in the striatum between the development of dopamine and neuropeptide systems studied. Further, these studies may be of relevance to the basal ganglia related neurologic and neuropsychiatric diseases in which dopamine may play a role. [Supported by grant PHS 5 SO7 RR 53711 (SPS), NS 21345 and HD 03110 (GRB)]

313.9 INTERFERON INDUCES DIFFERENTIAL REDISTRIBUTION OF THE 210KDALTON NEUROFILAMENT SUBUNIT IN CULTURED DORSAL ROOT GANGLIA CELL BODIES FROM NORMAL AND TRISOMY 16 MICE. D. MacFabe*, J. Lew*, B. Scott and A. V. Plioplys Surrey Place Centre and Div. of Neurology, Dept. of Pediatrics, Hosp. for Sick Children and Univ. of Toronto; 2 Surrey Place, Toronto, Ont., Can., M5S 2C2

The human 21st chromosome, as the mouse 16th chromosome, codes for the alpha and beta interferon receptors. Cultured fibroblasts from both the human and mouse trisomic conditions have an exaggerated response to exogenously applied interferon. To test whether neurons may display a similar sensitivity to interferon, dorsal root ganglia (DRG) were dissociated and grown in tissue culture. They were taken from gestational age E19 normal and phenotypic trisomy 16 mouse fetuses, and normal 3 month old swiss mice. The cultures were grown on a rat tail collagen matrix in CMRL-1415 medium supplemented with 10% FCS for 42 days prior to interferon treatment. Cultures from each fetus or adult were divided into an untreated group or treated with identical medium with 6700 units of alpha and beta mouse interferon per ml for 48 hours. After fixation with cold methanol and acetone, the cultures were reacted with monoclonal antibody (mab) N210 which recognizes the phosphorylated 210 Kdalton subunit of neurofilaments. The reaction product was revealed using direct PAP techniques with 4-chloro-1-naphthol as colorant. Statistical comparisons were done with the chi-square test.

In normal fetuses and adult mice there was a significant ($p < 0.005$) increase in the percentage of DRG cell bodies stained with mabN210. Trisomic DRG's displayed a similar response to interferon ($p < 0.05$). However, the percentage of staining of untreated trisomic DRG cell bodies was similar to the interferon treated normals.

These results suggest that neurofilament antigen expression may in part be regulated by interferon. Also, trisomic DRG cell bodies express greater mabN210-immunoreactivity than do normals. These findings may be related to the observation of precocious neurofilament antigen expression in Down's syndrome early in life (A. V. Plioplys, J. Neurol. Sci., in print).

MabN210 was kindly provided by Dr. R. B. Hawkes.

313.10 ^{3}H-PCP BINDING AND PCP-INDUCED LOCOMOTOR ACTIVITY AND ATAXIA AFTER PRENATAL EXPOSURE TO PHENCYCLIDINE. T.A. Fico and C. VanderWende. Rutgers University, Dept. Pharm. and Tox., P.O. Box 789, Piscataway, N.J. 08854.

There are very few studies regarding the postnatal consequences of prenatal exposure to phencyclidine (PCP). PCP abuse by pregnant women has been reported, raising questions of safety for the fetus. There are reports of prenatal exposure to methadone enhancing postnatal sensitivity to that drug. Therefore, it was of interest to examine whether prenatal exposure to PCP during different prenatal periods modified postnatal (1) ontogeny of ^{3}H-PCP binding in discrete brain areas and (2) PCP-induced ataxia and locomotor activity.

Pregnant CF-1 dams were injected daily with either PCP (5, 10 or 20 mg/kg) or saline, sc, during mid (E6-15) or late (E12-18) gestation. The litters were culled to 6 males and cross fostered to untreated dams that had delivered within 24 hours. Animals were weaned at postnatal day 21 (P21) and housed in single sex litter groups until P36. On P34-36 four male offspring from each litter were randomly choosen and each received either 0, 2.5, 5.0 or 7.5 mg/kg PCP, sc. Immediately following the injection, the mice were placed on an activity meter and their activity recorded in 6 min. intervals for 1 hr. Ataxia was scored in 15 sec. observation periods at 5 min. intervals for 50 min. using the rating scale of Castellani and Adams (Eur. J. Pharmacol. 73; 143-54, 1981). Another group of animals, prenatally treated as described, and not used in any behavioral test were used in the ^{3}H-PCP binding study. Five - six males of a litter were decapitated on either P21 or P35 and the striatum, hippocampus and cortex were dissected out. Tissue from the members of a litter were pooled. The ^{3}H-PCP binding assay of Zukin et al. (Brain Res. 258; 277-84, 1983) was used.

There was no significant effect of prenatal treatment or period of treatment on total locomotor activity or the temporal pattern of locomotor activity. Male offspring of dams treated in late gestation had greater ataxia scores than did untreated offspring and both had greater scores than did mid gestation offspring. There was a dose-dependent increase in postnatal PCP-induced ataxia with increasing dose of PCP administered prenatally, although this did not reach significance. These results are discussed in respect to the ontogeny of ^{3}H-PCP binding in prenatally treated and untreated offspring.

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313.11 ALTERATION OF EFFECTS OF PRENATAL EXPOSURE OF RATS TO MORPHINE OR CLONIDINE BY CROSS-FOSTERING PROCEDURES. E. Lacrosse*, B. Consoliver* and B. Culver Neurosci. Prog., School of Pharm., Univ. Wyoming, Laramie, WY 82071.

The consequences of prenatal drug exposure are likely the result of a complex interaction of indirect drug effects as well as direct pharmacological effects on the developing organism. Perinatal undernutrition, neonatal withdrawal abstinence and alterations in maternal physiology and/or behavior have been suggested to contribute to postnatal effects of in utero exposure of animals to narcotic drugs. We have described a number of parallel effects produced in offspring of rats exposed to either morphine or the α_2-adrenergic agonist clonidine during the last week of pregnancy. However, it is not clear wheather these effects are caused by drug-induced alterations of endogenous opiate and adrenergic systems or by indirect effects. As a first aproach to this problem we report here the results of a study comparing postnatal effects produced by in utero exposure to morphine or clonidine in animals reared by dams that have been administered these drugs or by control dams.

Pregnant rats were assigned to one of 4 treatment groups on gestation day (gd) 14. One group (CL) was implanted with osmotic mini-pumps which infused clonidine at a rate of 2µg/hour; paired controls (CO) were subjected to identical surgical procedures. Rats in the MS group were implanted s.c. with a silastic morphine sulfate (100 mg) pellet on gd 14 and 2 more pellets on gd 17 while paired controls (LA) were implanted with silastic lactose pellets. At parturition entire litters of drug-treated rats were cross-fostered (XF) to paired control dams and vice versa, or were left with their natural dams (ND).

For MS groups, mortality of ND (68%) and XF (62%) was not significantly different. However, the possibility that postnatal rearing factors may affect mortality was suggested by findings of lower mortality in CL pups in the XF (4%) compared to ND (32%) group; by higher mortality of LA pups when reared by MS dams (XF= 14% ND=4%); and by higher mortality of 5-14 day-old CO pups reared by CL dams (XF=5%; ND=2%). Results of tailflick (TF) tests in 2-3 mo. offspring show that postnatal factors are enduring and that effects are seen in adult rats in that drug-treated offspring XF to control dams show attenuated TF latencies while control off spring XF to drug-treated dams show enhanced TF latencies.

These studies indicate the need to examine indirect factors associated with prenatal drug administration. The possibility exists that direct pharmacological effects are not fully operational at early stages of development and thus are less important determinants of the consequences of prenatal drug exposure than are indirect factors.

313.12 MORPHINE ANALGESIA IN NEONATAL RATS AS ASSESSED BY FOREPAW, HINDPAW AND TAIL RETRACTION FROM A THERMAL STIMULUS. M. S. Fanselow[1]*, E. M. Blass[2], and C. P. Cramer[1]. [1]Deparment of Psychology, Dartmouth College, Hanover, NH 03755; [2]Deparment of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

The neonatal period is characterized by dramatic changes in opioid physiology. Less is known about the dynamics of behavioral responsivity to opiates during this period. Therefore, we explored the ontogeny of opiate analgesia in neonatal rats. The analgesic efficacy of morphine sulfate (MOR) was compared on 3 reflexive measures of nociceptive reactivity in rats ranging from 2 to 21 days of age. The response measures were the latency to retract either the front paw, hind paw or tail from a hot-plate. Rats were manually supported by an experimenter (blind to conditions) above the hot-plate and only the tested limb was allowed to contact the heated surface. Even 1-day-old rats were competent to perform these responses, and analgesia was indicated by an elevation in latency. The first experiment tested Long-Evans rats at 2, 7, 14, and 21 days post partum. Five independent groups received 1, 2, or 4 mg/kg MOR or saline (SAL), ip. Latencies were taken for each of the 3 measures (order counterbalanced) for each subject between 20 and 60 min following injection. The hot-plate temperature was 52° C and a 15 s cut off latency was employed. MOR produced a dose dependent analgesia at all ages on all measures. The rats at the 2 intermediate ages were the most responsive. Tail retraction was the least sensitive measure of MOR analgesia at early ages but the most sensitive measure at older ages. The second experiment compared the effects of MOR (.25 mg/kg), naloxone (.25 mg/kg) and SAL on Sprague-Dawley rats at 2, 6, 9 and 21 days of age. A 48°C hot-plate and 25 s cut-off were employed. Response measures were varied in a between groups fashion and drug treatment was counterbalanced across age of testing. MOR produced effects similar to those of the first study. Naloxone treated animals never differed from SAL. The data indicate that neonatal rats have behaviorally functional opiate analgesic systems.

313.13 NIGROSTRIATAL BUNDLE LESIONS IN NEONATES: INCREASED 5-HT BUT DECREASED MODULATION BY 5-HT OF CHOLINERGIC INTERNEURONS
D. Jackson*, J.P. Bruno, M.K. Stachowiak, and M.J. Zigmond. (SPON: R.W. Keller, Jr.) Dept. of Behavioral Neuroscience and Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA

6-Hydroxydopamine (6-HDA) destroys dopamine (DA) terminals. However, while adult-lesioned rats show profound neurological deficits, these effects are not observed when lesions are made at 3 days of age. When given to neonates, 6-HDA increases the density of serotonin (5-HT) terminals in striatum. Since DA and 5-HT have several similar effects on striatal function, this additional 5-HT might be responsible for the behavioral sparing. To further explore the role of 5-HT in animals given intraventricular 6-HDA as neonates, we examined striatal acetylcholine (ACh) release, a process that is under the inhibitory control of both DA and 5-HT. Coronal slices (350 uM) of adult striatum were incubated for 20 min at 37°C in Krebs buffer containing tryptophan (5 uM) and either [3H]choline (0.1 uM) or [^{3}H]5-HT (0.1 uM), superfused, and tritium efflux into the superfusion fluid used as a measure of ACh or 5-HT release. Slices were depolarized with bipolar pulses (2 msec, 18 mA, 1 Hz) both 85 min (S_1) and 125 min (S_2) after a 60 min equilibration period. Drugs were introduced 20 min prior to the S_2. Fluoxetine (1 uM), an inhibitor of 5-HT uptake, reduced ACh stimulation-evoked tritium overflow in control slices from caudal striatum by 16% but had no effect on overflow from rostral striatum, a region which contains 62% less 5-HT. This is consistent with an inhibitory influence of endogenous 5-HT on ACh release. However, fluoxetine failed to inhibit ACh overflow from the caudal striatum of lesioned slices, despite an 81% increase in tissue 5-HT content. Moreover, fluoxetine also had no effect on ACh overflow from lesioned slices from rostral striatum, although 6-HDA had increased 5-HT in that region by 337%. Quipazine (1 uM), a direct 5-HT agonist, which inhibited ACh efflux by 20% in both caudal and rostral control slices, had no effect on slices from lesioned animals. Finally, neonatal lesions reduced the stimulation-induced overflow of [^{3}H]5-HT from slices of lesioned striatum by 95%. Taken together these results suggests that while neonatal 6-HDA produces a serotonergic hyperinnervation of striatum, the ability of these 5-HT terminals to release 5-HT is impaired and the sensitivity of cholinergic neurons to 5-HT is markedly reduced. These results may help to explain our recent observation that pharmacological disruption of serotonergic function does not impair behavior in rats given neonatal 6-HDA. (Supported in part by USPHS grant NS19608.)

313.14 METABOLIC MATURATION OF THE BRAIN: A STUDY* OF LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE CAT. H. T. Chugani, D. A. Hovda, M. E. Phelps, and J. R. Villablanca. Depts. of Neurology, Pediatrics, Psychiatry and Anatomy, and Division of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles, CA 90024, USA.

We have previously shown, using positron emission tomography, that the anatomical distribution of brain glucose utilization in man undergoes dramatic changes during the first year of life consistent with behavioral, neurophysiological and anatomical changes (Science 231: 840-843, 1986). Furthermore, local cerebral metabolic rates for glucose (LCMRglc) in most regions increase to reach adult rates by 2 years, after which LCMRglc exceeds adult values until adolescence, when LCMRglc gradually declines to approximate adult values again by the end of the second decade (Ann. Neurol., in press). In order to develop an animal model of neurometabolic maturation, we measured LCMRglc of 49 brain regions in 22 kittens during normal postnatal development using [14C]-2-deoxyglucose autoradiography, and compared these rates to those of 5 adult cats. The following developmental time points (in days) were selected: 7 (N=2), 15 (N=3), 30 (N=3), 45 (N=2), 60 (N=3), 90 (N=5), 120 (N=4). LCMRglc at 7 days (range 16-49 umol/min/100g) were generally 50% below adult values, with a lesser distinction among gray matter structures and between gray and white matter than in adults. Although the maturational course varied among regions, LCMRglc in most areas increased from 7 to 45 days to reach adult values and subsequently exceeded adult rates with a peak at 90 days, when LCMRglc in many regions were almost twice adult rates and displayed considerable heterogeneity among structures (range 27-205 umol/min/100g). At 120 days, LCMRglc was generally lower than at 90 days, but still exceeded adult rates. LCMRglc in 9 measured regions (e.g. cerebellum, red nucleus, mammillary nucleus) did not exceed adult rates during development. This developmental profile of LCMRglc is consistent with the neurodevelopmental milestones of the cat in that most neurological functions in the kitten mature during the first three postnatal months (Dev. Psychobiol. 12:101-129, 1979). Furthermore, sensory structures (including visual, auditory and somatosensory regions) show a LCMRglc rise earlier than motor areas (including neocortex, basal ganglia, cerebellum), which also fits well with an earlier development of sensory functions versus fine motor components of locomotion and limb placing reactions. Thus, as in humans, LCMRglc values in the cat are age-specific, at least for most brain regions, and show a period of relative hypermetabolism. These normal maturational metabolic data will serve as baseline values with which to compare neonatal lesion-induced alterations of LCMRglc in assessing functional plasticity in the CNS (USPHS grants HD-05958, 5RO1-MH37916, 2PO1-NS15654; DOE AM03-76-SF00012).

DEVELOPMENT OF INVERTEBRATES II

314.1 LACK OF AN OOCYTE TO NURSE CELL VOLTAGE DIFFERENCE IN DROSOPHILA.
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There have been several reports suggesting a voltage difference between the nurse cell and oocyte in insect ovaries (e.g. Nature 286:84, 1980; Devel. Biol. 108:102, 1985). Such a gradient might establish embryonic polarity or be responsible for transport of cytoplasmic materials from nurse cells to oocyte.

Previous studies asserting (J. Cell Biol. 58:172, 1973) or denying (J. Cell. Sci. 81:207, 1986) a voltage difference for these cells also reported low resting potentials of 21mV in Drosophila and 30 to 50 mV for the larger cells of Hyalophora (a moth). These differing results and low potentials suggested that the membrane might have been damaged. We found similarly low resting potentials when the egg chambers were dissected by tungsten needle from the ovary. However, much higher potentials (82 to 92 mV) were obtained when the egg chambers were freed from the ovary with collagenase (1 mg/ml for 5-10 minutes).

An egg chamber consists of an oocyte, 15 nurse cells and an enveloping layer of follicle cells. Measurements were taken with the follicular epithelium intact. When prepared as above the potassium dependence of the membrane potential was log-linear from 2.7 to 100mM with a slope of 52mV for a 10 fold change in $[K]_o$. An extrapolation to zero potential indicates $[K]_i = [K]_o$ at 150mM.

No significant voltage differences were found between oocyte and nurse cell at any time from stage 5 to 10 (stages from King, 1970). Our data do not indicate that a potential difference between oocyte and nurse cell can be developmentally significant at these stages.

Stage	Oocyte mV ±S.D.	Nurse Cell mV ±S.D.	p
5	81.9 ±5.7 (n=7)		
6	90.3 ±5.7 (n=4)	90.3 ±4.5 (n=11)	p>0.5
7	90.0 ±3.3 (n=15)	91.9 ±3.6 (n=41)	p>0.2
8	90.5 ±4.9 (n=19)	91.4 ±3.7 (n=35)	p>0.9
9	90.0 ±4.2 (n=8)	88.1 ±6.2 (n=21)	p>0.5
10	85.2 ±5.0 (n=27)	86.2 ±5.7 (n=58)	p>0.5

At stage 5 the oocyte and nurse cells are not morphologically distinguishable. One value is reported for both. The lower potential at this stage may be due to greater penetration damage related to the small cell size at this stage (10u).
Saline (mM): Na, 50; K, 2.7; Ca, 5; Mg, 17; Cl, 25; glutamate 50; glucose, 10; Hepes, 5; sucrose to adjust osmolarity to 300.

314.2 BETA-GALACTOSIDASE AS A LINEAGE TRACER. R. D. Streck*, S. T. Bissen and D. A. Weisblat (SPON: B. Holton). Dept. of Zoology, University of California, Berkeley, CA 94720.

Knowledge of the lines of descent of the cells that comprise a mature organism is crucial for the understanding of developmental biology and neurobiology. One way to follow cell lineages is to microinject blastomeres with benign, easily detected substances (lineage tracers) that are subsequently inherited by all of the progeny of the injected cell. Horseradish peroxidase (HRP), fluorescent peptides, fluorescent dextrans and biotin-conjugated dextrans have all been used as lineage tracers. Unique properties of various tracers make them advantageous for different lineage tracing applications. We report here that the enzyme beta-galactosidase (β-gal) can also be used as a lineage tracer and that it too has properties that are advantageous for certain experimental applications.

β-gal (10 mg/ml) was pressure injected into identified blastomeres of the embryo of the leech Helobdella triserialis. After development to a later stage, the embryos were fixed and the distribution of β-gal was visualized by staining with "Bluogal" (BRL) for 6-12 hr at 37°C. The dark blue opaque reaction product can be viewed in wholemount or in sections of plastic-embedded specimens. The intensity and distribution of the reaction product is not affected by illumination or during ethanol dehydration and embedding in glycol methacrylate. By comparison with other lineage tracers, we conclude that the β-gal reaction product is confined to known descendants of the injected blastomere and does not perturb normal development; this enables β-gal to be used as a lineage tracer.

In addition, the enzyme or its reaction product is localized primarily in the cell cortex, so that microinjecting a lower concentration (1.0 mg/ml) of β-gal yields embryos in which the interiors of labeled cells are essentially unstained while their borders are clearly delineated by β-gal staining. This makes it relatively easy to discern the shapes of cells, compared to when other tracers are used. Moreover, it facilitates certain types of double label experiments, such as those entailed in studying the cell cycles of identified cells. For this purpose, S phase cells in embryos bearing β-gal-labeled cell lines were pulse-labeled with bromodeoxyuridine (BrdU) and identified by visualization with a peroxidase-antiperoxidase complex, after a biphasic fixation and intervening staining for β-gal (for details, see abstract by Bissen and Weisblat). The identification of S phase cells (brown nuclei) in the line of interest was facilitated because they were outlined by the blue β-gal reaction product.

314.3 CELL CYCLE ANALYSIS IN EARLY LEECH EMBRYO. S.T. Bissen and D.A. Weisblat. Dept. of Zoology, Univ. of Calif., Berkeley, CA 94720.

Segmental tissues of the leech Helobdella triserialis comprise five iterated sets of cells arising from longitudinally arrayed columns or bandlets of mesodermal (m) and ectodermal (n, o, p and q) blast cells. Blast cells are produced by five bilateral pairs of stem cells (M, N, O/P, O/P and Q teloblasts) at the rate of one per hr; older cells lie distal (future rostral) to younger ones in strict accordance with their birth rank. Each m, o and p bandlet contains one class of blast cell, as defined by their definitive fates, whereas there are two alternating classes of blast cells in each n and q bandlet (Weisblat and Shankland, Phil. Trans. R. Soc. B, 312:39-56, 1985). The two alternating classes of blast cells in the n bandlet may differ from the time of their births (Bissen and Weisblat, J. Neurobiol., in press). Moreover, the duration of the first cell cycle and the geometry of the first mitosis differ for each class of blast cell (Zackson, Dev. Biol., 104:143-160, 1984).

To determine what accounts for the variations in cell cycle duration, nuclei of cells in S phase were pulse labeled by incorporation of tritiated thymidine triphosphate (TTP) or bromodeoxyuridine triphosphate (BrdUTP). Embryos containing labeled cells were examined autoradiographically (TTP) or immunohistochemically (BrdUTP) making use of the precise rostrocaudal gradient of development. The cell cycles of the different classes of ectodermal blast cells (21 to 33 hr) differ in the length of their G_2 phases (17 to 29 hr) because they have very short or nonexistent G_1 phases and S phases of approximately equal duration (4 hr). The cell cycles of the mesodermal blast cells also seem to lack G_1 phases and have S and G_2 phases of one and nine hr, respectively.

Cells actively synthesizing RNA were labeled by incorporation of tritiated uridine triphosphate (UTP) and then identified by autoradiography. All seven classes of blast cells commence synthesis of RNA immediately after birth and continue to do so throughout their cell cycle until they enter M phase. The synthesis of RNA in these cells was drastically reduced in embryos treated with a low concentration of α-amanitin (1-2 μM), which suggests that the blast cells are synthesizing some mRNA.

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314.4 IDENTIFICATION OF HOMEO BOX GENES IN THE LEECH, HELOBDELLA TRISERIALIS. D.J.Price*, C.J.Wedeen* and D.A.Weisblat. Department of Zoology, University of California Berkeley, California 94720.

Many genes affecting the spatial organization of tissues in the fruitfly, Drosophila melanogaster, contain a homologous nucleotide sequence, called the homeo box, that encodes a putative DNA-binding protein domain of about 60 amino acids. The homeo box seems to have been highly conserved in evolution; genes containing sequences homologous to the homeo box are found in a wide variety of organisms ranging from yeast to humans. As well as being structurally homologous, it is conceivable that homeo box-containing genes from widely separated animal groups have functional homology. Thus, the presence of the homeo box may serve to identify developmentally important genes in organisms other than Drosophila. This would be especially true for organisms that are phylogenetically close to insects, e.g. annelids.

We are interested in the development of the leech Helobdella triserialis. One advantage of studying the leech embryo is that it comprises identifiable and experimentally accessible cells. Therefore it should be possible to examine how genes effect developmental changes at the level of individual cells. We have screened Helobdella for putative homeo box-containing genes by the cross-hybridization of Drosophila homeo-box probes to restriction fragments of Helobdella genomic DNA. Probes were obtained from Drosophila genes with maximally divergent homeo box sequences, including zen, engrailed and Sex combs reduced. Minimal amounts of the unique sequence surrounding the homeo box were included in the fragments used as probes, and we avoided incorporation of repeated sequences other than the homeo box (e.g. the M-repeat, or OPA sequence, a poly-glutamine encoding sequence found in many Drosophila genes).

Results from these experiments demonstrate the presence of six or more genes in Helobdella that cross-hybridize to the homeo box. We are now attempting to clone these genes from a genomic library of Helobdella constructed in the lambda phage vector, EMBL3.

314.5 ACQUISITION OF CENTRAL TARGETS BY OLFACTORY SENSORY NEURONS IN HOMEOTIC ANTENNULES: FUNCTIONAL CONSEQUENCES. DeF. Mellon, H.R. Tuten*, L.M. Hurley*, and M.E. Easley*. Dept. of Biology, University of Virginia, Charlottesville, VA 22901

Biramous homeotic antennules can be generated in the crayfish, Procambarus clarkii, by surgically removing an eye in posthatch juveniles. We have studied the anatomy, central connections, and functional consequences of the olfactory sensory neurons associated with the aesthetasc (chemosensory) hairs on one flagellum of the homeotic antennules. The number of aesthetascs on the homeotic antennules is similar to the normal antennules in all cases examined. Light and electron microscopical studies indicate that aethetascs on the homeotic antennules have a normal compliment of primary sensory neurons. Axons from these neurons terminate within the ipsilateral olfactory centers of the brain, as determined from the uptake and transport of tritiated leucine by olfactory sensory neurons, and the deposition of the radioactive tracer within glomeruli of the olfactory lobe. Crayfish possessing homeotic antennules exhibit a significant lateral bias in olfaction-triggered searching behavior, suggesting that the central connections of supernumerary olfactory sense organs are anatomically and functionally appropriate and are additive to the normal input.

Computer-aided 3-dimensional reconstructions of olfactory lobes from histological sections show that normal growth of the brain is accompanied by increases in the size of existing olfactory glomeruli, by which means normal growth-related additions in the number of primary sensory axon terminations must be accommodated. Our studies show that doubling of the afferent input due to the presence of homeotic antennules results in asymmetric olfactory lobe growth with the side supporting input from the homeotic antennule being up to 25% larger in volume than the contralateral side. The growth can be accounted for by an increase in volume of existing structures, since roughly the same number of glomeruli are found in the olfactory lobes of juvenile and adult crayfishes, and in animals possessing homeotic antennules. Because many crustaceans grow continuously throughout their lifespan, accommodation of additional sensory input from expansion of the peripheral receptive field normally must be an ongoing process. Details of the central reorganization through which this process occurs will be of considerable interest.

314.6 **LEECH GANGLIOGENESIS: IDENTIFIED PERIPHERAL AND SEROTONIN NEURONS ASSUME POSITIONS OF MISSING CONTRALATERAL HOMOLOGUES.**

Duncan K. Stuart, Margaret I. Law, and Steven A. Torrence*
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How do neurons come to lie in the proper position during development? Prior work has shown that when a major subset of neurons is prevented from forming on one side of the glossophoniid leech nerve cord, their contralateral homologues are distributed over both sides of the nerve cord (Blair 1983, Dev. Biol. 95:65; Stuart et. al. 1987, J. Neurosci. 7:1107). On either side of the embryo a column of cells, the n bandlet, gives rise to these neurons, which can be either labeled or prevented from arising by injecting the bandlet's precursor blastomere with lineage tracer or DNase, respectively. We show that identified neurons that differentiate on the opposite side still occupy normal positions.

In normal embryos of the leech Theromyzon rude none of the neurons derived from a labeled n bandlet crosses the ventral midline, and almost all lie within the chain of segmental ganglia. In each segment three peripheral neurons, nzl-3, are also derived from the n bandlet.

When only one n bandlet was present, the neurons nzl-3 that it produced could be found on either side of the ventral midline and occupied appropriate peripheral positions on either side. In segments where one or more nzl-3 crossed to the opposite side, they were missing on the side of origin.

In embryos with an n bandlet missing, any neuron (or its precursor) derived from the surviving n bandlet was capable of crossing the ventral midline and assuming a position on the contralateral side. The number of neurons found on the opposite side varied among segments, from none to all. Most or all of this crossover occurred early in gangliogenesis. Antibody against 5-HT identified five of these neurons: the anteromedial (am), Retzius (R), ventrolateral (vl), dorsolateral (dl), and posteromedial (pm) 5-HT neurons, which, except for pm, are normally paired in the segmental ganglia. In embryos with an n bandlet missing no more than one of these neurons was produced per segment. The am, R, and pm still occupied relatively normal positions along the ventral midline with axonal projections typical of neurons either on the left or right. In some cases the normally ipsilateral projection of R was bilateral. When one or two lateral 5-HT neurons, vl and dl, crossed over to the other side, they were typically found in the normal positions with the normal projections of their contralateral homologues.

Thus, we find in embryos with a missing ectodermal n bandlet that the remaining n derived neurons differentiate into the expected neurons occupying normal positions on either their normal or the contralateral side. Furthermore, the identity of these neurons is not determined by final position, and neither the commitment to differentiate as a given neuron nor differentiation itself requires a normal spatial arrangement of the clone of cells from which they arise. Since most ganglionic neurons arise from these n bandlets, the mesoderm is an alternative candidate for providing the required spatial information.

314.7 LEECH GANGLIOGENESIS: UNILATERAL MESODERM ABLATION AFFECTS NEURON POSITIONING DIFFERENTLY IN ANTERIOR vs. POSTERIOR SEGMENTS
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Unilateral ablation of the mesoderm in leech embryos induces the majority of ganglionic neurons, or their precursors, on the ablated side of the body to cross the ventral midline (Blair,1982, Devel.Biol.89:389-386) This transposition was examined in detail using embryos of the glossiphoniid leech, Theromyzon rude.

Ectodermal and mesodermal tissues on each side of the leech embryo arise from five columns of cells called bandlets. Four of these, n,o,p and q, form ectoderm, with n giving rise to the majority of ganglionic neurons. The remaining m bandlet forms the mesoderm. Unilateral ablation of the mesoderm was accomplished by injecting the m bandlet precursor blastomere with DNAse before gangliogenesis began and before the last mitosis of any neuroblast. To follow the development of the overlying ectoderm, various bandlets were labelled with rhodamine or fluorescein lineage tracers by injecting their parental blastomeres.

Ganglionic neurons or their precursors of the n lineage reliably crossed the ventral midline in embryos with unilateral mesoderm ablations, while ganglionic neurons or their precusors of the o,p and q lineages did not. To examine the distribution of crossed neurons, two lineage tracers were used to differentially label n-derived neurons from the left and right n bandlets. Unilateral ablations were performed after a period of normal development had ensued generating an anterior control region dubbed zone 1. In regions of mesoderm ablation, two patterns of crossover were observed: zones 2 and 3. In the more anterior zone 2 the majority of n-derived ganglionic neurons or their precursors from the ablated side crossed the ventral midline and intermingled with the native n-derived neurons. Three n-derived peripheral neurons (nz1-3) from the ablated side also crossed to the opposite side and found their appropriate peripheral positions. Therefore, crossed n-derived neurons in zone 2 appear to locate their correct, mirror-symmetrical mediolateral position.

In zone 3 the organization of n-derived neurons was disrupted on both sides of the nerve cord and gangliogenesis was abnormal. The zone 2/3 border appeared to be located in the same segmental location regardless of the number of segments of zone 2 anterior to zone 3.

Therefore, mesoderm is necessary for neurons to locate their appropriate positions. Moreover, the mediolateral position of ganglionic neurons appears to be under extrinsic rather than intrinsic control. One possible candidate to provide such positional information is the mesoderm. In addition, the occurrence of zone 3 suggests that the manner in which positional information is either aquired or interpreted in posterior segments of the embryo may be different than in more anterior segments. In normal development such differences could be involved in generating the normal posterior segmental variations.

314.8 LEECH GANGLIOGENESIS: ECTODERM-MESODERM INTERACTIONS IN THE PATTERNING OF IDENTIFIED NEURONS
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Individually identified cells occupy stereotyped positions in the bilaterally symmetrical segmental ganglia of the leech. Previous work has demonstrated that extensive morphogenetic movements, including cell migrations, occur in the normal development of this pattern. Studies reported in companion abstracts suggest that normal position in a ganglion is not required for the differentiation of 5-HT neurons, and that interactions between prospective neural ectodermal cells and the mesoderm are necessary for the genesis of the stereotyped architecture of the leech nerve cord.

To investigate the role of ectoderm-mesoderm interactions in the development of identified neurons, we studied the effects of ablating the mesoderm from the left side of embryos on the 5-HT neurons of the leech Theromyzon rude. Ablations were effected by injection of the lethal enzyme DNase into a precursor blastomere, or by laser irradiation of cells labeled with a photosensitizing lineage tracer dye. The 5-HT neurons were visualized by indirect immunofluorescence. Whether a given neuron originated from the left or right side of the embryo was revealed by differential labeling with fluorescent lineage tracers.

In normal development, all neurons differentiate on their side of origin, and in experimental embryos some 5-HT neurons did differentiate on the mesoderm-deficient left side. However, these cells were not organized in any recognizable spatial pattern.

Many 5-HT neurons (or their precursors) that originated on the left crossed the ventral midline to differentiate on the unablated right side of the embryo. Most dorsolateral and ventrolateral 5-HT cells that crossed occupied positions correct for their new side, and shared these positions with the homologous neurons native to that side. Thus, extra identified neurons from the left were incorporated into the normal organization of the right hemiganglia.

As in normal ganglia, Retzius neurons were found near the ventral midline. Both right and left Retzius neurons were abnormal however, in being found in all possible anteroposterior positions, including the interganglionic connectives. Apparently unilateral mesodermal ablation disrupted control of anteroposterior position for midline cells.

We conclude that the presence of a normal ipsilateral mesoderm is not required either for commitment of the ectodermal precursors of 5-HT neurons to their fates or for their differentiation, but that interactions between ectoderm and mesoderm are required to generate the normal spatial patterning of the neurons. A complete complement of ectoderm was present in these experiments, so interactions among ectodermal cells are not sufficient for such spatial patterning. We propose that during gangliogenesis in the leech, cells that are already committed to specific neural fates use positional cues derived from the mesoderm to find positions appropriate to their fates.

314.9 STEREOTYPED CELL MIGRATION AND THE FORMATION OF THE INSECT ENTERIC NERVOUS SYSTEM IN VIVO AND IN EMBRYO CULTURE.
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Stereotyped cell migration is a common feature in the formation of nervous systems in many organisms. It facilitates the local innervation of distal targets and influences the differentiation of the translocated neurons. We have examined the development of the enteric nervous system (ENS) of the moth Manduca sexta and have observed patterns of extensive neuronal migration that are multi-phasic and proceed along stereotyped pathways. We have characterized the sequence of normal neurogenesis and migration that gives rise to the ENS, and we have established a method of culturing these embryos such that both cell lineage analyses and surgical manipulations can be applied.

The ENS of Manduca includes two small anterior ganglia and a diffuse set of neurons that are distributed along the major longitudinal muscle bands of the midgut. At about 35% of embryogenesis, a region of columnar epithelium evaginates from the dorso-posterior margin of the foregut, which then delaminates into a discrete cluster of about 200 presumptive neurons -- the Enteric Crest neurons. Over the next 20% of development, these cells undergo a slow phase of migration, during which the Enteric Crest spreads to encircle the foregut. Dye fills of individual cells revealed relatively simple bipolar morphologies with short processes confined to the Enteric Crest; these processes gradually become longer as circumferential migration progresses. Then between 55-60% the Enteric Crest disperses, as a majority of the cells initiate a rapid longitudinal phase of migration and move in 8 small groups onto the midgut. Some of these neurons subsequently express neuropeptide phenotypes, although the onset of this expression commences only after cell migration is complete and appears to be a function of final soma position along the gut.

The small number of Enteric Crest neurons and the relative simplicity of their cellular environment on the developing gut make this an attractive system in which to analyze neuronal migration and its consequences at the level of individual cells. For these studies we have developed methods that permit extended embryonic development in culture. Under these conditions, embryos dissected out of their egg shells and membranes at 25% of development will undergo complete ENS neurogenesis and cell migration in vitro. Observations both in vivo and in vitro suggest that individual Enteric Crest cells are not pre-specified to follow particular migratory routes and do not appear to be uniquely identifiable. Rather, their final distribution and spacing, and perhaps their phenotypic diversity, may reflect probablistic developmental events. Supported by an NIH Fellowship to P.F.C. (F32NS07957) and NIH grant to P.H.T. (NS21749), a Sloan Fellow.

314.10 ISOLATION OF TISSUE AND STAGE SPECIFIC cDNA CLONES FROM THE NERVOUS SYSTEM OF THE MOTH, MANDUCA SEXTA.
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We are studying mechanisms of neuronal differentiation in the embryonic nervous system of the moth, Manduca sexta. We have focussed attention on the Frontal Ganglion (FG), an assembly of ~50 neurons that is a part of the enteric nervous system, lies atop the foregut and has many features associated with the larger, more complex segmental ganglia of the CNS. For example, two of the largest neurons (F1 and 2) have stereotyped positions, axon projections and transmitter phenotypes (Copenhaver and Taghert, 1986, Soc. Neurosci. 12, 318). These cells can be visualized by their immunoreactivity with a monoclonal antibody directed against the molluscan neuropeptide SCP_B. By about 40% of embryogenesis, the FG has acquired nearly its full complement of neurons; by 50%, many axons have been extended towards peripheral targets; by 55%, the onset of transmitter expression has begun (as indicated by immunostaining with anti-peptide antibodies). We wished to complement these on-going cellular observations with studies at the molecular level and have adopted a +/- screen in attempting to isolate FG-specific transcripts.

RNA was extracted from the FG (the positive tissue) and abdominal ganglion 1 (the negative tissue: it lacks SCP_B-immunoreactive neurons) from ~3500 Vth instar larvae. Radiolabeled cDNA was used to probe duplicate lifts of a Manduca brain cDNA library that was made in λGt11. After three rounds of purification, we found five individual clones that were moderately to greatly enriched in the FG probe. Cross-hybridization experiments suggest these represent three distinct mRNAs.

In addition to this tissue specificity (i.e., heterogeneous expression within the nervous system), we find evidence of developmental regulation in some of these transcripts. During adult development in Manduca, a general re-organization takes place within the nervous system that includes neurogenesis and programmed cell death. F1 and 2 are no longer SCP_B-immunoreactive by Day 7 of metamorphosis and in fact may have died. We probed the FG-enriched cDNA clones with RNA taken from the Day 7 developing adult FG and found that the relative levels of expression among the clones had changed dramatically. The previously most abundant clone was now barely above background. In addition to sequencing these clones, we are currently performing in situ hybridization to help identify the specific neurons that express the correspondent transcripts.
Supported by a Fellowship from the NIH to PFC and grants from the Monsanto Corp. and from the Washington University Center for Cellular and Molecular Neurobiology to PHT who is a Sloan Fellow.

314.11 EMBRYONIC DEVELOPMENT OF SEROTONIN-LIKE IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE SNAIL LYMNAEA. R. Marois, B.J. Chiasson* and R.P. Croll. Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

Although an extensive body of literature exists on early embryogenesis of the pond snail Lymnaea stagnalis and on the organization of the adult nervous system, little is known about embryonic or post-embryonic neural development in this species. In this study immunohistochemical techniques were used to identify a subpopulation of cells expressing serotonin in the central nervous system (CNS) of Lymnaea at hatching and to begin to examine the embryonic origin(s) of these cells.

The fully autonomous hatchling possesses about 250 serotonin-like immunoreactive (SLIR) cell bodies distributed throughout the ganglia of the CNS as either single identifiable cells or as members of identifiable cell clusters. Each cerebral ganglion contains about 20-25 SLIR cell bodies partitioned into five clusters. Within these clusters certain cells can be recognized as identifiable neurons such as the metacerebral giant (MCG) cell. The axonal projection of the MCG can be seen entering the cerebro-buccal connective and eventually the buccal ganglia where it appears to provide the only source of serotonin. At hatching, the pedal ganglia have the highest number of SLIR somata of any of the central ganglia. These cells are largely located within three discrete clusters or within a more diffuse population extending primarily over the anterior and medial surfaces of the ganglia.

Except for the large asymmetric cell (LPeD.1) all identifiable cells and cell clusters of the pedal ganglia form bilaterally symmetric pairs. The pleural ganglia do not contain any SLIR cell bodies although numerous SLIR en passant fibers are detected. The right parietal ganglion contains about 10-14 SLIR cell bodies at hatching whereas the smaller left parietal ganglion exhibits variable labelling of only a few SLIR cells. Within the visceral ganglion 10-20 cells can usually be seen at hatching.

Neural development was also studied and is expressed as a percentage of the total duration of development (about 15 days at 21-23°C). The first detectable expression of SLIR cells at 46-48% of development appears to be delayed by about 7% from the initiation of gangliogenesis. The SLIR somata are initially detected in the cerebral ganglia, then in the pedal ganglia and finally in the remaining suboesophageal ganglia. Shortly after the appearance of SLIR somata, fibres can also be detected within most connectives and commissures. Thus the double ring structure of the adult nervous system is detectable early in development using these techniques.

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314.12 POST-HATCHING DEVELOPMENT OF SEROTONIN-LIKE IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE SNAIL LYMNAEA. B.J. Chiasson* and R.P. Croll (SPON: D. Treit). Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

Post-hatching development of the central nervous system of Lymnaea stagnalis was studied with the aim of bridging the gap in our understanding of the adult central nervous system (CNS) and work in progress on the embryonic CNS. The post-hatching developmental period, which involves about a 20 fold increase in the linear dimensions of ganglia comprising the CNS, was examined using a sub-population of cells exhibiting serotonin-like immunoreactivity (SLIR). All clusters of SLIR cells present in the adult CNS were observed in the hatchling snail, as were all single, identified cells. However, all clusters, with the exception of one in the cerebral ganglion, demonstrated addition of SLIR cells during post-hatching development, although different clusters added cells at different periods. Some clusters only added SLIR cells shortly after hatching, whereas other clusters showed a continual increase in cell number during development. The I clusters of the pedal ganglia underwent another form of SLIR cell addition. Both the left and right I clusters showed gradual increases in cell numbers from hatching (shell length, 1.6mm) until the shell length reached 10-12mm. Cell number within the left I cluster then remained constant at 12-14, whereas the right I cluster increased to about 60 SLIR cells near the commencement of sexual maturity (shell length, 19-23mm). The overall increase in SLIR cell number is from 200-250 in the hatchling to 450-500 in the adult CNS. In addition to increases in cell numbers, post-hatching development in Lymnaea also involves individual cell growth. All cells examined showed continuous growth during post-hatching development. Certain identified cells were observed to have different growth rates. Growth rates of unidentified cells comprising the various clusters were also determined through measurements of both the largest and the smallest cells of each cluster and were found to be less than those of most identified cells.

This study suggests that at least two factors contribute to the large increase in linear dimensions of the ganglia of the CNS. Firstly, SLIR cells approximately double in number from hatchlings to animals approaching sexual maturity. These additions are variable from cluster to cluster. Secondly, individual SLIR cells undergo 3-6 fold increases in some diameters during post-hatching development. It remains to be seen if the expression of neurotransmitter phenotype is a good indicator of cell addition and if these findings can be generalized to neuronal development of the entire CNS.

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314.13 DEVELOPMENT OF THE ASCIDIAN LARVAL NERVOUS SYSTEM: THE SPECIFIC STAINING OF NEURONS. W.-G. Jia and I.A. Meinertzhagen. Dept. of Psychology, Dalhousie University, Halifax, N.S., Canada B3H 4J1.

Despite its chordate affinities, the larva of ascidians (Urochordata: Ascidiacea) has a nervous system which contains relatively few neurons (about 100); these arise from an embryo amply demonstrated to be generally mosaic. They therefore offer the prospect to study the cell-by-cell development of a nervous system which is widely held to be close to the ancestral form of, and thus may provide a model for, that of the vertebrate. In the sea-squirt, Ciona intestinalis, an acetylcholinesterase (AChE) staining technique (Bear, M.F. et al., J. Comp. Neurol., 234:411, 1985) and Met-enkephalin (ME) immunocytochemistry have been utilised to examine the nervous system in the normal larva. Eight to 10 primary sensory cells arranged bilaterally in pairs in the dorsal epidermis and a pair of neurons in the visceral ganglion were found to be AChE-positive, whilst a single caudal neuron in the nerve cord of the tail showed ME-immunoreactivity. This is the first demonstration in the ascidian larva of AChE-positive and ME-immunoreactive neurons and the latter is also the first evidence for neuronal perikarya in the caudal nerve cord, previously thought exclusively ependymal. The evidence reveals the existence of identifiable neurons but not whether the nervous system is entirely determinate. To explore the latter question, experimental embryological studies have been directed towards the CNS.

The mosaic nature of the ascidian embryo has been inferred especially from the results of deletion experiments, which result in the production of partial larvae (Cohen, A. & Berrill, N.J., J. E. Z., 74:91, 1936). Here, "half larvae" have been obtained by ablating the blastomeres of one side of the two- or four-cell stage embryo and the resultant larval CNS cells counted. The number was less in the "half"-larva than half the cells in the intact larva, even though the cell number in other organs was generally exactly half normal. In addition to these numerical findings there exist qualitative departures from mosaicism. The pigment cell from either otolith or ocellus, both of which originate from the right wall of the neural tube in the normal embryo (Willey, A., Q. J. Micr. Sci., 35:295, 1893), was invariably found in the half-larvae, no matter from which embryonic half the latter was derived; more than one larva had both pigment cells. It has been postulated that the development of the ascidian CNS depends upon evocation (Reverberi, G. et al., Acta Embryol. Morph. Exp., 3:296, 1960) from notochordal or entodermal cells. Our results suggest the existence either of some more profound regulative factor affecting CNS differentiation or of some departure in the half larva from the determinate patterns of mitosis (Nicol, D. & Meinertzhagen, I.A., Soc. Neurosci. Abstr. 11:648, 1985) by which the nervous system normally arises.

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314.14 IMMUNOCYTOCHEMICAL STUDIES OF AN ASCIDIAN LARVAE. C.D. Johnson, R.J. Crowther*, and J.R. Whittaker*
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The free swimming larvae of ascidians - sea squirts (subphylum Urochordata, class Ascidiacea) are comprised of only a relatively small number of cells (there are probably less than 100 neurons) which are organized in a body plan which shares many features with vertebrates. Studies of larval development, both classical and recent, have focussed on lineage commitment and on the roles of oogenic cytoplasmic determinants in lineage commitment. In modern studies, enzyme histochemical staining and ultrastructural features have been used as markers of differentiation in Ciona intestinalis (review, J.R. Whittaker, Amer. Zool. 27).

We sought to identify additional cell specific probes, particularly those for the larval nervous system, by screening antibodies which bind to putative neurotransmitters, to peptides or to other know proteins. Ciona larvae (18-24) hrs post-fertilization) were labelled in whole mounts; 50-200 animals were used for each different antibody or condition. Animals were fixed either with glutaraldehyde or with formaldehyde and, in some cases were post-fix treated with pronase before exposure to primary antibodies and then to secondary antibodies linked to HRP. Useful reagents identified so far include:

1) Antiserum specific for GABA labels two groups of bilaterally symmetrical neurons in the head, one just posterior to the sensory vesicle, the other in the hind brain region. In addition two neurons in the dorsal nerve of the tail are labelled.

2) Antiserum specific for glutamate labels fiber tracts anterior to the sensory vesicle including cell bodies within the tracts and some cell bodies within the brain.

3) Antiserum to Aplysia small cardioactive peptide B (SCPB) labels granules in mesenchymal cells located in the posterior region of the head.

4) Antiserum to human prolactin labels granules in endodermal cells of the gut.

5) Antiserum to Ciona adult tropomyosin (generated by T.H. Meedel) labels larval muscle fibrils lightly. In late larvae the circular muscle fibrils of the developing adult siphons are intensely labelled.

6) A monoclonal antibody to acetylated α-tubulin labels neural cilia in the sensory vesicle, cilia in dorsal nerve cord and cilia extending from neurons located in the epithelium of the tail.

7) A monoclonal antibody to β-tubulin appears to label all neural fiber tracts.

314.15 REGIONAL DIFFERENCES IN DROSOPHILA WING EPITHELIUM DURING THE TIME OF AXON OUTGROWTH REVEALED BY MONOCLONAL ANTIBODY. M.A. Murray and J. Palka. Department of Zoology, University of Washington, Seattle, WA 98195

Experimental studies (reviewed in J. Palka, 1986, J. Neurobiol., 17:581) show that the axons of sensory neurons in the developing wing of Drosophila behave as if a specific band of epithelial cells, those that will ultimately form the third of the five longitudinal veins, form a preferred growth substrate. We have initiated a search for monoclonal antibodies that might help to reveal a basis for this axon-guiding property of the L3 epithelium and describe here the characteristics of the first antibody recovered, 6G7.

Light microscopic sections of growing wings indicate that 6G7 binds to a component of the epithelial basement membrane. In wild-type animals, staining appears as early as mid-third instar, and is nearly gone by 24 hr after pupariation (AP). From 0 to 6 hr AP the pattern of staining is irregular, occupying about one half the area of the growing wing along its longitudinal axis. By 8 hr AP the pattern is dramatically vein-like, staining longitudinal pre-veins 3,4, and 5, with the strongest staining along L3. Binding persists through the period of wing inflation (12 to 18hr AP) and diminishes considerably by 24 hr AP.

Important events in the transformation of the wing imaginal disc to a mature wing include eversion, elongation, vein formation, sensilla development, axon outgrowth, and cuticle production. From the overall pattern of staining, the 6G7 antigen is a candidate for involvement in elongation and/or vein formation. Initial investigations support this hypothesis. In several mutants in which major segments of the veins of adult wings are missing, we have found a lack of 6G7 binding in the corresponding pre-vein regions of pupal wings.

6G7 staining is strongest along vein L3, the only route taken by axons in the middle of the wing; furthermore, the antigen is present throughout the period of axon outgrowth (0 to 18 hr AP) and is lost thereafter. However, we do not know whether the antigen is related to outgrowth in an instructive way, is simply permissive, or is irrelevant. Culturing wings in the presence of the antibody has no effect on axon outgrowth, but this does not constitute strong evidence since monoclonal antibodies often fail to block the function of their antigens. However, 6G7 does reveal a molecular heterogeneity in the wing which appears to be developmentally regulated, and which may well be involved in one or several important developmental events.

314.16 ANTIGEN 5B12, AN EXTRACELLULAR GLIA-ASSOCIATED PROTEOGLYCAN OF THE ADULT INSECT NERVOUS SYSTEM, IS EXPRESSED UPON EPITHELIAL CELL FILOPODIAL PROCESSES IN EMBRYONIC SENSORY STRUCTURES. M.R. Meyer, P. Brunner*, and J.S. Edwards. Dept. of Zoology, NJ-15, University of Washington, Seattle, WA 98195.

Monoclonal antibody (MAb) 5B12, generated from a glial-enriched crude membrane immunogen, labels a glia-associated antigen located only within the adult nervous system of the cricket Acheta domesticus (Meyer et al., J. Neurosci. 7:512, 1987) and that of other orthopteroid insects. In the adult terminal abdominal ganglion (TG), MAb 5B12 binds to glia of the cortex-neuropile interface and to glia associated with peripheral (e.g. cercal sensory nerves) and central nerve tracts. Immunogold-EM localization techniques demonstrate that the 5B12 antigen has a complex extracellular distribution within the TG; the antigen is abundant in the interface, but is restricted to discrete central neuronal processes within the ganglionic neuropile. These findings suggest that the antigen may be involved in the organization of fiber tracts within the CNS.

The 5B12 antigen is expressed relatively early during embryonic development (ca. 25%), where it is distributed along the luminal surfaces of sensory appendages (cerci, antennae) and limb buds; within the CNS, the antigen is associated with the anterior and posterior commissural tracts, and with regions of germinal neuroepithelium. This distribution pattern persists through mid-development; however, by 95%, reactivity is spread throughout the neuropile and the luminal surfaces no longer bind the antibody. Immuno-EM analysis of the 5B12 distribution in the embryonic cercal appendage demonstrates that the antigen is mainly associated with epithelial cell filopodial processes which are localized at the luminal surface in close apposition with basal lamina and developing nerve fiber tracts.

Western blot analysis of total adult TG proteins characterizes the 5B12 antigen as a M_r 185 kD macromolecule that has the properties of a proteoglycan (e.g. marked sensitivity to hyaluronidase and periodate treatments). Interestingly, although a lower MW component can be resolved in whole embryo immunoblot preparations, the 185 kD band is not readily detectible, suggesting extensive biochemical modification of the antigen with development. Thus, 5B12 antigen expression is developmentally regulated both in terms of its spatial distribution and biochemical nature, and we are currently exploring the possible interrelations between these processes and key events in neural development such as axon outgrowth and guidance. Supported by NIH #NB-07778.

314.17 DEVELOPMENT OF CONTROL OF CILIARY LOCOMOTION IN A GASTROPOD VELIGER. S.A. Arkett* (SPON:R.W. Skelton). Dept. of Biology Univ. of Victoria, Victoria, B.C. V8W 2Y2 CANADA

The ciliated velum of veliger larvae is a temporary structure used for locomotion during the short planktonic stage and discarded upon metamorphosis. The locomotory, pre-oral cilia of competent veligers beat in laeoplectic metachronal waves, but are periodically interrupted by coordinated, velum-wide ciliary arrests. These ciliary arrests have been shown to be caused by a single, regenerative action potential in the electrically-coupled, pre-oral ciliated cells of competent veligers of the gastropod Calliostoma ligatum (Arkett and Mackie 1986). These spikes result from summing EPSPs, which originate from the CNS. Pre-oral ciliated cells arise from prototrochal cells in the trochophore stage and are in turn descendents of the trochoblasts in the gastrula stage. I describe here the development of synaptic activity and action potentials in prototrochal and pre-oral ciliated cells and the rhythmical ciliary arrest behavior of a gastropod veliger, C. ligatum.

Intracellular electrode recordings from ciliated prototrochal cells as early as 45 hours post-fertilization (larvae were raised in the lab at 10-12°C) show a resting potential of -55 to -60mV and small (1-3mV), arhythmic, spontaneous EPSPs. Prototrochal cilia beat asynchronously and show no ciliary arrests. By 50h, arhythmic depolarizations are larger (3-6mV), the characteristic metachronal wave is established, but no spontaneous ciliary arrests are seen. However, injection of a brief pulse of depolarizing current causes a spike and a velum-wide ciliary arrest. This spike and ciliary arrest is identical to those observed in competent veligers. By about 58h, the depolarizations are larger still (12-15mV) and rhythmical, occurring at about 0.5Hz, yet are still subthreshold for ciliary arrest. Spontaneous ciliary arrests are first observed at this stage and these are caused by action potentials that are identical to those recorded from competent veligers. Pre-oral ciliated cells from veligers older than this show rhythmical spiking and ciliary arrests. It appears that prototrochal ciliated cells are capable of spiking very early in development, and that these spikes can cause coordinated, velum-wide ciliary arrests, yet spontaneous arrests are not seen. Neuro-ciliary synapses appear to be established before the velum expands and becomes functional for locomotion, but the 'strength' of these synapses is insufficient to generate suprathreshold EPSPs and no ciliated cell spikes are generated. The development of CNS control of locomotion and the capability of the velum to function as a locomotory organ appear to be closely linked.
Supported by NSERC grant to G.O. Mackie.

314.18 REMODELING OF LEG MOTOR CIRCUITS DURING POSTEMBRYONIC DEVELOPMENT OF THE MOTH MANDUCA SEXTA. K.S. Kent and R.B. Levine, ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

As part of our study of the postembryonic development of leg motor circuits in the moth Manduca sexta, we are continuing to examine the alterations that take place in sensory and motor components as the insect metamorphoses from larva to adult. Previously (Soc.Neurosci.Abs. 12:928), we described sensory and motor neurons of the larval prothoracic leg and reported that larval leg motorneurons (MNs) are retained and are present in the adult. To determine whether these retained larval leg MNs are also adult leg MNs, we are using a persistent, fluorescent tracer (Fluoro-Gold) to label larval MNs and a secondary fluorescent label (Texas Red) for adult MNs. Identified larval leg MNs are retrogradely labeled by injection of Fluoro-Gold into specific larval muscles. After metamorphosis is complete, adult leg MNS are labeled by intracellular injection or by backfilling leg nerves with Texas Red. Double-labeling indicates that certain adult leg MNS are indeed retained larval leg MNs.

To further identify and characterize prothoracic leg MNs in the adult, we have used intracellular recording and dye-injection methods. Like larval leg MNs, adult leg MNs have arborizations in a lateral region of neuropil but have more extensive arborizations than their larval counterparts. Examination of the dendritic arborizations of identified adult leg MNs suggests that certain features are common to particular classes of MNs. For example, flexor MNs innervating muscles controlling distal leg segments (pretarsus, tarsus, tibia) have many, long, sparsely branching processes in the lateral region of neuropil. Extensor MNs and MNs innervating muscles controlling proximal leg segments (femur, trochanter, coxa) have stouter, more densely branching processes in this lateral region and in some cases, extremely dorsal processes that extend to the midline of the ganglion.

We are currently investigating the nature of sensory inputs to these adult leg MNs by recording intracellularly from identified MNs while stimulating mechanosensory and proprioceptive elements of the leg. As in the larva, adult leg MNs are influenced by external mechanoreceptors. Tactile stimulation can elicit coordinated movements of several leg segments. In addition, adult leg MNs are influenced strongly by internal proprioceptors.

Cobalt staining of sensory neurons innervating specific groups of leg sensilla (hairplates, scattered hairs) demonstrates specific patterns of central projections characteristic of the type of sensilla and location on the leg. The apparent overlap at the light-microscope level between the dorsal-most extent of certain sensory processes and the ventral-most extent of MN dendrites suggests the possibility of direct sensory input to certain MNs.

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314.19 EFFECT OF AUDITORY DEAFFERENTATION ON THE SYNAPTIC CONNECTIVITY OF IDENTIFIED INTERNEURONS IN ADULT CRICKETS. P.D. Brodfuehrer and R.R. Hoy. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

Previous investigations have shown that chronic auditory deprivation of nymphal crickets, T. oceanicus, causes the dendrites of an identified auditory interneuron, Int-1, to sprout and form new functional connections in the contralateral auditory neuropil (Hoy et al., Proc. Nat. Acad. Sci. 82:7772, 1985). This neuropil is normally innervated by Int-1's homolog. In this system there is the potential for competition to occur between Int-1 on the deafferented side and its unaffected homolog on the intact side for synaptic sites in the remaining auditory neuropil. However, nymphal crickets are deaf because their ear appears only after the final molt to adulthood. Thus the neural circuitry of the auditory system may not be completely established when the deafferented Int-1 is forming its new connections. We were interested in determining whether the formation of new connections by the deafferented Int-1 affects the synaptic connections of the intact Int-1 in adult crickets where the auditory circuitry is fully established.

To test this we first demonstrated that auditory deafferentation of adult crickets also causes the medial dendrites of Int-1 to sprout and form new functional connections. These new connections form 4-6 days following deafferentation, and by 28 days following deafferentation these connections have restored most of Int-1's normal physiological responses to auditory stimuli. Secondly, in adult crickets we compared the strength (number of spikes/stimulus) and latency of the response elicited in both Int-1s by 30 kHz sound pulses, and the Int-1 tuning curves as a function of the number of days following deafferentation. If synaptic competition occurs between the Int-1 cell pair, the strength of Int-1's response should systematically decrease with number of days following deafferentation, while the latency and threshold intensities should increase.

In deafferented adult crickets, the strength of the Int-1 response increases with stimulus intensity, while the latency decreases. There was however, no systematic reduction in the strength of the intact Int-1 response to 30 kHz tones or an increase in latency with number of days following deafferentation. Moreover, there was no increase in threshold intensity for the intact Int-1 following deafferentation. Thus our physiological results suggest that competition is not occurring between the Int-1 homologs for synaptic connections in the remaining auditory neuropil.

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314.20 HYDROXYUREA-INDUCED EFFECTS PARALLEL RADIATION-INDUCED EFFECTS ON THE DEVELOPING OLFACTORY GLOMERULI IN *MANDUCA SEXTA*. Lynne A. Oland and Leslie P. Tolbert. Dept. of Anatomy & Cell Biology, Georgetown Univ., Washington, DC 20007.

The synaptic neuropils of first-order olfactory centers are organized into discrete glomeruli, thought to have functional roles in the processing of olfactory information. In the antennal lobe of the moth's brain, glomeruli form only in the presence of olfactory afferent axons and are enveloped by glial processes. Our previous studies have suggested that construction of the glomeruli from an early homogeneous neuropil depends upon changes in glial cells and that these changes are induced by the arrival of afferent axons from the antenna. Last year we reported experiments using irradiation to reduce the number of glial cells in the lobe in order to test the hypothesis that glial cells have a necessary role in the formation of glomeruli. We used gamma-irradiation to interfere with glial proliferation at a stage (4/5) when glia are dividing but neurons of the antennal system are all postmitotic and glomeruli have not yet developed. When the number of glial cells was severely reduced, the antennal-lobe neuropil failed to develop glomeruli despite the presence of antennal afferent axons. However, the presence of mild cuticular abnormalities in the treated animals raised the possibility that our findings in the antennal lobe might reflect direct cellular damage rather than the effect of reduction of glial numbers.

In order to circumvent the lack of possible internal controls for irradiation, we have now interfered with glial proliferation using hydroxyurea (HU; 9.5 mg/gm wt.; Truman & Booker, J. Neurobiol. 17:613, 1986) injected through the thoracic cuticle at stage 4/5.

External cuticular features develop in the normal sequence in HU-injected animals, but leg spines and claws are often mildly deformed and the animals' heads, wings, and antennae, though well formed, lack scales. These are the same abnormalities that characterize irradiated animals. Their presence in both experimental groups suggests that they reflect interference with mitotically active epithelial elements and cannot, in the case of irradiated animals, be attributed to radiation damage.

In the antennal lobes, injection of HU results in the desired reduction in number of glial cells. Reduction of the number by more than 2/3 results in a neuropil resembling that of irradiated lobes with an equivalent reduction in glial numbers, i.e. the neuropil lacks normal glomerular organization even though afferent axons are present. The experiments with HU confirm the results obtained with radiation and lead us to conclude that the organization of the antennal-lobe neuropil into glomeruli during development not only depends upon afferent axons, but also requires the presence of adequate numbers of glial cells. Our working hypothesis is that glial cells act as intermediaries in developmental interactions between afferent axons and antennal-lobe neurons. (Supported by NIH grants #NS 07602 to LAO and #NS 20040 to LPT.)

314PO ORIGIN OF SEGMENTAL DIFFERENCES AND BILATERAL ASYMMETRIES IN THE EMBRYO OF THE LEECH. M. Shankland and M.Q. Martindale*. Dept. of Anatomy & Cellular Biology, Harvard Med. Sch., Boston, MA 02115.

The segmental tissues of the leech arise from a set of 5 bilaterally paired embryonic teloblasts which give rise to stereotyped complements of adult tissue. A given teloblast generates a chain of primary blast cell daughters which represent segmental precursors for its particular cell line. For example, the O teloblast gives rise to a series of o blast cell daughters, each of which contributes a homologous set of descendants to a different body segment. These descendants are restricted to only one side of the midline, and bilaterally homologous structures arise from blast cells generated by the contralateral O teloblast.

Using fluorescent lineage tracers we have examined the origin of segmental differences in embryos of the leech Helobdella triserialis. Segmentally homologous o blast cells give rise to descendant clones of nearly identical cellular composition, with the only known exception being that these blast cells contribute the most distal cell of the nephridial tubule in abdominal segments 2-5 and 8-18, whereas no comparable cell is found in the mature leech in other segments that lack nephridia. By examining early stages of embryogenesis, we found that o blast cells generate a distal tubule cell homologue in every abdominal segment, and that this cell dies prior to terminal differentiation in segments that lack the remaining, mesodermal portion of the nephridium. Using a published method for shifting blast cells along the body axis, we were able to study the development of o blast cells which took part in the formation of ectopic segments, i.e. segments inappropriate for their cell lineage. In such cases the survival or death of the distal tubule cell did not depend upon its normal fate, but rather correlated precisely with the identity of the host segment. Thus, a lineally identified distal tubule cell could either be rescued or murdered by altering the segment into which it was born, and the segmental differences observed in normal embryos seem to derive from a positionally determined pattern of cell death.

There are also segmental differences in the distribution of neurons which stain with a commercial antibody against molluscan small cardioactive peptide B. In H. triserialis, the antibody only stains neurons in the head and abdominal ganglia 1-4. Once the teloblast of origin has been determined, we will ask whether or not the precursor blast cells will generate immunoreactive neurons if shifted to more posterior segments. Among the immunoreactive neurons is an unpaired cell located at the lateral edge of the ganglion. This unpaired cell is found on the right side of ganglia 1 and 3, and on the left side of ganglion 2. Additional experiments should reveal whether this unpaired neuron is the survivor of an initially bilateral cell pair, and to what degree the final pattern of asymmetry is dependent upon cell lineage and cell interaction.

315.1 LIGHT-MICROSCOPIC ANALYSES OF ORGANOTYPIC HIPPOCAMPAL EXPLANTS. J. Fowler, Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

Organotypic hippocampal explants have been used as models for studying hyperexcitability (Gahwiler,'83; Fowler et al., '86), innervation by catecholaminergic fibers (Dreyfus et al., '79) & development of granular & granular-like neurons (Zimmer & Gahwiler,'84; Fowler & Crain,'86; Fowler,'86). In the studies reported here, development & maintenance of pyramidal, granular & other neuronal types were assessed with Golgi, HRP & thionin labeling after 1d-8wks in culture. Generally, pyramidal & granular cells were arranged in organotypic arrays approximating field cytoarchitecture in situ & were not displaced or stimulated to migrate (to a significant degree) by dissection trauma, ongoing reorganization, cell death or maturation. When distinctive infra- & supra-pyramidal blades were not evident, the dentate formed a triangular wedge of cells, obscuring the hilar area. Neurons were labeled in all major subfields, including CA1-CA3, subiculum, "molecular layer" & dentate. Pyramidal & granular cells resembled in situ counterparts, with the exception cell bodies (pyramidal) were slightly smaller & dendritic branching not as elaborate as reported for cells (in situ) in juvenile & adult rodents by other authors. Granular & granular-like neurons often had multiple dendrites (2-3; apical, basilar or lateral) that persisted for the duration of the culture period(up to 8 wks). Dendritic spines were evident on pyramidal & granular neurons, although apparent spine density was never as great as reported for juvenile & adult rodents. As in situ, spine shapes were variable & in some cases formed small lumpy enlargements, nodulations or thin needle-like processes (pedunculate). On some neurons, 1 dendrite might have a relative abundance of spines whereas on adjacent branches spines were sparse. While the majority of pyramidal & granular cell bodies resembled similar neurons in situ, a number of features were observed that appeared atypical. A few neurons had large, irregular nodular protrusions, a lumpy, nodular cell body or stubby, foot-like outgrowths. Although most pyramidal-like neurons had apical & basilar dendrites, a few neurons were labeled in which basilar processes were not evident. Whether these unusual features associated with neurons in explant culture are related to immaturity, suboptimal nutrient medium or synaptic reorganization subsequent to denervation & explantation remains to be assessed.

315.2 DEVELOPMENTAL AND GROWTH-ASSOCIATED REGULATION OF THE LIMBIC SYSTEM ASSOCIATED MEMBRANE PROTEIN IN BRAIN EXPLANT CULTURES. F. Keller* and P. Levitt (SPON: M.R. Celio). Inst. of Pharmacology, Univ. of Zurich, CH-8006 Zurich, Switzerland, and Dept. of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

The limbic system associated membrane protein (LAMP) is a 60 Kd glycoprotein that is expressed early in development in cortical and subcortical limbic regions and associated fiber tracts. In the adult, LAMP remains in the cellular areas on somata and dendrites but is no longer detectable on axons. In the present study, we used the anti-LAMP monoclonal antibody and immunohistochemistry to investigate the distribution and time pattern of development of LAMP in explant cultures. Septal, hippocampal and amygdala cultures, prepared from newborn rats, contained high amounts of the protein. Within the explants, the distribution of LAMP-immunoreactivity was organotypic: e.g. in the amygdala, the staining was restricted to the amygdaloid nuclei, whereas the adjacent pyriform cortex was unstained. In addition, immunoreactivity was distributed on the surface of neurons as well as fibers, similar to that seen during development in situ. Fiber staining was prominent in the outgrowth zones of young cultures and in intraexplant pathways (e.g. between the fascia dentata and the Ammon's horn in hippocampal cultures) as well as in extrinsic pathways (e.g. the commissural fibers in double hippocampal explants). The fiber staining was transient in the explants, comparable to our previous findings in situ. The early neurites growing out from the explants in the first days in culture were strongly immunoreactive, gradually disappearing after the first week in vitro, at a time when fibre maturation proceeded, as shown by acetylcholinesterase histochemistry. An unexpected finding was the decrease in LAMP-immunoreactivity in single explant cultures, beginning at the 2nd of 3rd week in vitro. This phenomenon is not observed in situ, because limbic regions of the adult brain continually express LAMP. The decrease of LAMP in vitro was observed after aldehyde fixation, but not in unfixed or acetone fixed cultures, and seems to reflect antigen modification rather than antigen loss. It was further observed that once LAMP had disappeared from the explants, mechanical tissue damage rapidly induced immunoreactivity in the immediate vicinity of the lesion and on regenerating fibers which grew across the lesion cavity.

These observations suggest that 1) LAMP may be associated with the development of connections in the limbic system, 2) maintenance of LAMP in the mature brain may require intact specific connections between nerve cells, and 3) LAMP may be associated with the repair and reorganization processes that occur after brain injury.

315.3 PROTEIN SYNTHESIS IN GUINEA-PIG HIPPOCAMPAL SLICES: AN AUTORADIOGRAPHIC STUDY USING LIGHT AND ELECTRON MICROSCOPY. P. Lipton & S. Feig*, Depts. of Physiol. and Anat., Univ. of Wisconsin, Madison,WI53706.

The long-term goal of these studies is to examine regulation of protein synthesis in hippocampus by neurotransmitters and by electrical activity. Because of the marked heterogeneity of brain tissue, it is essential to be able to measure synthesis in clearly defined regions. Autoradiography offers the only reasonable way of doing this.

To measure protein synthesis it is essential to remove free radiolabelled precursor (3-H leucine). We have examined the effects of different fixatives on the washout. For electron microscopy it is essential to wash out precursor in warm buffer for 90' prior to fixation. In this case less than 5% of the tissue radioactivity is unincorporated precursor.

Three preincubation techniques have been tried to obtain tissue which synthesizes protein optimally: putting slices immediately into normal buffer with 2.4 mM Ca or 1.2 mM Ca or into OCa/10 mM Mg-containing buffer for 45' prior to switching to 1.2 Ca buffer. Slices from the last method showed distinctly improved morphology and more uniform protein synthesis. This is especially true in the principle cell layers. Interneurons, glia and endothelial cells are well labelled in all three preparations. We are now routinely using this method, after cutting the slices on a vibratome at 450 u. Slices are labelled with 3-H leucine for 90'. Cycloheximide included with the 3-H leucine, reduces observed grain density by at least 90%.

In the optimally prepared slices, observed by L.M., grains are present in all somata, and are also present in neuropil. In the neuropil, observed by E.M., grains are primarily localized in dendritic elements, including spine regions; there may be some localization in pre-synaptic elements. We are examining relative grain densities in different cell types; oligodendroglia are very heavily labelled.

A major aim of the study is to measure synthesis within dendrites. In order to do this dendritic flow must be prevented. The only reliable method here is to sever the dendrites from the somata. We examined the effect of small cuts in the apical region of the CA1 pyramidal cell layer, made immediately after 3-H-incorporation, on the morphology of and grain distribution in the apical dendrites. The surgery caused profound disruption of the dendritic microtubules and some morphological changes. Most importantly, silver grains are present in these dendrites, and are distributed similarly to those in the control dendrites. Thus, it should be possible to study protein synthesis occurring within the dendrites.

315.4 THE TIME OF ORIGIN OF SOMATOSTATIN-IMMUNOREACTIVE NEURONS IN THE HIPPOCAMPAL FORMATION OF THE RAT. P.R. Rapp and D.G. Amaral. Developmental Neurobiology Laboratory, The Salk Institute, La Jolla, CA 92037.

Somatostatin-immunoreactive (SSIR) neurons and fibers are present in the hippocampal formation of many vertebrate species including man. While the hippocampus has been widely used as a model for cortical ontogeny, little is known about the development of specific cell types in the hippocampus. It is now possible to determine the time course of neurogenesis of specific neurochemical systems using a combination of immunohistochemistry and [3H]-thymidine autoradiography. Experiments using flattened hippocampal preparations were therefore conducted to determine the time of origin and anatomical distribution of SSIR neurons in the hippocampal formation of the rat.

Timed pregnant Sprague-Dawley rats were given a single I.V. injection of [3H]-thymidine on embryonic day (E) 13, 14, 15, 16, 17, or 18. At approximately 2 months of age the offspring were sacrificed and prepared for immunohistochemistry. Dissected hippocampi were gently flattened and coronal sections (20 µm) were cut on a freezing microtome throughout their rostro-caudal extent. Free floating sections were incubated for 24 hrs. at 4° C in antiserum (SS 309) directed against somatostatin 28. Tissue was then processed according to the avidin-biotin-peroxidase method. Mounted sections were subsequently prepared for autoradiographic visualization of [3H]-thymidine labeled neurons. The distribution of [3H]-thymidine labeled and somatostatin positive (THY-SSIR) cells was plotted using a microscope linked computer digitizing system.

Most hippocampal THY-SSIR cells are generated on E13 and E14. The majority of these cells are generated on E14 and are preferentially added to septal and temporal ends of the hippocampus. Fewer SSIR cells are generated on E15 and only occasional SSIR neurons are born at later embryonic stages. The overall pattern of SSIR neurogenesis also reflects a subiculo-dentate gradient such that SSIR cells generated on E13 are preferentially distributed to the subicular complex, E14 generated cells tend to be positioned throughout the CA1-CA3 fields of the hippocampus, and the majority of cells born on E15 are located in the hilar region of the dentate gyrus. These results, in parallel with previous studies on the neurogenesis of GAD-positive cells, demonstrate that hippocampal SSIR cells are generated during a narrow period between E13-E15. In addition, the present findings demonstrate that computer-assisted quantitative analysis of neurogenesis in the flattened hippocampus can reveal subtle gradients of development which are not apparent in standard preparations.

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315.5 BEHAVIORAL MODULATION OF THE INDUCTION OF LONG-TERM SYNAPTIC PLASTICITY IN THE HIPPOCAMPUS. C.R. Bramham* and B. Srebro*. Dept. of Physiol., Univ. of Bergen, N-5000 Bergen, Norway. (SPON:ENA)

The efficacy of neuronal transmission in the major hippocampal pathways changes with the behavioral state of the animal. We investigated the possibility that the ability of neurons to undergo long-term potentiation (LTP) of the perforant path-granule cell system is also modulated by behavioral state.

Fifteen male Sprague-Dawley rats were anesthetized and electrodes cemented in position to allow chronic stimulation of the perforant pathway with recording of evoked potentials and EEG from the dentate hilus. Experiments started at least one week after surgery in environmentally habituated animals and were performed between 8 A.M. and 1 P.M.. Standard field potential measurements were collected in a still-alert state (SAL), slow-wave sleep (SWS), and during rapid eye movement sleep (REM), as defined by behavioral and EEG criteria. On the first 3 days of testing, stimuli were delivered during SAL at regular intervals throughout the experimental period to detect possible fluctuations in the response, and stimulus strength-response curves were collected in SAL and SWS to establish the behavioral modulation pattern of the field potential. On day 4 animals received a high-frequency stimulus train (400 hz, 20 ms, 8 sequences with at least a 10 s rest interval) during either SAL or SWS. A second train was given on the next day (after the response decayed to the baseline level) during the other behavioral state, with the current intensity adjusted to evoke an epsp equivalent to that of the first train. Six of these animals were later tetanized during REM sleep.

The following results were obtained: 1) the pattern of behavioral modulation of field potentials (SAL versus SWS) was as described by others and did not change after tetanization, 2) induction of LTP was more likely to occur during SAL and REM sleep, as compared to SWS, 3) animals which failed to demonstrate LTP in SWS sustained typical LTP when tetanized in SAL, 4) after tetanization in SWS, a long-term depression of the epsp-spike relation occurred as often as LTP, but was not seen in SAL.

The results suggest that the capacity to sustain long-term synaptic plasticity is not a static property of hippocampal neurons but is dynamically modified according to the behavioral state of the animal.

315.6 LONG-TERM POTENTIATION DECREASES DEPENDENCE OF HIPPOCAMPAL DENTATE GRANULE CELL RESPONSE ON THE FREQUENCY OF PERFORANT PATH INPUT. J.R. Balzer*, R.J. Sclabassi and T.W. Berger (SPON: P. Jannetta). Departments of Behavioral Neuroscience and Neurosurgery, Univ. of Pittsburgh, Pittsburgh, PA 15260.

High-frequency stimulation of perforant path (PP) afferents to the hippocampal dentate gyrus (DG) is known to produce long-term potentiation (LTP) of PP-DG synaptic efficacy. We previously have shown that LTP is associated with a decrease in nonlinear properties of the PP-DG projection when those properties are evaluated using the same stimulation intensity pre- and post-LTP (Berger et al., Soc. Neurosci. Abstr., 1984), i.e., when the number of presynaptic elements activated is held constant. To determine whether this represents a saturation phenomenon, we examined nonlinear properties of the PP-DG response using stimulation intensities that equated the number of granule cells activated pre- and post-LTP.

Nonlinear characteristics of the PP-DG projection were determined using systems analytic techniques. For these procedures, a train of 4064 impulses (0.1 ms duration) with randomly varying inter-impulse intervals (Poisson distribution, λ = 500ms) was delivered to the PP of chronically implanted rabbits. Amplitude of the DG population spike was measured in response to each impulse in the train. Stimulation intensity was varied both pre- and post-LTP; one random train was delivered at one intensity each day. Intensities were those producing 5%, 10%, 30%, 50% or 100% of maximum spike amplitude based on I/O curves generated by single impulses delivered at 0.1 Hz.

First order kernels, which reflect the average population spike response to each impulse in the train, were unaltered as a result of LTP. Second order kernels, which reflect the modulatory influence of a previous impulse on the activity evoked by the current stimulus, revealed pronounced decreases in nonlinear characteristics post-LTP. Facilitation, which averages 70-80% pre-LTP when low stimulation intensities (5-10%) are used, is greatly reduced when the number of granule cells activated is equated using low intensities post-LTP. Third order kernels values, which reflect the modulatory influence of the preceding pair of intervals on the activity evoked by the most current stimulus, also were greatly reduced post-LTP. When pre-LTP intensities reached 50% or more, nonlinearities were equivalent to those observed post-LTP, i.e., second and third order kernel values approached zero. These results indicate that LTP is associated with a decrease in the dependence of DG output on the frequency and pattern of PP input, and that post-LTP nonlinear characteristics are independent of the intensity of stimulation. Supported by The Whitaker Foundation and the Office of Naval Research.

315.7 CALCIUM-INDUCED LTP IN THE HIPPOCAMPUS: REGIONAL DIFFERENCES BETWEEN CA1 AND CA3. P.P.H. Leung*, K.G. Baimbridge and J.J. Miller. (SPON.: J.P.J. Pinel) Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada. V6T 1W5

The N-methyl-D-aspartate (NMDA) receptor has been implicated in the induction of long-term potentiation (LTP). It has also been suggested that there is a regional disparity in the distribution of the NMDA receptors within the rat hippocampal formation favoring the regio superior over the regio inferior (Monaghan et al., J. Neurosci., 5:2909, 1985). Furthermore, Harris et al. (in: Excitatory Amino Acid Transmission, 1987) demonstrated that the NMDA antagonist, D-2-amino-5-phosphonovaleric acid (D-APV), blocked the mossy fibre but not the commissural/association activated frequency-induced LTP in guinea pig slices. In view of these differences, we have examined the ability to induce LTP simply by raising extracellular calcium (Ca^{2+}-LTP) (Turner et al., Neuroscience, 7:1411, 1984) in the CA1 and CA3 regions and the effect of D-APV on these responses.

Transverse hippocampal slices prepared from male Wistar rats were allowed to recover in normal artificial cerebro-spinal fluid (CSF) containing 1.5 mM Ca^{2+} and 1.5 mM Mg^{2+}. Stable synaptically activated population spike responses from CA1 (via stratum radiatum) or CA3 (via mossy fibres) were obtained from different slices incubated together in the same chamber to ensure that both were exposed to near identical experimental conditions. We have confirmed our previous report that 5-6 min exposures of artificial CSF containing 4 mM Ca^{2+} and 1 mM Mg^{2+} produced a consistent (200-500%) long lasting potentiation in CA1. However, the same treatment failed to induce a potentiation, and in some cases, actually resulted in a depression of the responses in CA3.

Ten minute pre-treatment by D-APV at 25 uM did not alter the responses in CA3 but exerted a consistent, slight depression (up to 50%) of the control synaptic responses in CA1. This pre-treatment failed to affect the potentiation induced by the high Ca^{2+} media especially over the early potentiation phase (30-45 min post high Ca^{2+}). We did however observe an attenuation of the potentiation response over the extended period which requires further investigation.

These results suggest that the NMDA receptors might partly participate in the normal synaptic responses of the stratum radiatum evoked CA1 responses but not in the mossy fibre evoked CA3 responses in the rat. In view of the failure of D-APV to affect Ca^{2+}-LTP, at least for the early phase, it can be suggested that the induction of LTP involves more than just the NMDA receptor and may be pathway-specific.
Supported by Canadian MRC Program Grant to K.G.B. and J.J.M.

315.8 KINDLING ALTERS A CALCIUM DEPENDENT POTASSIUM CONDUCTANCE AND SYNAPTIC TRANSMISSION IN AMYGDALA NEURONS. Po-Wu Gean* and Patricia Shinnick-Gallagher. Department of Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston, TX 77550.

Kindling is the development of seizures following repeated electrical stimulation of certain brain structures. Amygdala kindling is of clinical interest because the behavioral seizures that develop are limbic in nature and related to common forms of human complex partial seizures. The purpose of this research was to analyze normal electrophysiological characteristics of neurons in the basolateral amygdala (BLA) nucleus and to determine the changes that occur in those properties at the membrane level as a result of kindling. Standard electrophysiological recording methods were used in an in vitro rat amygdala slice preparation.

Kindling was produced by daily administration of a subconvulsive stimulus through an electrode implanted in the BLA nucleus. After 11 days, a stage 5 kindled seizure was produced with the same stimulus. A contralateral brain slice was prepared 4-6 weeks after kindling was complete. Data were obtained from 28 control implanted rats and 36 kindled rats.

In the normal and implanted control animals, BLA neurons accommodated in response to injection of a long depolarizing current. An afterhyperpolarization (AHP) due primarily to calcium activated potassium conductances followed the termination of the cathodal current injection. Stimulation of the stria terminalis, an afferent pathway to the BLA, evoked an excitatory postsynaptic potential (EPSP)-inhibitory postsynaptic potential (IPSP) sequence.

Resting membrane potential, input resistance, membrane time constant, and spike amplitude and threshold were not different in control-implanted and in kindled cells. However, in kindled cells, intracellular injection of long depolarizing current pulses elicited action potential firing which did not accommodate. The amplitude of the AHP was unchanged but the 1/2 decay time was significantly shortened in kindled cells (178 $\pm$ 91 ms, n=10) compared to control implanted cells (635 $\pm$ 330 ms, n=6). These data suggest that a Ca-dependent K conductance, an active membrane property, is altered in this chronic model of epilepsy. In addition, stria terminalis stimulation in kindled animals evoked epileptiform discharge in 50% of the neurons and 'extra' synaptic potentials in the remaining neurons. IPSPs were not recorded in kindled cells.

These results suggest that chronic epileptiform activity induced in vivo is maintained in the in vitro amygdala brain slice and that alterations in excitatory transmission and loss of IPSPs as well as a Ca-dependent K conductance contribute to epileptogenesis in the amygdala.

315.9 EFFECTS OF ANOXIA ON SYNAPTIC TRANSMISSION AND CALCIUM CURRENTS IN IMMATURE HIPPOCAMPUS.

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In hippocampal slices from adult animals 2-4 min periods of anoxia cause a rapid loss of excitability and synaptic transmission, accompanied by a marked increase in K conductance and some hyperpolarization (Hansen et al. Acta Physiol. Scand., 115, 301, 1982). In addition, voltage clamp studies have revealed a strong but also reversible suppression of Ca current (Krnjević and Leblond, J. Physiol, 1987, in press).

In the present experiments, we have recorded in hippocampal slices from Wistar rats aged 0-15 days (as well as adult rats). The slices were superfused with ACSF at 33-34° gassed with either 95 % O_2 and CO_2 or 95 % N_2 and CO_2. Recording was mainly in CA1 (less often in CA3), spikes and PSPs being evoked by stimulating stratum radiatum.

Anoxia rapidly caused a loss of excitability but synaptic transmission was more resistant in immature than in adult slices and recovery more rapid on reintroducing O_2. In most cases, exposure to N_2 for 2-4 min produced only a partial reduction of EPSPs recorded extra or intracellularly.

In voltage clamp studies, Ca inward currents could be readily demonstrated after treatment with tetrodotoxin. Cs^+ and TEA (in some cases recording electrode contained 2M CsCl instead of the usual 2M KCl). As in mature slices at least two types of Ca current could be distinguished : one transient fast-inactivating, seen exclusively at holding potentials (V_H) of -70 to -90 mV was relative insensitive to Ba^{2+} and Cd^{2+} but blocked by Ni^{2+} (100 uM) : a more sustained current was seen exclusively at holding potentials (V_H) -40 to -50 mV, that was much enhanced by 2 mM Ba^{2+} and blocked by Cd^{2+} (50-200 uM). Both types of Ca current were reversibly depressed by anoxia, but the suppression of the sustained current was much more rapid, being largely complete within 2 min : the transient current disappeared only after 4 or more min.

[1] On leave from Mc Gill University, Montréal, with support from Fondation de la Recherche en Santé du Québec.

315.10 EPILEPTOGENIC REGIONS OF HUMAN MESIAL TEMPORAL LOBE SHOW ALTERED CONNECTIVITY AS MEASURED BY STIMULATION-EVOKED FIELD POTENTIALS. C.L. Wilson, M. Isokawa-Akesson, and T.L. Babb Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024.

On the basis of field potentials evoked by electrical stimulation of amygdala and hippocampus, Buser and Bancaud (EEG J., 55:1, 1983) suggested that connections between these mesial temporal lobe structures may undergo modification under the pathological conditions of temporal lobe epilepsy. In the present study, patients were studied during telemetry monitoring of EEG from depth electrodes chronically implanted for recording spontaneous seizure onsets required for the identification of resectable areas of epileptogenesis in medically intractable complex-partial epilepsy patients.

Electrodes stereotactically implanted in amygdala (AM), pes hippocampi (HP), presubiculum and parahippocampal gyrus were were used to deliver biphasic rectangular pulses of 0.1 msec duration at current densities ranging from 5 to 50$\mu C/cm^2$/ph, at a frequency of 0.1 Hz or less. Recordings were made from electrodes in adjacent structures as each site was stimulated with single pulses (imperceptible to patients using the above parameters). Averages based on 3 to 50 stimuli evoked field potentials ranging from 20-50uV at threshold to 0.5-3.0mV. Of the 52 patients tested preoperatively, data from 38 patients who came to surgery as a result of lateralization of an epileptogenic site or region were examined to determine which AM and HP sites evoked field potentials in the adjacent structure.

On the side contralateral to the site of epileptogenesis, AM stimulation evoked clear field potentials in the HP of 15 out of 24 patients (63%), and HP stimulation evoked clear field potentials in the AM of 19 out of 26 patients (73%). AM stimulation ipsilateral to the seizure onset side evoked HP responses in only 5 of 21 patients (24%) and HP stimulation evoked responses in the AM in 9 of 27 (33%) patients. The probability of such a difference in HP response between epileptogenic vs. non-epileptogenic sides during stimulation of AM occurring by chance is $p<.01$ ($\chi^2=6.88$, 2-tail, df=1), and in AM during HP stimulation is $p<.05$ ($\chi^2=5.31$, 2-tail, df=1).

These results agree with the study cited earlier in the significant reduction of amygdala input to a hippocampus associated with epileptogenesis, but differ in the reduction of hippocampal output we found as compared to a facilitation of output which they reported. Our results support a hypothesis of epileptogenic alteration of connections associated with neuronal loss rather than a facilitation of synaptic routes suggested by others, since hippocampal neuronal loss has been documented in this same population of patients on the side of seizure onset (Babb et al., Epilepsia , 25:729, 1984).
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315.11 MOCHA: A NEUROLOGICAL MUTANT EXPRESSING PERSISTENT THETA SYNCHRONIZATION OF CORTICAL AND HIPPOCAMPAL NEURONS. J. L. Noebels and R. L. Sidman, Dept. of Neurology, Section of Neurophysiology, Baylor College of Medicine, Houston, TX 77030 and Div. of Neuroscience, Children's Hospital, Boston, MA 02115.

Phenotypic survey of mapped single locus mutations in the mouse reveals the existence of genetic loci which control specific patterns of excitability in central neurons. We find that the coat color mutant mocha (gene symbol mh, Lane and Deol, 1974, chromosome 10, recessive), inherits an enhanced rhythmic pattern of activity normally associated with learning and exploratory behavior. Electrocorticographic (ECoG) recordings from the cerebral cortex and hippocampus in unrestrained mice revealed a persistent and exclusive pattern of high amplitude (>300 μV), 7-7.5 Hz waves (theta rhythm) unresponsive to sensory stimuli. Unlike other inbred mouse strains, the mocha rhythm is not intermixed with fast desynchronized activity. The mutant ECoG phenotype was present bilaterally in 11/14 adult homozygotes. Several mh/mh mice showed the mutant pattern over only a single hemisphere and a high frequency, desynchronized contralateral pattern. In younger mice studied, two showed the trait at 4 weeks postnatal, but one did not display the synchronized rhythm until 6 weeks of age. Heterozygous mh/+ mice (6/6) did not display the persistent theta trait. The littermate control mice, homogygous for the grizzled mutation and wild type at the mocha locus, showed either little theta activity at all (3/6), or steady but low amplitude rhythms. A second neurological deficit of mocha homozygotes (unilateral head tilt due to otolithic degeneration) is also incompletely penetrant, and also exhibits clear axial asymmetry, but is not concordant with the abnormal ECoG trait. Atropine (100 mg/kg) caused a long-lasting disorganization of the mocha ECoG pattern, blocking virtually all theta activity, just as it did in control mice of several strains. A second allele C3H-mh^{2J} can also show a similar background ECoG rhythm.

The mocha mutant thus reveals that the predominance of a specific spectral frequency of brain rhythm can be a heritable trait. Although difficult to breed, mocha offers a singular genetic opportunity to define cellular mechanisms controlling activation of neuronal theta synchrony and to determine the effects of this inherited error of brain signaling patterns on synaptic circuitry underlying learning and memory.
Supported by NS 11535, RR 05425, Pew Foundation Scholars Award (JLN) and NS 20820 (RLS).

315.12 ALTERED CHOLINERGIC FIBERARCHITECTURE AND SELECTIVE SURVIVAL OF ACHE-POSITIVE HILAR NEURONS WITH HIPPOCAMPAL SCLEROSIS IN HUMAN TEMPORAL LOBE EPILEPSY R.C. Green*, H.W. Blume, S.B. Kupferschmid*, M-M. Mesulam Harvard Medical School, Boston, Massachusetts 02215

Temporal lobe epilepsy (TLE) is a common subtype of human epilepsy which is treated by anterior temporal lobectomy in some medically intractable cases. The pathology of the surgically resected tissue is varied, but the most common finding is hippocampal sclerosis, consisting of neuronal cell loss and gliosis in a characteristic distribution with relative sparing of neurons in the CA2 subregion. We analyzed surgically resected temporal lobe tissue from four patients with TLE who were found to have hippocampal sclerosis on Nissl staining. Adjacent sections were stained for acetylcholinesterase (AChE) with a modification of the Koelle technique. The distribution of AChE fibers in the hippocampi of the patients with TLE was compared to that previously described in the normal human hippocampus (Green et al., Soc. Neurosci. Abst. 12:356, 1986)

In all four cases, the normal distribution of AChE fibers in the hippocampus was disrupted. AChE fibers were lost in a pattern that closely matched neuronal dropout in each case, and fibers were preserved in regions where there was relative sparing of cells. In each case, AChE positive polymorphic neurons within the hilum of the dentate gyrus were relatively spared despite severe loss of the immediately adjacent CA4 pyramidal neurons. In two of the four specimens, the normally heavy juxtagranular staining of AChE along the inner edge of the molecular layer of the dentate gyrus was absent while AChE staining was conspicuous in the outer two-thirds of the molecular layer of the dentate gyrus.

The AChE-rich septo-hippocampal pathway is the major source of cholinergic input into the hippocampus, projecting to neurons in Ammon's horn and to the proximal portion of the dentate granule cell dendritic tree. Loss of the juxtagranular AChE fibers in the molecular layer of the dentate gyrus may reflect a loss of cholinergic input from the septum, suggesting the possibility of septal pathology in some patients with hippocampal sclerosis. The residual AChE fibers in the outer portion of the molecular layer of the dentate gyrus could represent afferents from surviving acetylcholinesterase positive cells within the hilum.

Supported by the Wilder Penfield Fellowship of the Epilepsy Foundation of America (RCG).

315.13 INTERRELATIONSHIPS BETWEEN HIPPOCAMPAL CA1 AND DENTATE GYRUS: COHERENCE AND PHASE MEASUREMENTS. J.D.Bronzino, M.Grewal*, R.J.Austin-LaFrance* and P.J.Morgane. Dept. of Engineering and Computer Science, Trinity College, Hartford, CT 06106 and The Worcester Foundation for Experimental Biology, Shrewsbury, MA. 01545.

A number of studies have indicated that two regions within the dorsal hippocampal formation, the dentate gyrus and region CA1, are responsible for generation of the 4 -11 Hz rhythmic slow activity (RSA) known as theta rhythm (Bland, B.H. et al., Brain Res., 94:199, 1975; Bland, B.H. and I.Q. Wishaw, Brain Res., 118:259, 1976). In the present study we have utilized power and spectral analysis techniques to quantitatively describe the interrelationships which exist between the electrical activity recorded from these two regions during various vigilance states.

High coherence values (> 0.85) and a consistent phase difference of approximately 170° were found to exist within the theta range of frequencies during REM sleep. Coherence and phase spectrum computed during the vigilance states of slow-wave sleep and quiet waking showed no consistent relationship at any of the frequencies studied. These results agree with previous studies which indicate that whenever theta rhythm is present, the signal obtained from CA1 is consistently 180° out of phase with that obtained from the dentate gyrus (Krug et al., Brain Res. Bull., 6:5, 1981; Leung, W.S., J. Neurophysiol., 52:1051, 1984).

Except for the studies mentioned above, the evidence describing this phase difference has been determined primarily by qualitative observation of the EEG. Our results provide quantitative measures of the degree of interaction of the cellular activity which occurs between CA1 and the dentate gyrus during generation of theta rhythm in the adult rat.

Since the theta rhythm does not appear in the EEG until approximately 8 - 10 days of age, and there is a significant difference in the timing of both the neuro- and synaptogenesis within these hippocampal fields, (i.e., the pyramidal cells of region CA1 are formed entirely during the prenatal period while the granule cells of the dentate gyrus are formed largely postnatally), we intend to follow up these studies in developing rats in an effort to determine whether the measures of coherence and phase spectra are affected by the differential development of these two structures. These measures may then serve as markers of normal EEG development to assess the effects of various brain insults, such as prenatal protein malnutrition, on the development of theta activity within the hippocampal formation.

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315.14 PAIRED PULSE INHIBITION IN THE DENTATE GYRUS OF THE POSTNATAL RAT: AN IN VITRO STUDY. A.V.Nowicky, P.G. DiScenna,& T.J. Teyler. Neurobiology Dept., Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Extrinsic inhibition of hippocampal pyramidal cells develops late in rat and rabbit CA1, relative to excitatory synaptic events (Mueller et. al., 1984 and Harris & Teyler, 1983). The granule cells (GC) of the dentate gyrus (DG) are generated later than the CA1 pyramids, the majority leaving the mitotic cycle during the week after birth. Therefore we are interested in examining the the expression of extrinsic inhibition in rat pup DG. Orthodromic and antidromic stimulation (OS,AS) of the GCs are used in a paired pulse (PP) paradigm to study this question. Hippocampal slices were obtained from 6-10 day (PN6-PN10) rat pups and maintained under standard conditions. Small concentric bipolar electrode was placed in the developing molecular layer (OS) and in the hilar region (AS). A glass micropipette (2M NaCl, 4-8MOhm) was placed in the GC layer to record extracellular field potentials. The waveforms collected in this manner (OS) commonly had the basic triphasic pattern of the adult DG (P1,N1,P2)., however the potentials were much smaller (<1-2mv.). A stable stimulus at 50-75% max. was used for homosynaptic paired pulse series (.04Hz: 10 to 2000ms ISI). When a good antidromic response was available, a second series was recorded with AS as the conditioning pulse and OS , the test pulse. The results are based on experiments from 33 slices. As expected, the homosynaptic paired pulse series produced a complex pattern. Generally, strong depression of the N1 component was found across 10-40ms ISI (often N1 was completely eliminated). Preliminary analysis of the putative field EPSP slope (P1) also demonstrates a depression of the test pulse, which is similar to that reported for medial perforant path PPs in adults (McNaughton,1980). By PN7, antidromic stimulation inhibits the N1 component of the test pulse, without affecting the EPSP slope. This inhibition is not as strong or as long-lasting as the homosynaptic depression, but still can eliminate the N1 component of the test pulse at >20ms. ISI. This strongly suggests the capacity for feed-back inhibition. At longer ISIs, the antidromic pulse has minimal effects on the test pulse, usually depressing the P2 component. The homosynaptic PP however demonstrates effects on the test pulse out to 2000ms ISI. Bicuculline (1 nl. spritz to cell body) shifted the paired pulse response (short delays) from inhibition to faciliation in a PN9 slice, further supporting the presence of inhibitory circuitry in this relatively young system. Since the CA1 region has a low seizure threshold, the presence of the postnatal dentate inhibitory circuitry may provide protective attenuation of the excitatory input to the trisynaptic circuitry.

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315.15 INHIBIITION OF RADIATUM AND ORIENS EVOKED POTENTIALS BY STIMULATION OF THE DISTAL APICAL DENDRITIC FIELD IN RAT AREA CA1. P.G.DiScenna, A.V. Nowicky and T.J.Teyler. Neurobiology Department, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

A few studies have described the inhibition of orthodromically evoked population spikes (area CA1) by a preceeding conditioning stimulus, to neighboring afferents, that is below spike threshold. (Lynch etal,Exp.Neurol.71:527 & others) These findings suggest the operation of feed-forward inhibition (FFI) onto hippocampal pyramidal cells. A number of studies have provided anatomical and physiological evidence for hippocampal FFI (Buzsaki, Prog.Neurobiol.22:131: Somogyi et al,Brain Res.332:143). We report that stimulation in stratum lacunosum-moleculare (LM) can significantly inhibit population spikes evoked by stimulation of stratum radiatum or stratum oriens, in a paired pulse paradigm (area CA1). Our LM stimulation does not produce an observable population spike in CA1, CA3 or the subiculum, but appears at the cell body layer (extracellularly) as a short positivity (variable) followed by a 25-40ms negativity. This response is generally less than 0.5mv amplitude. Stimulation across the hippocampal fissure, in the dentate molecular layer, does not produce this effect. The inhibition can last over 200ms, but is most effective in the range of 10-50ms. where the test pulse spike can be inhibited by 75-100%. By delivering a short burst of LM stimulation (200Hz, 20ms or 40ms) the test pulse inhibition can be extended to beyond 500ms. LM stimulation can also decrease the amplitude of sub-spike threshold test pulses. An initial attempt at observing this phenomenon at the intracellular level was successful. The LM stimulus produces a pure hyperpolarizing event in a pyramidal cell and inhibited the cell's firing to orthdromic stimulation. Presently we are continuing the intracellular investigations, examining in detail the effects on synaptic activity, and computing one-dimensional current source density analysis of the LM stimulation. It is interesting to speculate on the nature and use of this inhibition. The entorhinal cortex (ECx) is a major source of afferents to LM, part of the so-called temporo-ammonic projection. It is not known whether these fibers are collaterals of the entorhinal projection to the dentate gyrus, or whether they arise from a separate neuronal population. Perhaps the ECx plays a dual role in the trisynaptic circuit, shaping output by excitation and inhibition, capable of providing a margin of safety through FFI during periods of intense activity.

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315.16 ISOLATION REARING LOWERS DOPAMINE METABOLITES IN RAT MESIAL PREFRONTAL CORTEX AND INCREASES TIMIDITY IN OPEN FIELD. R.R. Holson*, B.J. Gough*, A.C. Scallet and S.F. Ali (SPON: Kang Fan). Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Isolation rearing in rats has been reported to alter forebrain monoamine concentrations and activity in the open field. The precise nature of either effect is as yet uncertain, both as to direction and reproducibility of effects. Two isolation experiments are reported here. In the first, two sets of animals were reared in rigorous isolation (metal hanging cages) or in social housing (3/cage). At 90 days of age, one set of animals was sacrificed. Brains were dissected into five regions: mesial frontal cortex (MFC), hypothalamus, amygdala (Am), entorhinal cortex, and nucleus accumbens, then assayed for serotonin, dopamine and their metabolites by high pressure liquid chromatography with electrochemical detection. At the same age, a second set of animals was exposed for three minutes daily for four consecutive days to the open field. Thereafter, cage conditions were reversed for four months, following which the animals were sacrificed, and MFC and Am were assayed as above. In the second experiment, isolates and socially-reared animals were sub-divided into two groups, one of which received brief handling (5 secs twice a week) after weaning. All subjects were tested as above in the open field. Results were that isolation rearing produced alterations only in MFC, and in this region only the acidic metabolites of dopamine (homovanillic acid and dihydroxyphenylacetic acid) were reduced by 20% in isolates. Four months after reversing housing conditions, the two groups showed identical levels of MFC dopamine metabolites, dopamine, serotonin and its metabolite. Isolates in both experiments displayed strong symptoms of fear in the open field, including heightened defecation and prolonged freezing. Brief handling completely protected against this syndrome. Moreover, the first unhandled socially-reared animal to be taken from the home cage for testing displayed the same fear-like behavior as did isolates, while subsequent animals from the same cage did not. It is concluded that isolation rearing selectively lowers dopamine metabolites in prefrontal cortex. This appears to be a true developmental effect, not simply an acute reflection of differential housing, since four months of reverse housing did not differentially alter MFC dopamine metabolites. Isolation effects on activity are probably due to a lack of social facilitation from odor cues of familiar rats, and hence may be unrelated to isolate MFC dopamine abnormalities.

315.17 MICROCOMPUTER BASED UNIT ACQUISITION AND ANALYSIS SYSTEM. D. ZENDZIAN*, K. AUSTIN*, P.J. MORGANE AND J.D. BRONZINO (SPON C. Siok) TRINITY COLLEGE, HARTFORD CT 06106 AND THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY, SHREWSBURY, MA 01545.

Recently there has been considerable interest in determining the cellular events underlying specific types of EEG activity (Busaki, G. et al., Brain Res. Rev., 6:139, 1983). With this in mind, we have developed a microcomputer based unit acquisition and analysis system capable of detecting the occurrence of unit activity based upon a variety of specific waveform criteria (such as spike amplitude, spike width, etc.). This neuronal biosensor provides a specific set of measures including: 1) the time of occurrence at either or both the high and low threshold settings, and 2) the width of the action potential at each threshold, as well as extrapolated to baseline. Unit activity based upon any of these parameters may then be displayed in a variety of histograms which include 1) interspike interval (ISI) histograms, and 2) spike width histograms.

From these histograms the user may then select a particular range of values for one specific criterion (e.g., ISI, spike width), and the system will generate a spike train representing the firing rate of all the units satisfying the selected criterion along with the corresponding EEG. Once the resultant spike train and EEG is obtained, convolution and cross correlation procedures (Leung L.S. and G. Buzsaki, EEG and Clin. Neurophysiol., 56:688, 1983) may be employed to determine the degree of correlation between the cellular events depicted in the spike train and the corresponding EEG activity.

This system, therefore, provides the neuroscientist with a versatile tool to study the interrelationships between specific cellular events and the EEG. Of particular interest is its use in developing a better understanding of the role of neuronal populations in the areas CA1 and dentate gyrus in the generation of the theta rhythm observed in the hippocampal EEG during specific behaviors. This system offers great promise in the classification of different types of cells based upon electrophysiological measures. Efforts along these lines need to be extended to include detection and classification of a wide variety of bioelectric events. Supported by NSF Grant# E41688416708.

AGING AND DEMENTIA: PLAQUES, TANGLES, AMYLOID

316.1 LACTOFERRIN IMMUNOREACTIVITY IN NEURITIC PLAQUES AND NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE: LOCALIZATION TO THE RHINENCEPHALON AND ADJACENT CORTEX.

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Although several major components of the neuritic plaques (NP) and neurofibrillary tangles (NFT) that constitute the pathological lesions of Alzheimer's Disease (AD) have been extensively characterized, the etiology and molecular pathways involved in their initiation and formation are largely unknown. During studies of the distribution of iron and iron binding and iron storage proteins, we have observed the selective presence of lactoferrin (LF) immunoreactivity on both NP and to a lesser extent NFT in AD brain. Using affinity-purified antibody to human LF and avidin-biotin-peroxidase methodology, we have detected LF determinants on NP and NFT in amygdala, hippocampus and entorhinal cortex from 16 of 18 AD cases examined. AD cases included 8 males (age 70-83) and 10 females (age 56-88); LF immunoreactivity was absent from 5 age matched controls without neurological disease (age 61-88) and from 2 AD cases with extensive cortical plaques. Immunochemical specificity was confirmed by antigen inhibition and the use of multiple antisera and affinity-purified antibody at high dilution.

LF immunoreactivity was absent from or considerably reduced in other regions of brain that contained large numbers of NP or NFT as revealed by silver stains, indicating that this protein is not an essential component of these lesions. LF is normally a major protein in seromucous secretions and the granules of polymorphonuclear leukocytes and is a trace constituent of plasma, arising there from leukocyte degranulation. The presence of high levels of lactoferrin in nasal mucous has been described, indicating that the protein may be reaching the brain by anterograde transport along the olfactory tract. Indeed LF immunoreactivity was localized to those regions of brain that receive major input from the olfactory system, most notably in the cortical nucleus of the amygdala and entorhinal cortex. The presence of LF immunoreactivity in both the olfactory bulb and tract was confirmed immunohistochemically.

These observations support the contention that the extensive involvement in AD of those regions of brain that receive primary input from the olfactory system indicates an important role for this pathway in the pathogenesis of AD (Pearson et al. Proc. Nat. Acad. Sci. USA, 1985, 82:4531; Roberts Neurobiol. Aging, 1986,7: 561-567). Furthermore the iron binding sites of LF and reactivity with cellular receptors may involve this protein in the transport of metal ions and metal complexes such as aluminum or aluminosilicates in a majority of cases of AD. (Supported by grants from the NIH [NS23634] and the Robert H. Cole Foundation).

316.2 **LOCALIZATION OF BRAIN SPECTRIN IN ALZHEIMER SENILE PLAQUES.** **L.S. Perlmutter, P. Seubert, H.C. Chui*[1], M. Baudry and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717 and University of Southern California[1], Los Angeles, CA 90089-0191.**

Alzheimer disease is apparently a disease of the cytoskeleton. Abnormal accumulations of actin, tubulin, and neurofilament and microtubule-associated proteins have been reported. Abnormalities in brain spectrin, a structural protein which links cytoskeletal elements to the plasma membrane, have yet to be investigated. The present study examines the localization of brain spectrin in relation to thioflavin S-positive amyloid deposits in human brain.

Autopsy samples from Alzheimer diseased (n=4) and, at present, one Parkinsonian patient were immersion fixed in a mixed aldehyde fixative. Regions known to be affected in Alzheimer disease (areas 9, 17, 22, hippocampal formation) were sectioned on a vibratome at 50 *um* thick. Brain spectrin was immunocytochemically localized using a rabbit anti-rat brain spectrin and the biotin-avidin peroxidase technique (Vectastain). Tissue sections were then counterstained with thioflavin S, a fluorescent marker for amyloid proteins. The same tissue section could thus be examined for both brain spectrin-like immunoreactivity (BSLI) and amyloid.

BSLI was seen to line somatic, dendritic and axonal plasma membrane. No BSLI could be identified intracellularly, i.e., in relation to the paired helical filaments. There were, however, extracellular dark circular plexuses of densely immunoreactive processes. These processes formed a fibrous network that was highly distinguishable from the surround. This fine meshwork could be contrasted to the thick knob-like processes seen with an antibody to the microtubule-associated protein, Tau (Wood *et al., PNAS*, 1986, *83*, 4040; personal observations). BSLI plexuses appeared in two forms: (1) immunoreactive processes encircled the amyloid core; (2) immunoreactive processes formed a reticulum over the entire plaque. In these latter cases, the fluorescence was diminished or totally blocked (as determined by comparison with adjacent sections of thioflavin-stained sections from which the primary antibody was omitted). These staining configurations could result from the brain spectrin immunopositive processes forming a sphere around the amyloid core; alternately, they may signify plaques at different stages of development. There were neither BSLI circular plexuses, nor amyloid fluorescence in the Parkinsonian tissue.

These results suggest that brain spectrin is associated with the Alzheimer senile plaque, but not with the neurofibrillary tangle. In light of the recent finding of a protease inhibitor in the plaque (Abraham *et al., J Cell Biochem*, 1987, Suppl. 11D, 193), this staining pattern may reflect a build-up of unproteolized brain spectrin. (Tau antibody was a generous gift from Dr. L.I. Binder. Supported by NIA AG05373 to LSP and AG00538 to GL. This work done in collaboration with the Alzheimer Disease Research Center Consortium.)

316.3 INCREASED DETECTION OF LYSOSOMAL PROTEINASE ANTIGENS ASSOCIATED WITH THE NEUROPATHOLOGY OF ALZHEIMER'S DISEASE. A.M. Cataldo*, R.A. Nixon, C.Y. Thayer*, F.M. Benes and T.R. Wheelock* (SPON: N. Fleming). McLean Hospital, Harvard Medical School, Belmont, MA 02178

Previous studies in non-neural tissue indicate that alterations in the distribution of lysosomal enzymes may play an important role in mediating cell death in response to certain metabolic insults. The proposed mechanism involves the release of latent hydrolytic enzymes, including proteases, into the cytoplasm where certain of these enzymes may act on subcellular structures and lead to irreversible cellular damage. In the nervous system, lysosomes and lysosomal proteinase activities increase during normal aging; however, relatively little is known about lysosomal proteolysis in normal and degenerative states. To investigate the possible involvement of lysosomal proteolysis in neuronal cell death in Alzheimer's disease (AD), we have used specific polyclonal antisera to human brain cathepsin D (CD) and human cathepsin B (CB) to localize these antigens in the prefrontal cortex and hippocampus of postmortem human brain from individuals with AD and age-matched neurologically normal controls. Premortem clinical diagnoses were confirmed neuropathologically. CD and CB immunoreactivity was most abundant within pyramidal cells of neocortical layers III and V and in the large pyramidal cells and dentate neurons of the hippocampus in control and AD brains. At the subcellular level, CB and CD immunoreactivity was localized exclusively to lysosomes that were concentrated in the perikaryon and proximal portions of apical and basal dendrites. In certain neurons from AD brains, immunostaining was especially intense in the lysosomes of basal processes. Unlike neurons, glial cells contained relatively few immunostained lysosomes, and staining in the neuropil was negligible. Qualitative assessment of CB and CD staining suggested an increased immunoreactivity of neurons in AD brains. Preliminary microdensitometric analyses of CD staining in 7 control and 9 AD brains suggested that neurons of all sizes in neocortical layer III of AD brains contained increased immunoreactivity. In addition, senile plaques in AD brains were intensely stained by antisera to both CB and CD. Typically, plaques contained a diffuse ground substance that stained relatively homogeneously and irregular bodies of varying sizes that stained darkly. Some plaques contained one or more neuronal perikarya in various stages of degeneration. In these cells, immunoreactivity was not confined to lysosomes. These results are consistent with the possibility that, in AD, increased expression of lysosomal proteases may be an early response of the neuron to the disease process and that release of cathepsins from lysosomes may mediate aspects of neuronal cell death. Furthermore, lysosomal proteinases appear to increase in one or more cell types during or in response to plaque development and may persist as constituents of the neuritic plaque. Support: NS17535, AG05134, Anna and Seymour Gitenstein Foundation, Inc.

316.4 ALZHEIMER PAIRED HELICAL FILAMENT IMMUNOREACTIVITY IN DOWN SYNDROME BRAIN, L.A. Mattiace[1], D.R. Sparkman*[1], and C.L. White, III[2], Departments of Neurology[1] and Pathology[2], Southwestern Medical School, Dallas, Texas, 75235.

Down syndrome (DS) patients over the age of 40 demonstrate many of the histopathological markers which are associated with Alzheimer disease (AD), including neuritic plaques and neurofibrillary tangles (NFT). Although occurring in both cortical and subcortical structures, the presence of NFT in the cortex appears to correlate clinically with the expression and severity of dementia in both AD and DS (Wisniewski et al., Ann. Neurol., 17:278, 1985).

Ultrastructurally, NFT are comprised of proteinaceous paired helical filaments (PHF), which are morphologically identical in DS and AD (Rubenstein et al., Brain Res., 372:80, 1986). Although previous staining of NFT has been demonstrated by either silver impregnation or thioflavine S or T in both DS and AD tissue, polyclonal antibodies to PHF provide a more specific method for detecting the presence of PHF-containing NFT. Using a monospecific polyclonal antibody to Alzheimer PHF, we studied the immunoreactivity of NFT in a 34 year old DS patient.

PHF protein was isolated at autopsy from the brain of a patient with neuropathologically confirmed AD, and polyclonal antibodies were produced in rabbit. This antiserum has been well characterized in previous studies of AD brain (White et al., J. Neuropathol. Exp. Neurol., 44:368, 1985). Optimal dilution of the antiserum was determined by checkerboard titration.

Brain tissue obtained at autopsy from a patient (age 34M) diagnosed clinically with DS was immersion-fixed in 10% phosphate buffered neutral formalin. Thirty micron floating sections from the temporal neocortex were stained for PHF using a modified peroxidase-antiperoxidase procedure. Negative controls included non-immune serum and antiserum absorbed against both AD and young/normal brain. Adjacent areas were embedded in paraffin and 5 micron sections were stained with hematoxylin and eosin and by the Sevier-Munger silver method for NFT and neuritic plaques.

PHF immunoreactivity was present in tangle-bearing neurons as demonstrated in the silver-stained sections. This study confirms the antigenic similarity between NFT of AD and DS. Immunohistochemical staining using monospecific polyclonal antibodies to PHF provides an additional method for detecting the presence of NFT in DS.

316.5 ALZ-50 APPEARS TO BE ABLE TO DISTINGUISH BETWEEN THE NEURITIC PLAQUES OF ALZHEIMER'S DISEASE AND THOSE OF NORMAL AGING. A. Scicutella*, B.L. Wolozin and P. Davies*. (SPON: C.S. Raine) Depts. of Pathology and Neuro-science, Albert Einstein College of Medicine, Bronx, NY, 10461.

One of the hallmark lesions of Alzheimer's Disease, the neuritic plaque, is also found in relatively small numbers in the brains of normal aged humans and animals (see Selkoe et al, Science, 235,873-876, 1987). One major component of the plaque, the amyloid deposit, appears to be identical in both Alzheimer's Disease and in the normal aged human and animal. These observations have led to the concept that the difference between aging and Alzheimer's Disease is quantitative rather than qualitative. Alz-50 is a monoclonal antibody that reacts with a neuronal antigen present in brains of patients with Alzheimer's Disease but essentially absent from normal brain. In samples of tissue from cases of Alzheimer's Disease, the antibody reacts with both neuronal perikarya and neurites, and is particularly strongly reactive with clusters of neurites surrounding amyloid cores. Staining of neurites in neuritic plaques has been observed in all cases of Alzheimer's Disease examined (more than 50 cases). However, brain tissue from two elderly patients with Parkinson's Disease in which small numbers of plaques were present showed no staining with Alz-50, raising the possibility that plaques produced as a result of normal aging did not contain the Alz-50 antigen.

We have investigated this possibility by examination of brain tissue from patients whose mental status prior to death was well established by neuropsychologic testing. Double staining with Alz-50 and thioflavine S (a very sensitive reagent for the detection of neuritic plaques) was used to determine what percentage of plaques contained the Alz-50 antigen. In 5 cases of Alzheimer's Disease, over 90% of plaques (250 out of 270) detected by thioflavine S were also stained by Alz-50. A number of large clusters of dystrophic neurites stained by Alz-50 but not by thioflavine-S were also apparent in some cases. In tissues from cases with mild memory dysfunction (possible early Alzheimer's Disease), smaller numbers of plaques were found and a variable and lower percentage (25 out of 65) contained Alz-50 reactive neurites. In individuals aged over 80 with normal mental status, plaques were found with no detectable Alz-50 reactivity in cerebral cortex. These results suggest that plaque formation in Alzheimer's Disease proceeds by a process that involves expression of the Alz-50 antigen, while formation as a result of normal aging does not.

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316.6 RE-EXPRESSION OF A DEVELOPMENTALLY REGULATED ANTIGEN IN ALZHEIMER'S DISEASE AND DOWN'S SYNDROME. B.L. Wolozin, A Scicutella*, and P. Davies*. Departments of Pathology and Neuroscience, Albert Einstein College of Medicine, Bronx, New York, 10461.

Alz-50 is a monoclonal antibody prepared by immunization of mice with ventral forebrain tissue from cases of Alzheimer's Disease. By immunocytochemical techniques, abundant neuronal reactivity is present in tissues from patients with this disorder, but little or no staining is found in normal adult tissues. Biochemical studies are consistent with immunocytochemical results, and show that the antibody reacts with a 68,000 dalton protein (A68) in tissue from cases of Alzheimer's Disease. Brain tissues from cases of Down's Syndrome aged over 40 years at death also show abundant staining with Alz 50, and Western blot analysis reveals the antigen to be A68 in these cases.

In attempts to study when Alz-50 reactivity first appears in the brains of cases with Down's Syndrome, we have examined tissue from cases ranging in age from 16 weeks of gestation up to 62 years. Temporal cortex from 5 out of 6 fetal brains (16 to 24 weeks) were entirely negative for Alz-50 reactivity, despite the use of frozen sections and several different fixatives. The 6th cases, a 16 week Down's fetus, did show small numbers of reactive neurons diffusely distributed through cortex. At later developmental stages, all cases examined revealed positive neuronal staining in temporal cortex: stained neurons became obvious at 34 to 36 weeks gestation, and were detected in both Down's and normal brains in the first year of life. Reactive neurons had the appearance of small interneurons, and were located predominantly in layers 5 and 6 of the cortex, as well as in the underlying white matter. Approximately 50 neurons per cm^2 were found, and the number of positive cells did not differ between normal and Down's brain tissue. Both perikarya and neuronal processes were stained, the latter frequently showing a very beaded, varicose appearence. Numbers of stained cells dropped very markedly in tissues from cases aged 2 years or older: less than 1 neuron per cm^2 is found in normal adult brain. Reactive neurons and occasional clusters of reactive neurites reappear in tissue from Down's Syndrome cases aged 20 to 30 years, and in cases of Alzheimer's Disease at more advanced ages. Thus Alz-50 appears to detect a protein with some role in normal brain development, and this protein is re-expressed in both Down's and Alzheimer's Disease.

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316.7 IMMUNOHISTOCHEMICAL DEMONSTRATION OF ALZ-50 ANTIGEN AND UBIQUITIN IN PICK BODIES AND NEUROFIBRILLARY TANGLES. S. Love, S. Quijada, T. Saitoh, P. Davies, and R. D. Terry(SPON: R. Katzman). Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093 and Departments of Pathology and Neurosciences, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

The monoclonal antibody Alz-50 and antibodies to ubiquitin react with neurofibrillary tangles in Alzheimer's disease (Wolozin, B. L., et al Science 232:648, 1986; Mori, H., et al Science 235:1641, 1987). In the present study, these antibodies were used to immunostain sections of brain from cases of Pick's disease, Kuf's disease with neurofibrillary tangles, and from elderly non-demented patients with occasional neuritic plaques and neurofibrillary tangles in the hippocampus. Alz-50 and anti-ubiquitin both immunostained the neurofibrillary tangles and the neuritic components of plaques in the brains from non-demented individuals, the neurofibrillary tangles in Kuf's disease, and Pick bodies. The levels of Alz-50 antigen and ubiquitin were measured by immunoblot analysis of serially diluted brain extracts, obtained within a few hours of death, from 13 cases of Alzheimer's disease, two cases of Pick's disease, and 6 age-matched normal controls. Whereas the amount of Alz-50 antigen in the cytosolic fractions was increased several-fold in Alzheimer's disease and Pick's disease, the level of ubiquitin was similar to that in controls. The concentration of ubiquitin within neurofibrillary tangles and Pick bodies is thought to be due to its conjugation to proteins that are resistant to digestion rather than to increased ubiquitin synthesis.

316.8 DIFFERENTIAL SENSITIVITY OF THE MICROTUBULE-ASSOCIATED PROTEIN, TAU, IN ALZHEIMER'S DISEASE TISSUE TO FORMALIN FIXATION. N.J. Pollock and J.G. Wood* Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

The microtubule-associated protein, tau, is a family of proteins reported to be normally concentrated in axons. Neuritic plaques (NPs) and neurofibrillary tangles (NFTs) which pathologically characterize Alzheimer's disease (AD) tissue, are located in the neuropil and cell body of affected neurons, respectively. We have previously demonstrated that NFTs and NPs contain a modified form of tau (PNAS, 83:4040-4043, 1986). Immunocytochemistry of formalin-fixed human autopsy AD tissue using two monoclonal antibodies, Tau-1 and Tau-2, shows intense staining of NFTs and NPs while normal axonal staining is less apparent. A biochemical approach was used to assess the effects of formalin on the antigenic sites of tau isolated from human and rat tissue. Highly enriched tau samples isolated from histopathologically confirmed AD tissue were separated by gel electrophoresis and tansferred to nitrocellulose paper. Blots were incubated in 10% neutral-buffered formalin and then processed for Tau-1 immunoreactivity following digestion with alkaline phosphatase. Controls included no formalin fixation of blots and digestion with heat-inactivated alkaline phosphatase.

A progressive decrease in Tau-1 immunoreactivity of the tau bands was observed with increasing times of formalin fixation. Phosphatase digested blots, however, after formalin fixation, demonstrated an increase in Tau-1 immunoreactivity of a subset of bands, as compared to control blots. These results mimic the phosphatase-sensitive Tau-1 immunocytochemical staining of formalin-fixed AD tissue slices. Since the tau isolation proceedure used does not separate normal axonal tau from the pathology-associated modified tau (AD tau), we used rat brain white matter homogenates to test the formalin sensitivity of axonal tau to Tau-1 and Tau-2 staining. Formalin fixation of white matter homogenate blots decreases Tau-1 immunoreactivity of the tau bands. Digestion with alkaline phosphatase after exposure to formalin had no effect. Tau-2, which has a low affinity for rat tau in fixed tissue, does recognize the tau bands on a blot of white matter homogenates. In support of the tissue result, formalin fixation of those blots eliminates Tau-2 immunoreactivity. These results indicate that the lack of good axonal staining with Tau-2 of fixed rat tissue may be due to the formalin sensitivity of axonal tau. These overall results suggest that normal axonal tau has greater sensitivity to formalin than AD tau and support the hypothesis that a modification of AD tau in pathological structures protects it from the effects of formalin with regards to Tau-1 and Tau-2 antigenicity. NS-17731.

316.9 A2B5 ANTIBODY TO A NEURAL SURFACE COMPONENT LOCALIZES TO SITES OF ALZHEIMER DEGENERATION ASSOCIATED WITH BOTH NEUROFIBRILLARY TANGLES AND NEURITIC PLAQUES. C.A. Marotta and R.E. Majocha* (SPON: G. Hauser). Program in Neuroscience and Dept. of Psychiatry, Harvard Med. Sch.; Mailman Res. Ctr., McLean Hosp., Belmont, MA 02178; and Massachusetts General Hosp., Boston, MA 02114.

Gangliosides have been implicated in neuronal growth and regeneration under some conditions as well as in reducing neuronal degeneration caused by experimental lesions. Therefore, we have begun to examine the possible role of gangliosides in relation to the degenerative changes that are characteristic of the Alzheimer's disease (AD) brain. Monoclonal antibody A2B5 was originally developed against embryonic retinal cells and was shown to react with a neural surface component present in the ganglioside fraction of brain. Application of the mab to fixed sections of control hippocampus revealed only light background staining that was homogeneous and contained few formed elements. By contrast, similar processing of AD hippocampus revealed intense staining of numerous flame-shaped cells resembling neurons with neurofibrillary tangles (nft). The reaction product partly or completely covered the area circumscribed by the cell boundary. In some cases the antigen was localized in the same cells that were also shown to contain nft as revealed by co-staining with thioflavin S. Examination of adjacent sections stained either with mab A2B5 or with thioflavin S revealed that in a specified area there were 1.5 to 2 times greater numbers of neurofibrillary tangles than sites containing the A2B5 antigen; this suggests that high antigen levels were expressed in a subclass of degenerating neurons. In addition, neuritic processes associated with thioflavin-stained senile plaques also contained the A2B5 component. All immunostaining was eliminated by preincubation of the mab supernatant with a total ganglioside fraction from bovine brain. The role of the A2B5 antigen in neuronal degeneration is unknown and its expression may not be confined to the AD brain. Other neurodegenerative disorders are being examined. Although these early observations raise the possibility that expression of a unique ganglioside may have a protective function in injured or dying neurons, the results do not rule out the prospect that expression of the A2B5 antigen contributes to the degenerative process. Both possibilities are under investigation. Supported by AG02126, AG04522, AG05134, AHAF and McKnight Foundation.

316.10 NEUROFIBRILLARY TANGLES (NFT) OF PROGRESSIVE SUPRANUCLEAR PALSY (PSP) AND ALZHEIMER DISEASE (AD): INFLUENCE OF NEURONAL CELL LOCATION ON ANTIGENIC PROPERTIES. M. Tabaton*, V. Manetto*, L. Autilio-Gambetti, P. Gambetti, V. Fried*, G. Perry. Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106 and St. Jude Children's Research Hospital, Memphis, TN 38101.

This study was undertaken to investigate whether antigenic differences between two types of NFT can be attributed to their different location. Antigenic and solubility properties of subcortical NFT of PSP, which occur subcortically and are made of 15nm straight filaments, were compared to those of subcortical and cortical NFT of AD, composed mostly of paired helical filaments (PHF). Ten cases of PSP and 2 cases of AD were examined. An antiserum to PHF (Ihara et al, Nature 304:727-730, 1983), a monoclonal antibody (Mab) to tau, tau-1 (Binder et al, J Cell Biol 101:1371-1378, 1985), two Mabs to neurofilaments, SMI 34 (Sternberger-Meyer) and 1.1.1 (Autilio-Gambetti et al, Banbury Report 15, 1983) and 4-2D8, a Mab to ubiquitin, all of which stain cortical NFT in AD, were used. Immunostaining was performed on paraffin, vibratome and frozen sections from the pons in PSP cases and from the pons and hippocampus in AD cases. Except for 1.1.1, all antibodies immunoreacted with subcortical NFT in both PSP and AD cases. A quantitative analysis showed a difference in the number of subcortical NFT immunostained by these antibodies. Anti-PHF immunoreacted with 100%, tau-1 with 70%, 42D8 with 50% and SMI 34 only with 10% of the NFT, both in PSP and AD. All antibodies immunostained subcortical NFT after treatment with SDS. Colloidal gold immunoelectromicroscopy of PSP cases demonstrated that the antibodies recognized both the straight filaments and the amorphous material constituents of NFT of PSP. Subcortical NFT of AD were confirmed to consist mostly of PHF (Tabaton et al, Acta Neuropathol 68:218-223, 1985). Our results show that despite their different structure, the 15nm straight filaments of PSP and the PHF of AD, when located in the same brain region, share all their known antigens. They also show that NFT in AD are antigenically different according to their location: pontine NFT lack a neurofilament epitope that is consistently present in cortical NFT. These observations suggest that the antigenic differences between the straight filaments of PSP and PHF of AD are due more to their different location than to their different structure, and that different PHF are expressed by neurons in different locations.

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316.11 UBIQUITIN IN ALZHEIMER PAIRED HELICAL FILAMENTS: A STUDY WITH MONOCLONAL ANTIBODIES. G. Perry, P. Mulvihill*, V. Manetto*, V. Fried*, H. Smith*, I. Grundke-Iqbal*, K. Iqbal*, L. Autilio-Gambetti and P. Gambetti. Case Western Reserve Univ., Cleveland, OH 44106; St. Jude Children's Research Hospital, Memphis, TN 38101 and Inst. Basic Research in Developmental Disorders, Staten Island, NY 10314.

Some antibodies raised to paired helical filaments (PHF), the major cellular inclusion of Alzheimer disease, have been found to crossreact with tau or neurofilaments. However, other antibodies to PHF are thought to be directed to determinants unique to PHF, since they have been found not to react with any known protein. Biochemical and immunocytochemical studies have recently demonstrated that ubiquitin (Ub) is a component of PHF (Mori et al Science,235:1641, 1987; Perry et al, PNAS,84:3033, 1987). We have investigated whether antibodies previously thought to recognize unique determinants in PHF react with Ub. We found that 2 of 3 monoclonal antibodies (Mabs) and 1 of 5 antisera raised to PHF are directed to Ub. The Ub epitope recognized by Mab 5-25 resides in a peptide containing amino acid residues 64-76 and that recognized by Mab 3-39 in a Ub peptide containing residues 50-65. Ub epitopes in these regions are not recognized by other available Mabs to Ub. We also tested 8 Mabs to epitopes located between residues 34 and 53 of Ub, but that recognize different conformational determinants of Ub , dependent on the nature of the conjugate. Five of these 8 Mabs react with PHF. These findings indicate a) that Ub is one of the previously unidentified proteins recognized by antibodies raised to PHF, b)that several epitopes along residues 34 to 76 of Ub can be detected in PHF, c) that Ub is conjugated in PHF in various conformations.

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316.12 MODIFICATION OF HUMAN ANTINEUROFILAMENT ANTIBODIES WITH PHTHALOCYANINES. S.E. Kornguth, T. Kalinke* and W. Pietro*. Depts. of Neurology, Physiological Chemistry and Chemistry, University of Wisconsin, Madison, WI 53706.

Human antineurofilament immunoglobulins were attached covalently to cobalt tetracarboxy phthalocyanines (Pc) with retention of immunoreactivity. High titer (>1:1000) antineurofilament antibodies have been observed by us to occur in the sera of patients with visual paraneoplastic syndrome associated with small cell carcinoma of the lung and in patients with diffuse neuropsychiatric lupus erythematosus. The intense blue color of the Pc serves as an excellent marker for histological studies; the metal center of Pc permits applications in magnetic resonance imaging (MRI); the photolytic properties of Pc have applications in chemotherapy. The Pc was activated with a carbodiimide (EDC) and then modified with either diaminoethane (2 DAE:1 Pc) or with polylysine in the solvent dimethylsulfoxide. The diamine and the polylysine provide primary amines which serve as functional groups to crosslink the Pc with dextran or glycoproteins such as immunoglobulins. The amine modified Pc's were then coupled covalently to the periodate oxidized diethylaminoethyl dextran (DEAE dextran) which were then coupled to periodate oxidized immunoglobulins in aqueous systems. Alternatively, the amine modified Pc's were attached directly to the periodate oxidized immunoglobulins. The DEAE dextran maintains the Pc complex in solution in the aqueous solvent. The phthalocyanine-immunoglobulin complexes remain soluble and retain immunoreactivity as demonstrated on western blots. The western blots contained neurofilaments that were resolved on PAGE in SDS. This study now permits the use of phthalocyanines in MRI, photolysis and immunohistochemical procedures.

316.13 ALZ-50 RECOGNIZES AN EARLY NEURONAL ALTERATION IN ALZHEIMER'S DISEASE, INVOLVING NEURONAL PERIKARYA, DENDRITIC TREES, AND TERMINATION ZONES OF SPECIFIC PROJECTION NEURONS. B.T. Hyman, G.W. Van Hoesen, L.J. Kromer, and A.R. Damasio. Depts. of Neurology and Anatomy, University of Iowa College of Medicine, Iowa City, Iowa 52242.

Alz-50 is a monoclonal antibody that recognizes an antigen present in Alzheimer's disease brain but not in the brains of age compatible controls (Wolozin et al., Science 232:648, 1986). This antigen is distinct from the paired helical filament protein of neurofibrillary tangles and from the amyloid protein of neuritic plaques. Immunohistochemical staining with Alz-50 reveals immunoreactivity in Alzheimer brains in neurons that contain neurofibrillary tangles and in some neurons that do not, as well as in the neuritic portion of neuritic plaques and in the neuropil. Both types of neuronal alterations are located selectively in "at risk" lamina and cytoarchitectural fields. Some of the Alz-50 positive, neurofibrillary tangle negative neurons appear to contain immunoreactivity in the neuronal perikarya, throughout the dendritic tree, and in axons. Alz-50 positive neurons that contain neurofibrillary tangles appear to be at various stages of degeneration, and some completely destroyed neurons (tombstone tangles) lack Alz-50 immunoreactivity. These results are consistent with the hypothesis that the Alz-50 antigen precedes and accompanies the deposition of neurofibrillary tangles in neurons, and suggest that Alz-50 recognizes an early neuronal alteration in Alzheimer's disease. The possible localization of Alz-50 immunoreactivity in axons is of particular interest, because in certain areas areas a discrete immunoreactive zone occurs in terminal zones predicted by anatomic tracing studies in the nonhuman primate. For example, the pattern of Alz-50 neuropil immunoreactivity in the molecular layer of the dentate gyrus corresponds exactly to the predicted termination zone of the Alz-50 positive neurons and neurofibrillary tangles in layer II of entorhinal cortex (the perforant pathway). In addition, Alz-50 positive neuritic processes of neuritic plaques are located in this terminal zone. Alz-50 positive terminals corresponding to projections from Alz-50 positive neurons in the subicular cortices to layer IV of entorhinal cortex and to the mammillary bodies are also readily visualized. This staining pattern represents a direct demonstration of involvement of cortical and subcortical projections in Alzheimer's disease. (Supported by NS 14944 and PO NS 19632. We thank P. Davies and B. Wolozin, Albert Einstein College of Medicine, for generously providing Alz-50).

316.14 IMMUNODETECTION OF HUMAN SERUM AMYLOID P-COMPONENT IN ALZHEIMER'S DISEASE. A.B. Scheibel, E. Pommier* and T. Duong. UCLA Departments of Anatomy, Psychiatry and Brain Research Institute, Los Angeles, CA 90024

We are reporting the presence of human serum amyloid P-component in two characteristic hallmarks of Alzheimer's disease. This finding, contrary to results from previous studies, infers a concomitant impairment in the blood-brain barrier.

Human serum amyloid P component (235,000 MW), synthesized only in the liver, is both a normal plasma glycoprotein and a constituent of connective tissues. It has been found in all forms of systemic and localized amyloidosis so far investigated, where it forms a minor but constant constituent (6-14% of mass). In senile dementia of the Alzheimer's type (SDAT), intracerebral amyloidosis occurs in three characteristic microscopic lesions: cerebrovascular amyloid, senile plaques and neurofibrillary tangles. The exact composition of these amyloids is unknown. The presence of human serum amyloid P-component has been demonstrated in cerebrovascular amyloid but not in the senile plaques and tangles in cerebral tissues from patients with SDAT(Westermark et al, 1982; Rowe et al, 1983). Unlike previous researchers, we report here the consistent labelling of senile plaques and tangles, using a polyclonal antibody to human serum amyloid P-component. Our specimens consisted of brains from patients with a verified post-mortem diagnosis of Alzheimer's dementia and control brains from patients with no obvious neurological diseases. Various cortical areas and the hippocampus were dissected from coronal sections of fresh tissue (7-15 hours postmortem) and fixed by immersion. Cut sections were stained using a peroxidase-antiperoxidase method.

In accordance with previous reports, we found serum amyloid P-component positive reaction products in segments of pial arteries, penetrating and intraparenchymal arterioles. The reaction products are unevenly distributed from the tunica media outward and contribute to the lumpy-bumpy appearance of the abluminal portion of the affected vessels. Unlike previous reports, senile plaques in our specimens were also positive to the human serum amyloid P-component antibody. The distribution of reaction products was varied: in some plaques they formed small clumps distributed throughout the fibrillar and nonfibrillar portion. In others, they were mostly observed in the plaque core, which was either stained throughout its extent or only in a surrounding rim. Stained portions of vessels and/or swollen neurites were occasionally observed within the plaques. Unexpectedly, we also obtained antibody positive staining within the neurofibrillary tangles. The pattern of staining followed exclusively the intracytoplasmic distribution of paired helical filaments. The intensity of the antibody staining appeared to correspond to the amount of paired helical filaments within the neurofibrillary tangles. These antibody-positive tangles were also observed within senile plaques.

With a molecular weight of 235,000, serum amyloid P-component would not be expected to penetrate into the brain parenchyma. Our detection of the presence of serum amyloid P-component in brains from patients with SDAT suggests the possibility of an impaired blood-brain barrier. It also points to the universal association of this glycoprotein with all forms of amyloidosis and the possibility that cerebral amyloidosis in SDAT may be partially of extracerebral origin. Supported by the Dorothy Van Ness-Thompson Foundation and the David H. Murdock Foundation for Advanced Brain Studies.

316.15 STRUCTURAL DIVERSITY AND INFRASTRUCTURE OF AMYLOID DEPOSITS IN ALZHEIMER BRAIN. F.M. Benes, J. Reifel*, R. Majocha* and C.A. Marotta. Department of Psychiatry, Harvard Medical School and Mailman Research Center, McLean Hospital, Belmont, MA 02178.

Although the structure of amyloid in Alzheimer's disease (AD) is yielding to analyses carried out at the gene level, little direct information is available with regard to mechanisms related to amyloid accumulation at the cellular and tissue levels. To gain insight into putative regulatory processes, we applied monoclonal antibodies (mabs), directed against a known amyloid protein sequence, to the AD cortex and analyzed the resulting staining pattern of amyloid deposits by means of computer-enhanced imaging procedures. Three mabs were characterized and shown to react with parenchymal and vascular amyloid. The mabs allowed visualization of morphlogically distinct amyloid deposits in the prefrontal cortex that differed with respect to size, qualitative appearance and internal distribution of amyloid epitopes. Previously unappreciated infrastructural details of AD brain amyloid became accessible through computer imaging. All amyloid-stained areas showed gradients of stain density suggesting that either diffusion or coalescence of this protein may be involved in plaque formation. In spite of this characteristic appearance of internal density gradients, at least four structurally discrete morphologic types of amyloid staining were observed: punctate and macular types were most common; more rarely, ring and ring with core configurations were observed. Each type showed a distinct size distribution with punctate being smallest and rings, with or without cores, being largest. Macular amyloid deposits showed a broad distribution of size that overlapped with that of the ring-like deposits. The four groups exhibited further dissimilarity with respect to distribution within the cortical lamellae. Punctate amyloid densities were most numerous and were preferentially localized in layers I and II, while those with a ring-like appearance were least common and primarily found in layers V and VI. Macular amyloid deposits were most commonly found in layer III. Thus, detailed analyses of AD amyloid epitopes has demonstrated non-uniformity with regard to size, morphology and site-specificity. The dissimilarities suggest that the appearance and distribution of amyloid deposits are dependent upon the underlying laminar-specific organization of the cortex. Supported by National Institute on Aging grants #AG02126, AG04522, AG05134; American Health Assistance Foundation and McKnight Foundation awards.

316.16 DISTRIBUTION OF THE ALZHEIMER AMYLOID BETA PROTEIN mRNA IN THE HUMAN AND RAT BRAINS. E.A. Finch,* L.I. Benowitz, S.P. Finklestein, R.E. Tanzi,* E.D. Bird and R.L. Neve. Depts. of Pediatrics, Neurology, Neuropathology, Psychiatry, and Program in Neuroscience,, Harvard Med. Sch.; Dept. of Genetics, Children's Hospital; Neurology Service, Massachusetts General Hosp.; Mailman Res. Ctr., McLean Hosp., Boston and Belmont, MA.

The neuropathology of Alzheimer's disease is marked by deposits of the proteinaceous material amyloid, in the core of extracellular neuritic plaques and the walls of the cerebral microvasculature. Similar amyloid deposits occur in the brains of older Down syndrome patients and, to a much lesser degree, in association with the normal aging process. We have isolated amyloid beta protein cDNAs that were shown to hybridize with messenger RNA that is expressed normally in the brain and other tissues(Tanzi, et al., Science 235, 880, 1987). These findings suggest the possibility that amyloid deposits in Alzheimer's disease may result from either an abnormal expression or post-translational modification of a normal molecular constituent. In order to understand better the normal significance of the amyloid protein gene product, as well as its relevance to the pathophysiology of Alzheimer's disease and Down syndrome, we have used a cDNA to the amyloid beta protein gene (FB68L) to determine the distribution of the amyloid beta protein gene transcript in the mature rat and developing and mature human brains. Northern blot analysis of RNA's from various regions of the adult rat brain showed the 3.7 kb amyloid beta protein RNA to be expressed at highest levels in association (posterolateral) cortex and striatum; modest levels were seen in the midbrain, motor (anterior) cortex, brainstem (medulla), septum, and hippocampus, and very little in the cerebellum.

Strikingly, the amyloid beta protein mRNA is abundantly and homogeneously expressed across human fetal brain subregions. In the adult human brain, however the expression of the amyloid beta protein showed marked regional variation. Levels of mRNA were highest in the frontal pole (A10) and the anterior perisylvian cortex (A44). Moderate hybridization was observed in the posterior perisylvian cortex (A40), the inferior temporal cortex (A20/21), and the cerebellar cortex. Signal was weak in subcortical regions, and in striate, extrastriate, and motor cortices (A17, 18, A4) respectively.

Studies are currently in progress to determine the cellular distribution and relative abundance of the amyloid beta protein mRNA in various human and rat brain regions by in situ hybridization. Preliminary results indicate that it is expressed selectively in layer II of the adult rat and human cortex and in certain cells of the hippocampus. In situ hybridization to primary cell cultures is also being used to gain information about the developmental expression of this gene.

316.17 ANTIBODIES TO THE AMYLOID β-PROTEIN DETECT ~80 kDa BRAIN PROTEIN AT HIGHER LEVELS IN ALZHEIMER THAN NORMAL CORTEX BUT NOT IN CEREBELLUM. D. Selkoe,* A. Saperstein, M. Podlisny and L. Duffy. Center for Neurologic Diseases, Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115.

The formation of neuritic plaques containing central deposits of extracellular amyloid filaments is a more specific lesion for Alzheimer's disease (AD) than is the neurofibrillary tangle. Neuritic plaques are essentially restricted to 3 conditions: AD, Down's syndrome and normal aging. The hydrophobic 4-5 kDa amyloid protein (β-protein) found in the cores of neuritic plaques is also deposited in some cortical and meningeal microvessels in AD brain. Recent studies show that the β-protein is cleaved from an ~80 kDa precursor encoded by a gene on chromosome 21q near the familial AD gene. Northern analyses reported to date suggest that there are 2 closely spaced mRNAs of ~3.4 and 3.2 kb in human brain and that the message is present in a wide range of non-neural tissues. However, polymers of the cleaved β-protein (i.e., amyloid filaments) accumulate only in the brain, and within brain, only in certain regions. In an attempt to detect any regional differences in distribution or amount of the precursor, we used an antiserum raised to synthetic β-peptide residues 1-28 (not carrier coupled) to examine brain homogenates from 8 pathologically verified AD patients and 3 age-matched controls. On Western blots, the antiserum consistently detected a doublet of ~M_r 80 and 83 kDa in frontal cerebral cortex of all 8 AD patients. Using equal protein loading, this doublet was always present in lower amounts (or not detectable) in frontal cortex from the control patients. Frontal cortex from a case of Down's syndrome also showed the immunoreactive doublet comigrating with that in AD cortex. No immunoreactive bands were detected in cerebellar homogenates from the same AD cases showing the doublet in frontal cortex. Western blots of kidney homogenates from a pathologically-verified AD case and a control also showed no immunoreactive bands. The 80/83 kDa proteins were also detected in higher amounts in AD than control frontal cortex using an antiserum to synthetic β-protein residues 1-45. The immunoreactive proteins were present in the particulate but not the cytosolic fraction of the cortical homogenates. Further studies of their regional and subcellular distribution and attempts to identify the proteins will be presented. The apparent M_r of these proteins and their consistent increase in AD cerebral cortex but not cerebellum suggest that they could represent a cell-specific expression of the β-amyloid precursor protein(s) in brain regions prone to developing amyloid deposits in Alzheimer's disease.

316.18 AMYLOID FIBRILS IN HEREDITARY CEREBRAL HEMORRHAGE WITH AMYLOIDOSIS OF DUTCH ORIGIN SHARE AMINO ACID SEQUENCE WITH ALZHEIMER'S DISEASE β-PROTEIN. E.M. Castaño*, S.G. Van Duinen*, F. Prelli* and B. Frangione* (SPON: E. Simon). Dept. of Pathology, N.Y.U. Med. Ctr., New York, 10016.

Hereditary cerebral hemorrhage with amyloidosis of Dutch origin (HCHWA-D) is an autosomal dominant form of cerebral amyloidosis that has been described in four different families from The Netherlands. The disease is clinically characterized by recurrent strokes starting in the fifth decade of life. The pathological findings include amyloid deposits in the small arteries of the leptomeninges and cerebral cortex and in senile plaque-like structures. These lesions lack the dense amyloid cores and resemble the immature or early plaques described in cases of Alzheimer's Disease and Sporadic Congophilic Angiopathy. No neurofibrillary tangles are present.Immunoperoxidase on brain sections from two cases of HCHWA-D showed that the amyloid deposits were specifically stained by an affinity purified polyclonal rabbit antiserum raised against a synthetic peptide homologous to the 28-residue amino-terminal sequence of Alzheimer's Disease β-protein (anti-SP28). The amyloid fibrils were isolated from the leptomeninges and further characterized by SDS-PAGE, immunoblotting and partial amino acid sequence. The amyloid subunits had a molecular weigth of 4-6 kDa and reacted specifically with anti-SP28. The amino-terminal sequence in both cases revealed homology with Alzheimer's Disease β-protein. However, in contrast to the reported sequence of the vascular β-protein in Alzheimer's Disease, but similar to the β-protein isolated from the senile plaques and neurofibrillary tangles, HCHWA-D vascular amyloid protein had amino-terminal heterogeneity. Recently, it has been reported that neurofibrillary tangles in Guamanian Parkinson-Dementia are composed of an amyloid subunit identical to Alzheimer's Disease β-protein. In this disease, neither senile plaques nor vascular deposits are present.Therefore, β-protein cerebral amyloidosis comprises a spectrum of clinico-pathological entities including vascular types (HCHWA-D, Sporadic Congophilic Angiopathy) and neuronal types (Guamanian Parkinson-Dementia) of amyloid deposition.The full range of lesions is present in most cases of Alzheimer's Disease. Structural variants of β-protein and/or different processing of β-protein precursors synthesized in particular cell types may be responsible for the distinctive patterns of amyloid deposition in this group of degenerative neurological diseases.

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316.19 AMYLOID DEPOSITS IN SPORADIC CONGOPHILIC ANGIOPATHY AND HEREDITARY CEREBRAL HEMORRHAGE WITH AMYLOIDOSIS OF DUTCH ORIGIN ARE ANTIGENICALLY RELATED TO ALZHEIMER'S DISEASE β-PROTEIN.

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Amyloid deposition is a prominent feature of a number of brain disorders, differing in their neurological manifestations and clinical course, which range from acute vascular syndromes to progressive dementia. The most prevalent among these is Alzheimer's disease (AD) in which the amyloid deposits are mainly composed of a protein of 4kD (β-protein) that forms fibrillar structures within the wall of blood vessels, the neuropil (neuritic plaques) and neurons (neurofibrillary tangles). Because the nature of the amyloid deposits in other types of cerebral amyloidosis is not known, we have conducted immunocytochemical studies on brains from autopsied cases of AD (3 cases), Hereditary Cerebral Hemorrhage with Amyloidosis (HCHWA) of Dutch origin (2 cases), Sporadic Congophilic Angiopathy (1 case). Brains from two patients without neurological involvement were used as controls. Sections from these specimens were incubated with rabbit polyclonal antibodies against: a) a synthetic peptide of 28 residues (anti-sp28), homologous to the NH_2-terminal sequence of the β-protein, b) the main amyloid component of the HCHWA of Icelandic origin, a variant of Cystatin C, an inhibitor of cystein proteinases (Ghiso et al., PNAS, 83:2974, 1986), and c) a purified fraction of paired helical filaments. Bound antibody was revealed with immunoperoxidase. In all of these cases, anti-sp28 antibody stained amyloid deposits around leptomeningeal and cortical vessels and neuritic plaques, but not paired helical filaments. By contrast, anti-paired helical filaments antibodies stained only neurofibrillary tangles, and anti-Cystatin C variant antibody, as well as control preimmune rabbit serum, gave no staining. These findings demonstrate that the amyloid deposits of Sporadic Congophilic Angiopathy, HCHWA of Dutch origin and brain aging are composed of a protein antigenically similar to AD β-protein, and suggests that these three clinically and etiologically different morbid conditions are pathogenetically related. On this basis, they can be tentatively grouped as β-protein diseases.

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316.20 POLYPEPTIDE COMPOSITION OF CORPORA AMYLACEA PURIFIED FROM AGED HUMAN BRAIN. D. Gauvreau*, A. Steyaert*, S. Cissé*, M. Blum*+ and G. Lacoste* (SPON: J. Metuzals). INRS-Santé, Pointe-Claire, Canada H9R 1G6, +Dept. Biochemistry, Univ. of Toronto, Canada, M5S 1A2.

Corpora amylacea (CA) accumulation in the CNS is associated with normal ageing. CA are made up of glucose polymers. However, the presence of a small quantity of protein in CA is a consistent finding, although the identity of this protein material is unknown. We developed a method to purify CA from human brain, and we analysed their protein constituents. CA were purified from frozen human brain using sucrose gradient fractionation and density centrifugation on Percoll: CA preparations thus obtained are over 95% pure as judged by visual estimation of PAS-stained material. The protein content of CA was estimated to be 3% of the total CA fraction by weight. SDS-PAGE analysis of CA preparations showed four major polypeptide bands, of molecular weight 100 kd, 94 kd, 40 kd, and 24 kd; these polypeptide species were consistently enriched in all preparations of CA. Amino acid analysis was performed on the 94 kd species, and revealed a high content of glutamic acid, glycine, and aspartic acid.

The presence of the same set of polypeptides in all CA preparations makes it unlikely that proteins are randomly trapped in CA. Rather, it suggests a specific mode of formation for these polyglucosan bodies. Recent reports indicate a significant increase in the number of CA in the hippocampus and frontal cortex of AD patients compared to controls. We are now looking at the protein composition of CA prepared from AD brains. The study of the formation of CA in conditions of degeneration may provide clues to the cellular processes of ageing in the brain.

EPILEPSY: BRAIN SLICES

317.1 POTASSIUM-INDUCED TONIC-CLONIC SEIZURES IN THE MAMMALIAN IN VITRO HIPPOCAMPUS. G. David*, Y. Yaari and M.S. Jensen*. Department of Physiology, Hebrew University School of Medicine, Jerusalem, Israel, and Institute of Physiology, Aarhus University, Aarhus, Denmark.

Mammalian cortical slices have been used extensively for elucidating mechanisms of interictal epileptogenesis. Recently, experimental procedures for inducing ictal (i.e. seizure) patterns of epileptiform discharge in hippocampal slices have been described, allowing in vitro analysis of interictal-ictal transitions and seizure mechanisms.

Perfusing rat hippocampal slices with K^+-enriched (5-8.5 mM instead of normal 3.5 mM K^+) solutions induced interictal bursts in areas CA3 and CA1 of all slices (n = 60) and recurrent ictal episodes in area CA1 in about 50% of the preparations. Seizures were induced only if the divalent ion concentrations were maintained at normal physiological levels (Ca^{++} 1.2, Mg^{++} 1.2 mM), i.e., at approximately half the total divalent ion concentration normally used in slice experiments. Raising $[Ca^{++}]o$ and $[Mg^{++}]o$ to 2 mM suppressed all ictal activity and reduced interictal bursting. Lowering $[K^+]o$ to 3.5 mM abolished all epileptiform activity in CA3 and CA1.

A gradual buildup of $[K^+]o$ (above the already elevated baseline) and interictal afterdischarges precipitated each ictal episode. The seizure itself consisted of a tonic-clonic sequence. During the tonic phase, which lasted several seconds, CA1 pyramidal neurons exhibited sustained depolarizations (ca. 10 mV), marked depression in input resistance, and repetitive discharge coincident with, and apparently triggered by, synchronized population spikes. Concomitantly, the $[K^+]o$ at the stratum pyramidale rose to about 12 mM. Termination of the tonic phase was associated with neuronal spike inactivation and hyperpolarization, from which intermittent bursts gradually emerged, increased in size and duration, and then recurred for many seconds. This clonic phase was ultimately followed by a period of postictal depression, during which interictal bursts and synaptic responses in CA1 were reduced.

Thus, mammalian hippocampal slices rendered epileptic by elevated $[K^+]o$ can produce abnormal discharge patterns (interictal-ictal transitions, tonic-clonic seizures, postictal depression) remarkably similar to those observed in vivo, provided they are not partially anaesthesized by elevated divalent cation concentrations.

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317.2 DIFFERENTIAL CHOLINERGIC MODULATION OF INTERICTAL AND ICTAL EPILEPTIFORM ACTIVITY IN THE MAMMALIAN IN VITRO HIPPOCAMPUS. M.S. Jensen* and Y. Yaari. (SPON: S.G. Rayport) Institute of Physiology, Aarhus University, Aarhus, Denmark, and Department of Physiology, Hebrew University School of Medicine, Jerusalem, Israel.

Two patterns of abnormal neuronal discharge are typically manifested by epileptic cortex: (1) the brief, spatially restricted interictal bursts, and (2) the sustained, widely spread, ictal paroxysms (the seizure proper). In some models of experimental focal epilepsy, transitions from interictal "spiking" to maximal seizures are associated with progressive buildup of interictal afterdischarges, suggesting that interictal activity is a progenitor of seizures. We have examined this notion utilizing an in vitro model of hippocampal epilepsy, produced by perfusing rat hippocampal slices with K^+-enriched (5-8.5 mM), otherwise normal, physiological solutions. Slices which become epileptic exhibit interictal "spiking" in areas CA1 and CA3 and recurrent seizures in area CA1.

We found that each interictal-ictal transition in CA1 was associated with a progressive buildup of interictal afterdischarges, commencing several seconds before, and apparently leading to, seizure onset. Addition of neostigmine (1 μM) to the perfusing solution promptly suppressed all interictal bursts (in CA3 and CA1) and afterdischarges (in CA1). This effect was not accompanied by a marked suppression of synaptic responses in CA1. Despite losing their apparent interictal drive, ictal seizures continued to be generated in CA1. Muscarine (1 μM) and carbachol (10 μM) mimicked the effects of neostigmine, while atropine (1 μM) promptly reversed it, leading to the reappearance of interictal discharge in the CA3 and CA1 fields and apparent interictal-ictal transitions in CA1.

We conclude that in the mammalian hippocampus, interictal bursts are sensitive to blockade by activation of muscarinic cholinergic receptors. The finding that muscarinic suppression of interictal bursts does not concomitantly abolish seizures indicates that, at least in the in vitro hippocampus, interictal activity is not a progenitor of seizures.

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317.3 NONSYNAPTIC AND SYNAPTIC MECHANISMS GENERATE TONIC-CLONIC SEIZURES IN THE MAMMALIAN IN VITRO HIPPOCAMPUS. Y. Yaari and M.S. Jensen*. (SPON: E. Leike). Dept. of Physiology, Hebrew University School of Medicine, Jerusalem, Israel and Inst. of Physiology, Aarhus University, Aarhus, Denmark.

In clinical and experimental epilepsy, generalized seizures commonly consist of a short tonic phase (sustained mascular spasm) followed by a prolonged clonic phase (repetitive muscular jerks). This tonic-clonic sequence is associated with characteristic cortical electrical activity, indicative of massive neuronal recruitment and synchronization during both phases of the seizure episode. However, the neuronal mechanisms underlying tonic and clonic neuronal discharge are poorly understood.

An in vitro model of tonic-clonic seizures can be produced by perfusing rat hippocampal slices with K^+-enriched (5-8.5 mM), otherwise normal, physiological solutions. This leads to the development of interictal bursts in the CA3 and CA1 fields and to recurrent tonic-clonic seizures in CA1. We have begun to investigate in this model the interrelationships between interictal, tonic and clonic epileptiform activity and report the following: 1) Interictal bursts in CA3 precede by several milliseconds the interictal events, the tonic phase of seizure and each clonic burst in CA1. This may be interpreted as indicating that all epileptiform activity in CA1 is triggered by a "focus" in CA3. 2) However, exposure of epileptic slices to the glutamate antagonist pCB-ρZ (500 μM) or to cadmium (50-200 μM) abolished all interictal activity (in CA1 and CA3) and clonic bursts (in CA1), but did not antagonize the generation of tonic seizures in CA1. Cadmium concentrations of 500 μM, which totally suppressed synaptic responses in CA1, also did not block this form of seizure discharge. 3) Similarly, cutting between CA3 and CA1 in epileptic slices abolished interictal and clonic bursts, but not tonic seizures, in CA1. Repetitive stimulation delivered to the Schaffer collaterals of these isolated CA1 preparations at 0.5-1 Hz produced interictal-like bursts. Following a tonic seizure episode, the same stimuli evoked a series of clonic bursts. 4) Tonic seizures in isolated CA1 preparations were accompanied by a steep transient rise of $[K^+]_o$ to about 12 mM. The $[K^+]_o$ slowly returned to baseline following termination of the seizure episode. Synaptic activation after the tonic phase produced a clonic burst only if $[K^+]_o$ was still markedly elevated.

We conclude that in mammalian hippocampal slices interictal bursts and tonic seizures are produced by disparate mechanisms. As commonly assumed, interictal burst generation depends on synaptic interactions. In contrast, tonic seizures in CA1 are most likely nonsynaptically generated. Their underlying mechanism may be regenerative accumulation of $[K^+]_o$. Synergism between interictal (synaptic) excitation of CA1 and enhanced neuronal synchrony (produced by elevated $[K^+]_o$) may underlie the clonic phase of seizure.

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317.4 **NORADRENERGIC INHIBITION OF STIMULUS TRAIN-INDUCED BURSTING (STIB) IN AREA CA3 OF RAT HIPPOCAMPAL SLICES APPEARS TO BE MEDIATED BY ALPHA$_1$ RECEPTORS.** D.M. Taylor, A.C. Bragdon and W.A. Wilson. Depts. of Pharmacology and Medicine (Neurology), Duke University and VA Medical Centers, Durham, NC 27705.

Norepinephrine (NE) influences the development of epilepsy in a variety of experimental models. STIB is an in vitro model of epileptogenesis in which repeated delivery of stimulus trains (STs) leads to the progressive development of spontaneous population bursting in area CA3 of hippocampal slices (Stasheff et al., Brain Res. 344: 296-302, 1985). We previously showed that NE retards epiletogenesis in the STIB model, and presented evidence that this effect is mediated by alpha receptors (Soc. Neurosci. Abstr. 12: 80, 1986). In the presence of 1 uM timolol (TIM) to block beta receptors, 10 uM NE maximally increased the number of STs to spontaneous bursting from a mean of 4.4 to 7.6. This effect was completely blocked by the alpha receptor antagonist phentolamine (100 uM). We now present additional evidence that this anti-epileptogenic effect of NE is alpha-mediated, probably through alpha$_1$ receptors.

Slices prepared from temporal hippocampus were studied in ACSF containing (mM): KCl 3.3, $CaCl_2$ 1.8, $MgSO_4$ 1.2, NaCl 120, $NaHCO_3$ 25, NaH_2PO_4 1.23, and dextrose 10. Slices were stimulated in s. radiatum of CA3a, and extracellular fields were recorded in s. pyramidale of CA3b. Slices producing stable, healthy evoked potentials for at least 15 min were randomly assigned to control or treatment group, after which control or drug-containing ACSF was applied for the remainder of the experiment. After an additional 15 min equilibration period, STs (60 Hz for 2 sec) were applied every 10 min, and the number of stimulus trains needed to elicit spontaneous bursting (#STs to SB) were counted.

As before, NE (10 uM) plus TIM (1 uM) more than doubled the mean #STs to SB from 3.4 to 6.9. This effect was completely blocked by the alpha receptor antagonist phenoxybenzamine (10 uM), and partially blocked by the selective alpha$_1$ antagonist prazosin (1 uM), but was unaffected or even enhanced by the selective alpha$_2$ antagonists idazoxan (10 uM) and yohimbine (1 uM).

These results confirm that NE has antiepileptogenic effects mediated by alpha receptors. Additionally, they show that this effect is not mediated through alpha$_2$ receptors, and suggest it may be mediated through alpha$_1$ receptors.

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317.5 INITIATION AND SPREAD OF SYNCHRONIZED NEURONAL ACTIVITY IN NEOCORTEX DURING PARTIAL BLOCKADE OF SYNAPTIC INHIBITION. Y. Chagnac-Amitai* and B.W. Connors. Dept. of Neurology, Stanford Univ. Sch. of Medicine, Stanford, CA 94305.

When synaptic inhibition is strongly suppressed with high doses of the GABA$_A$ receptor antagonist bicuculline methiodide (BMI; $\geq$ 10 μM), slices of neocortex in vitro generate synchronized discharges that resemble the interictal spikes of epilepsy. These discharges have fixed (all-or-none) amplitudes and durations (hundreds of msecs), widely varying latencies to a threshold stimulus, and they spread horizontally without decrement (Connors, Nature, 310:685, 1984). We now report that much lower doses of BMI (i.e. partial suppression of inhibitory synapses) generate very different epileptiform activities.

Coronal slices of rat SI were maintained in vitro, field potentials were recorded simultaneously through 4 separate microelectrodes arrayed horizontally in layer 2/3, monopolar stimuli were delivered to layer 6, and the dose of BMI in the bath was slowly increased from zero to between 0.1 and 1.0 μM.

In control solution, stimuli evoked activity only in a narrow, vertically oriented column above the stimulating electrode. BMI doses between 0.1 and 0.6 μM caused the primary evoked response to spread farther in the horizontal directions, often asymmetrically. The threshold dose for evoked epileptiform activity was in the range of 0.4 to 1.0 μM. Spontaneous field potentials were not observed. These low-dose epileptiform events had clear stimulus thresholds, variable amplitudes, and widely shifting latencies (as long as 500 msec) and durations (10 to hundreds of msecs). Unlike the high-dose BMI state, low-dose epileptiform events were not necessarily initiated within the column above the stimulus, but arose from preferred sites as far as 4 mm away horizontally. These sites were usually within the medial aspect of SI. Horizontal propagation of epileptiform events was slow (about 15-65 mm/sec) and often partial; the distance that events spread could vary from trial-to-trial. Unlike high-dose events, propagating low-dose events were sometimes reflected, e.g. an event propagating from medial-to-lateral would, at a particular point, initiate both retrograde and anterograde events.

Low-dose epileptiform events are distinguished by their preferred sites of initiation, their variable durations and by their partial and reflecting propagation. The former imply spatial non-uniformities of either intrinsic neuronal properties or local circuitry. The data also suggest that the horizontal spread of cortical activity is constrained by GABA$_A$-mediated synaptic inhibition. We hypothesize that horizontally directed inhibitory circuits can strongly modify the movements of epileptiform events.

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317.6 STIMULATION-INDUCED STATUS EPILEPTICUS: ROLE OF THE HIPPOCAMPAL MOSSY FIBER PROJECTION. J.P. Vicedomini and J.V. Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Status epilepticus (SE) involves prolonged activation of limbic and related neocortical circuitry and ultimately leads to the degeneration of CNS neurons. We have used two different animal models to study SE: (1) repetitive electrical stimulation of specific hippocampal afferent pathways and (2) intracerebroventricular (i.c.v.) administration of convulsants, such as kainic acid (KA). Previous studies of the KA model suggested that an intact mossy fiber projection is essential for seizure-induced CA3 pyramidal cell death, but not necessarily for the induction of SE. We have now explored this issue further by use of the electrical stimulation model of SE.

Adult male rats were chronically implanted with a recording electrode in the hippocampal CA3 area and a stimulating electrode in the contralateral fimbria. Prior to electrode implantation, either the mossy fiber pathway of the rostral hippocampus was transected or dentate granule cells were destroyed with colchicine on the side of the brain ipsilateral to the recording electrode. After an appropriate postoperative recovery interval, subjects were electrically stimulated according to a protocol which consistently evokes prolonged SE (Vicedomini and Nadler, Exp. Neurol., in press). The electrographic, behavioral and neuropathological consequences of the electrical stimulation were determined.

Fimbrial stimulation eventually elicited at least 1 h of SE in all animals which had been subjected to a mossy fiber transection or in controls which had received no prior lesion. In contrast, none of the colchicine-treated animals developed SE, despite receiving nearly twice the number of stimulus trains delivered to the other subjects and despite having experienced as much as 145 min of afterdischarge. Although the CA3 area and other brain regions suffered extensive neuronal degeneration in subjects with a MF transection and in controls, no such degenerative changes were observed in colchicine-treated subjects. Finally, both MF-transected and colchicine-treated subjects exhibited far fewer and less severe limbic motor seizures than did control rats. These results (1) suggest that at least some mossy fibers must be intact for fimbrial stimulation to evoke SE; (2) support our previous conclusion that afferent pathway stimulation destroys CNS neurons only if it evokes a prolonged period of self-sustained seizure activity and (3) suggest that generalization of the seizure to motor centers requires a substantially intact mossy fiber pathway. The present findings may be reconciled with the somewhat different effects of mossy fiber lesions in the KA model by postulating that a specific interaction between the convulsant and the mossy fiber pathway augments the toxic effect of seizures on CA3 pyramidal cells. (Supported by NIH grant NS 17771.)

317.7 EPILEPTIFORM BURSTING DISPLAYED BY HIPPOCAMPAL NEURONES IN ORGANOTYPIC MONOLAYER CULTURE. C.J.McBain*, P.Boden* and R.G. Hill. Parke-Davis Research Unit, Addenbrookes Hospital Site, Cambridge CB2 2QB, United Kingdom.

The hippocampus in vitro has been shown to be a useful model for the study of epileptiform activity. Many previous investigations have relied however, on pharmacological manipulations to induce epileptiform activity. We show here that explants of neonate hippocampus in long term tissue culture display both spontaneous and evoked seizure-like discharges and paroxysmal depolarising shifts (PDS).

Organotypic monolayer cultures of hippocampal slices from 6-day neonate Wistar rats were prepared and maintained for periods of up to 80 days as described previously (Gahwiler 1981). Conventional intracellular recordings were made from the s. pyramidale of CA1 using K-acetate electrodes (3M, d.c. resistances 80-200MΩ). Cells were considered in two groups, (a) 1-12 days in vitro (DIV) and (b) 12 DIV onwards. Comparable resting membrane potentials were obtained between group (a) and group (b); 60.0±1.4mV (n=10), 61.0±1.4mV (n=11) respectively. The apparent input resistances of cells in group (b), 79±11MΩ (n=11), were twice those observed in group (a), 39±4 MΩ (n=10), which may be indicative of a decrease in membrane conductance. 7 out of 10 cells from group (a) displayed spontaneous and evoked activity comparable to that seen in conventional hippocampal slices prepared from 6 and 21 day old rats. These cells fired action potentials which overshot zero potential followed by either afterhyperpolarisations (AHP) or depolarising after potentials (DAP). Spontaneous and evoked EPSPs and IPSPs could be recorded from these cells. 8 out of 11 cells in group (b) and 3 cells in group (a) however displayed spontaneous or evoked epileptiform discharges. Seizure activity typically commenced with a large paroxysmal depolarising shift (PDS) of 40-50mV resulting in 20-50 action potentials which overshot zero potential. Each PDS had a duration of 1.0-1.5 seconds and occurred at a frequency of 0.08Hz. The PDS activity appeared to be associated with a loss of AHPs and GABA-mediated IPSPs, although cells showed no change in their post-synaptic sensitivity to GABA (100μM). Epileptiform activity was markedly attenuated by the NMDA antagonist D-APV (30μM), Agents which block synaptic transmission (Mg^{2+} (20mM) and tetrodotoxin (1μM)) abolished all epileptiform activity indicating that functional synapses are responsible for these events.

These results suggest that long-term culturing of hippocampal explants may lead to a loss of GABAergic inhibition, allowing the expression of an NMDA receptor mediated depolarisation which may contribute to the generation and maintenance of epileptiform activity. Gahwiler B.H. J.Neuroscience. Methods 4,329-342. (1981)

317.8 **THREE DIMENSIONAL TIME VARYING COMPUTER SIMULATION OF EPILEPTIFORM BURST GENERATION IN THE CA3 HIPPOCAMPAL SUBFIELD.** **E.J. Smith*, D.K. Terry* and J.W. Swann (SPON: R.A. Waniewski). Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201**

The epileptiform burst generated in rat hippocampal CA3 region was simulated by a time varying, three dimensional spatial graphics display on a digital computer. The experimental data used to generate the model were obtained from laminar field potentials analysed from rat hippocampal slices exposed to penicillin. The methods used to obtain these data have been reported previously (J. Neurophysiol. 56:1718-1738, 1986). Recordings were made at 25 μm intervals across the CA3 subfield on an imaginary line orthogonal to the pyramidal cell body layer. At each position recordings were obtained from a linear triple microelectrode array. These electrodes were spaced 100 μm apart. The center electrode of the array recorded the field potential while current source density at that location was computed electronically using the recordings from all 3 electrodes. The signals were collected on-line and stored in computer memory. From these data the amplitude of the signals across the CA3 laminae could be obtained for any given point in time. To date we have analysed data collected at 10 msec intervals before, during and after burst generation. Each of these laminar profiles were fit by a seventh order polynominal to yield a smooth function. These functions were plotted in elliptical coordinates which simulated the anatomy of the CA3 subfield. It was assumed that equipotential lines were elliptical in shape with an eccentricity of 0.66. At the present time the animated model consists of twenty sequential frames spaced at 10 millisecond intervals for both potential and CSD. The model provides a clear insight into the spatial distribution of the potential and CSD in the hippocampus and sharply defines the temporal evolution of current sources and sinks during epileptiform burst generation.

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317.9 **LOCALIZED SYNAPTIC INTERACTIONS MEDIATE THE SUSTAINED DEPOLARIZATION OF SEIZURE-LIKE DISCHARGES IN IMMATURE HIPPOCAMPUS.** **J.W. Swann, K.L. Smith* and R.J. Brady. Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201**

Experiments were performed on isolated segments of the CA3 subfield of 10-15 day old rat hippocampus. Prolonged (5-10 sec) seizure-like discharges were produced by bath application of penicillin. Like in intact slices CA3 pyramidal neurons underwent an intense sustained depolarization during the course of the synchronized afterdischarges. Simultaneously, a slow negative field potential was recorded in the CA3 basilar dendritic layer. We tested the hypothesis that these coincident slow events were produced by local circuit excitatory synapses on pyramidal cell basilar dendrites. To this end we locally applied microdroplets of kynurenic acid (0.5 - 1.0 mM) or MK 801 (50 - 100 μm) to the minislices.

When droplets of either drug were applied to a single site in the basilar dendritic layer of small minislices (500-700 μM along the cell body layer) afterdischarge generation ceased. Most often the PDS was unaltered. Drug application to the apical dendritic layer did not alter the duration of the seizure-like discharges. In larger minislices application of either drug to a single site in the basilar dendritic layer did not block afterdischarges. However, recordings from this site revealed the slow negative field potential to be suppressed and the sustained depolarization to be abolished. Indeed at these sites the synchronized afterdischarges recorded intracellularly now arose either from a resting or hyperpolarized membrane potential. Simultaneous extracellular recordings in the basilar dendritic layer remote from the site of drug application revealed the presence of a large slow negative field potential. We reasoned that at this time the seizure discharges were likely to be generated at such remote sites where excitatory synaptic transmission had been unaltered. In keeping with this scenario, the cells exposed to the drug now had become 'followers' of the distant population, which was being driven to afterdischarge by the tonic depolarization generated in their basilar dendrities. In support of this contention when we applied the drug to the remote site as well, seizure discharges ceased throughout the minislice. These results lead us to suggest: 1) that the sustained depolarization recorded during seizure-like discharges is a separate physiologic process from those of the synchronized discharges, 2) that the depolarization is the product of summated excitatory amino acid mediated synaptic events on basilar dendrites, and 3) that these synaptic potentials play a central role in seizure generation in immature hippocampus.

(Supported by NIH Grant NS18309)

317.10 EXTRACELLULAR CALCIUM MODULATES THE FREQUENCY OF ICTAL-LIKE DISCHARGES IN PENICILLIN-TREATED IMMATURE HIPPOCAMPAL SLICES. R.J. Brady, K.L. Smith* and J.W. Swann, Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201.

Penicillin-treated hippocampal slices taken from immature rats 9-19 days of age exhibit the capacity to produce prolonged ictal-like discharges. Simultaneous extracellular and intracellular recordings in the CA3 region show that these ictal-like events consist of a field epileptiform burst paired with a depolarization shift (DS) followed by a self-sustaining series of afterdischarges. This series can vary from no afterdischarges being present to a prolonged series of up to 30 sec in duration, producing an ictal-like event.

The amount of epileptiform activity in a given penicillin-treated hippocampal slice can be quantified by measuring the percent epileptiform discharge (PED). The amount of time epileptiform activity appears is accumulated and expressed as a percentage of the total collection epoch. In a penicillin treated (1.7 mM) hippocampal slice taken from a twelve day old animal the PED was 5%.

It has been shown that during ictal activity the levels of extracellular calcium decrease. Given the effects on changes in extracellular calcium on cellular excitability and reported effects of divalent cations on excitatory amino acid responses, we tested the effects of changes in extracellular calcium on the pattern of spontaneous afterdischarge generation in penicillin-treated immature hippocampal slices. We have found that decreasing calcium in the bathing media to 1 mM dramatically increased the number of afterdischarges seen during spontaneous epileptiform activity. The PED increased from a control value of 5% to, in 1 mM extracellular calcium, a value of 18.6% Each epileptiform event was an ictal-like discharge with a prolonged series of afterdischarges. Raising the level of calcium in the bathing media to 3.5 mM blocked afterdischarges leaving only the DS and associated field epileptiform burst. The PED decreased to 0.6%.

The mechanisms that underly the effects of a change in extracellular calcium on afterdischarge generation are under investigation. We have not been able to demonstrate a direct effect of calcium changes on CA3 pyramidal cell excitability. Decreasing calcium has been seen to increase the response of CA3 pyramidal cells to iontophoretically applied N-methyl-D-aspartate. However, this effect has not been observed in every cell studied. Increasing calcium has been observed to decrease the N-methyl-D-aspartate response.

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317.11 PROLONGED SEIZURE-LIKE DISCHARGES IN IMMATURE HIPPOCAMPAL MINISLICES CAN BE INITIATED BY SINGLE NEURONS. K.L. Smith* and J.W. Swann (SPON: M. Pierson). Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201

Miles and Wong (Nature 306:371:1983) have previously demonstrated that interictal epileptiform discharges can be triggered by action potential in single CA3 pyramidal cells in small isolated segments of mature hippocampus. In our laboratory, we have been studying mechanisms underlying penicillin-induced ictal-like discharges in immature hippocampus and wondered whether single CA3 neurons could initiate these prolonged seizure-like events.

Experiments were performed in minislices isolated from the CA3 subfield of 10-15 day old rat hippocampus. In a first series of experiments we attempted to determine the minimal neuronal population size that could support seizure-like discharges. We found that minislices bathed in penicillin and measured 500-700 μm along the pyramidal cell body layer routinely generated ictal-like discharges that were 5-10 sec in duration. These events occurred spontaneously or could be evoked by a single orthodromic stimulus.

Simultaneous intracellular and extracellular recordings from these isolated hippocampal segments showed that the seizure-like discharges were structurally very similar if not identical to those that occur in intact slices. When depolarizing current was applied to single pyramidal cells we found that 58% (22/38) were capable of initiating epileptiform discharges. Following each ictal-like discharge there was a prolonged period of refractoriness during which action potentials in any single pyramidal cells were unable to entrain the population. Suddenly bursts of spikes, which had previously been ineffective, could initiate a synchronous population discharge of up to 10 sec in duration. Interestingly we also found that 40% (4/10) of fast repetitively spiking neurons were also able to initiate the population discharge. We assume these cells are non-pyramidal type neurons.

Paired intracellular recordings (n=10) were made in order to study variations in synaptic interactions between neurons during the cycling in the effectiveness of individual neurons to entrain the neuronal population. Monosynaptic interactions were not observed between pairs of cells. Generally during the period of refractoriness spikes in a presynaptic neurons did not produce an observable response in the other cell of the pair. However, in most pairs a presynaptic train of action potentials resulted repeatedly in a depolarizing potential which immediately preceded depolarization shift generation and the coincident discharge of the population. (Supported by NIH Grant NSI8309.)

317.12 ANTICONVULSANT EFFECTS OF DEXTROMETHORPHAN IN A KINDLING MODEL OF EPILEPSY. H.R. Feeser, J.L. Kadis*, B.Y. Wong*, and D.A. Prince. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Dextromethorphan (DM), a non-prescription antitussive, has anticonvulsant properties in both in vivo and in vitro seizure models. Work in our lab has demonstrated that both DM and its metabolite dextrorphan (DX) block prolonged ictal afterdischarges and interictal spiking in neocortical slices perfused with low-magnesium artificial cerebrospinal fluid and that the effect of these drugs may be a result of their NMDA-antagonistic properties. DM has also been shown to be effective in blocking maximal electroshock seizures in rats (Tortella and Musacchio, Brain Research 383 (1986) 314-318). We now report the effects of DM on electrical kindling of the amygdala.

Bipolar electrodes were stereotaxically implanted into the left amygdala of male Sprague-Dawley rats. The animals were allowed to recover for one week, then stimulated five times weekly with one-second trains of 60 Hz, 250 μamp square wave pulses. Thirty to forty minutes prior to stimulation, treated animals received i.p. injections of 35 mg/kg DM, while control animals were injected with saline. Length of afterdischarge and seizure intensity following simulation were recorded for all animals. Once fully-kindled, control rats were treated with 35 mg/kg DM prior to stimulation, and the duration and intensity of their seizures following drug treatment were compared to those following saline treatment.

Results to date indicate that DM produces a statistically significant shortening of seizures both in animals treated with DM throughout the course of kindling and in fully-kindled control animals treated acutely with DM. More trials may be required to fully kindle DM-treated rats, but data are not yet sufficient to demonstrate statistical significance. A mild hind-limb ataxia was the only adverse reaction noted in DM-treated animals. These results, combined with the findings that DM and DX antagonize NMDA, suggest that DM may be a clinically useful member of a new class of anticonvulsants.

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317.13 EFFECT OF CA AND OTHER ION CHANNEL BLOCKING AGENTS ON THE HIGH AFFINITY BINDING OF DEXTROMETHORPHAN TO GUINEA PIG BRAIN. M. Klein* L.J. Santiago* and J.M. Musacchio. Dept. Pharmacology, N.Y.U. Medical Center, New York, NY 10016.

Dextromethorphan (DM), a dextrorotatory non-narcotic centrally-acting antitussive, binds in the CNS (Craviso and Musacchio, Mol. Pharmacol., 23:629, 1983) to high (Kd 53 nM) and low (Kd 20 μM) affinity sites. The high affinity binding is allosterically modulated by the anticonvulsant phenytoin (PHT). DM, carbetapentane and caramiphen, antitussives which displace [^{3}H]DM with high affinity, protect rats against maximal electroshock seizures (Tortella and Musacchio, Brain Res. 383:629, 1986; Fed. Proc. 46:708, 1987).

To explore the nature of the DM binding sites we tested the effect of several ion channel blockers on the binding of [^{3}H]DM. Prenylamine, cinnarizine and hydroxyzine were very potent competitors with higher affinity for the DM binding sites than [^{3}H]DM itself, with Ki values of 17 nM, 22 nM and 46 nM respectively. Cinnarizine and flunarizine, which have been shown to have anticonvulsant activity, have Ki values of 22 nM and 150 nM respectively. The diphenylalkylamines tested showed a higher affinity for [^{3}H]DM binding sites than for [^{3}H]desmethoxyverapamil sites. By contrast, phenylalkylamines inhibited the binding of [^{3}H]DM in the low micromolar to high nanomolar range. Nifedipine, a dihydropyridine, and diltiazem, a benzothiazepine, were much less potent. These findings suggested that DM sites probably are not related to either dihydropyridine or diltiazem sites.

The Na channel agents tested were very weak competitors for [^{3}H]DM binding sites. Tetrodotoxin showed no effect at concentrations as high as 10 μM. Veratridine and aconitine inhibited slightly at a concentration as high as 300 μM. These results suggest that DM binding sites probably are not associated with Na channels.

Among the K channel blocking agents tested, 4-aminopyridine and tetraethylammonium both inhibited the binding of [^{3}H]DM in their pharmacological range, with Ki values of 0.75 and 1.35 mM respectively. All of the antimalarial K channel blockers tested inhibited [^{3}H]DM binding in the micromolar range. Quinidine was the best competitor for [^{3}H]DM binding sites, with a Ki value of 0.57 μM. The Ki values of other agents were the following (μM): quinine: 9.0; primaquine: 7.3; chloroquine: 1.2; quinacrine: 1.9. Atracurium exhibited a Ki value of 1.9 μM. Pancuronium showed no effect at a concentration as high as 35 μM. These results suggest a possible association of DM binding sites with K channels. Supported in part by the Epilepsy Foundation of America and UPHS grants DA-02013, MH-29591 and MH-17785.

317.14 EFFECT OF ANTICONVULSANT DRUGS AND OTHER AGENTS ON THE HIGH AFFINITY BINDING OF DEXTROMETHORPHAN TO GUINEA PIG BRAIN. L.J. Santiago* M. Klein* and J.M. Musacchio (SPON: R.U. Margolis). Dept. of Pharmacol., N.Y.U. Medical Center, New York, NY 10016.

Dextromethorphan (DM), a dextrorotatory non-narcotic centrally-acting antitussive, binds in the CNS (Craviso and Musacchio, Mol. Pharmacol., 23:629, 1983) to high (Kd 53 nM) and low (Kd 20 μM) affinity sites. The anticonvulsant phenytoin (PHT) is an allosteric enhancer of the binding of [^{3}H]DM. DM, carbetapentane and caramiphen, antitussives which displace [^{3}H]DM with high affinity, also protect rats against maximal electroshock seizures (Tortella and Musacchio, Brain Res. 383:629, 1986; Fed. Proc. 46:708, 1987).

To investigate the interactions between PHT, the DM sites and the anticonvulsant activity of these drugs, we tested the effects of several anticonvulsant drugs on the binding of [^{3}H]DM. SC-13504, an anticonvulsant benzhydryl piperazine, produced a marked increase in the binding of [^{3}H]DM, similar to that previously reported for PHT. SC-13504 produced a 62 and a 200% increase in the binding of [^{3}H]DM at 1 and 10 μM respectively. These effects are due to a two fold increase in affinity without a change in Bmax.

Nafimidone has a Ki of 1 μM, which is compatible with the anticonvulsant ED_{50} in rats (7.2 μmoles/Kg, Walker et al., J. Med. Chem 24:67, 1981). Two other anticonvulsants, hibital and AHR 11748, decreased the binding of [^{3}H]DM 26 % at a concentration of 100 μM. The brain level of AHR 11748 measured at the anticonvulsant ED_{50} is 200 μM in mice, indicating that the anticonvulsant effects of these drugs could be mediated through the DM sites.

Felbamate and zonisamide had no effect at a concentration of 100 μM, and fluzinamide displaced only 10 % of the DM bound at a concentration as high as 1 mM. Ethosuximide, methsuximide, phensuximide and phenacemide had no effect on [^{3}H]DM binding at 100 μM, either. Analogs of PHT, such as mephenytoin, ethotoin and paramethadione were tested up to a concentration of 100 μM, and did not increase the binding of [^{3}H]DM. Competition studies carried out with the inhibitory amino acids GABA and glycine, with taurine, cysteine, aspartate, glutamate as well as kainate, NMDA and 2-amino 5-phosphonovaleric acid showed no effect on [^{3}H]DM binding, indicating that there is no apparent relationship between the site of action of these agents and the DM binding sites.

These findings suggest that the DM binding sites are involved in the mediation of the anticonvulsant activity of several drugs. The study of these sites may help to elucidate the mechanism of action of some anticonvulsant agents, and to discover new ones. Supported in part by the Epilepsy Foundation of America and UPHS grants DA-02013, MH-29591 and MH-17785.

317.15 A NEW ANTICONVULSANT, PR 934-423, ACTIVE AGAINST MAXIMAL ELECTROSHOCK SEIZURES I: MICE STUDIES. G.C. Palmer, R.C. Griffith*, M.J. Ordy, C.W. Becker*, J.M. Frankenheim* and M.L. Stagnitto*. Pharmaceutical Division, Pennwalt Corporation, Rochester, NY 14623

A major problem in treatment of epilepsy is the lack of structurally different, but highly effective anticonvulsants when the primary agent fails because of toxicity or refractoriness to the disease. The present compound (+)-2-amino-N-(1-methyl-1, 2-diphenylethyl) acetamide-HCl or PR 934-423A was developed from computer modeling experiments which demonstrated a potential three dimensional correlation with the pharmacophore pattern of known anticonvulsants. Initial pharmacological screening indicated that PR 934-423A did not produce prominent CNS side effects in mice until large oral doses were administered. PR 934-423A did not possess sedative properties and was found to be orally effective in protecting mice from MES (maximal electroshock seizures). An oral ED50 dose for MES protection was established at 33 mg/kg while an ED50 for neural impairment (failure of mice to climb an inverted screen) was 580 mg/kg. Thus a therapeutic ratio of 17.6 was determined which was less than corresponding ratios for the reference agent phenytoin, but greater than phenobarbital and carbamazepine. Of other compounds synthesized in the chemical series, PR 934-423A possessed a superior efficacy/safety ratio and was thus selected for further pharmacological profiling.

PR 934-423A was essentially ineffective orally against chemically-induced seizures elicited by: pentylenetetrazole (ED50 = 328 mg/kg), picrotoxin (ED50 = 417 mg/kg), bicuculline and strychnine (ED50s > 600 mg/kg).

The margin of safety for orally administered PR 934-423A in mice was 28.1 (lethal dose 50 of 926 mg/kg/ED50 of 33 mg/kg for MES protection). The margin of safety value was less than phenytoin but greater than phenobarbital. The margin of safety after intravenous administration was considerably narrower for PR 934-423A = 3.8 (LD50 of 56 mg/kg/ED50 of 14.7 mg/kg) when compared to phenobarbital.

The duration of action for oral protection of mice against MES was, however, longer than that for carbamazepine, but shorter than phenytoin and phenobarbital. The duration of action for PR 934-423A was, however, lengthened by increasing the dose (≈3 hour at 60 mg/kg and > 6 hour at 120 mg/kg). Both PR 934-423A, as well as the reference compounds had rapid onsets of action (45 minutes or less).

Tolerance to protection of mice against MES did not develop following oral administration for 5 days of either the ED50 or ED99 doses of PR 934-423A, phenytoin and phenobarbital.

Therefore it appears that PR 934-423A is a relatively safe and potent antiseizure drug that might be targeted for clinical effectiveness for treatment of generalized tonic/clonic seizures.

(Appreciation is expressed to R.H. Harvey, M.A. Knowles, L.A. Steiner, S. Phippen for technical assistance).

317.16 A NEW ANTICONVULSANT, PR 934-423, ACTIVE AGAINST MAXIMAL ELECTROSHOCK SEIZURES II: RAT STUDIES. P.M. Colombo*, G.C. Palmer, R.C. Griffith*, G.E. Garske*, C.W. Becker*, M.J. Ordy, J.C. Blosser, M.L. Coan*, and S.A. McCreedy*, (Spon: C.N. Latimer) Pharmaceutical Division, Pennwalt Corporation, Rochester, NY 14623

PR 934-423A or (+)-2-amino-N-(1-methyl-1,2-diphenylethyl) acetamide-HCl was found to be an effective oral anticonvulsant for protection of mice against seizures elicited by maximal electroshock (MES). PR 934-423A was selected for further pharmacological development in the rat. The oral ED50 for protection of male and female rats against MES was 19.8 mg/kg. In neural impairment tests (modified gang plank walking) the oral ED50 was 690 mg/kg. A therapeutic index of 34 was obtained which was superior to the reference agent, phenobarbital. The oral LD50 for PR 934-423A was 940 mg/kg yeilding a margin of safety (LD50/ED50 for MES protection) of 46, a value higher than phenobarbital. However, after iv administration the margin of safety became narrower (7.2) a value less than for phenobarbital.

The duration of action of oral PR 934-423A was determined. At the ED97 dose for MES, the time to peak action was 30 mins and 80% protection was sustained for 6 hrs. Following iv or lower dose oral administration, the plasma half life of PR 934-423A was relatively short (iv 10 mg/kg = t 1/2 of 0.36 hr with a clearance rate of 6.5 l/kg/hr and at total volume of distribution of 2.8 l/kg). At 80 mg/kg (po), drug concentrations in plasma peaked at 30 mins and the t 1/2 was 3.4 hrs. The total amount of drug absorbed was approximately 10%. This rapid metabolism and low rate of absorption most likely accounted for the short duration of action at low doses. When compared to phenytoin, 7 days oral administration of PR 934-423A at the ED97 dose did not shorten hexobarbital-induced sleep time. However, after acute administration PR 934-423A lengthened hexobarbital sleep time, despite its lack of sedative effects when given alone at high doses. The acute response probably represents competition with hexobarbital for metabolic degradation in the liver.

In mechanistic studies, PR 934-423A was evaluated for neurotransmitter receptor affinity in particulate fractions of rat brain. PR 934-423A was ineffective in preventing convulsions elicited by pentylenetetrazol.

Therefore, PR 934-423A appears to be an effective anticonvulsant against seizure spread and would be predicted to be useful in patients with generalized tonic/clonic seizures.

(Appreciation is expressed to L.R. Freedman, M.A. Barrantes, E. F. Cregan, and T.M. Wengenack for technical assistance).

317.17 A NEW ANTICONVULSANT, PR 934-423, ACTIVE AGAINST MAXIMAL ELECTROSHOCK SEIZURES. III: ELECTROPHYSIOLOGICAL STUDIES. L.R. Freedman*, R.C. Griffith and E.W. Harris. Pharmacology Dept., Pharmaceutical Div., Pennwalt Corporation, 755 Jefferson Road., Rochester, NY 14623

There is a great need for anticonvulsants in new structural classes and possessing new mechanisms of action. The compound (+)-2-amino- N-(1-methyl-1,2- diphenylethyl) acetamide. HCl (PR 934-423A) appears to be such a drug. PR 934-423A is effective against MES in mice and rats, with a larger therapeutic index than phenobarbital, carbamazepine or valproate (see Griffith, Palmer, Ordy, Becker and Stagnitto; this session). Computer modelling indicated structural similarities with phenytoin, and like phenytoin it is more effective against MES than chemically-induced seizures. The mechanism of action of PR 934-423A has been investigated using the in vitro hippocampal slice preparation.

Hippocampal slices were prepared from young adult male Sprague-Dawley rats using standard procedures. Slices were submerged in constantly refreshed medium in which compounds were dissolved to yield known final concentrations. Phenytoin (Sodium diphenylhydantoin) was first dissolved in 40% PEG/10% ethanol at 1000 times the desired final concentration. Extracellular synaptic responses were recorded using a 1M NaCl filled glass micropipette placed in CA1. Constant current electrical stimuli were delivered to stratum radiatum near CA2 to activate Schaffer collaterals.

Phenobarbital (up to 300 μM), phenytoin (up to 40 μM) and PR 934-423A (up to 150 μM) were compared in terms of effects on Schaffer evoked population spikes, paired-pulse potentiation of the population spike (25 m sec ISI), and the induction of long term potentiation of the population spike by high frequency stimulation (100 Hz, 100 pulses, 3 times). Recordings were made from stratum radiatum in CA1. Only phenobarbital significantly reduced paired-pulse potentiation and inhibited the induction of long-term potentiation.

In a second series of experiments, spontaneous epileptiform activity was elicited from hippocampal slices using high-potassium/penicillin-G). Phenytoin and phenobarbital both reversibly slowed spontaneous burst firing rate at concentrations at or just above clinically relevant concentrations (10 and 100 μM respectively). In contrast, PR 934-423A up to 100 μM (at least 20 times its estimated effective free levels in plasma) had no apparent effect on spontaneous firing rate.

These data confirm that PR 934-423A acts differently from phenobarbital on CNS tissue, and, insofar as effects in the penicillin-treated slice model reveal, this study also suggests that PR 934-423A acts differently from phenytoin to reduce seizure activity.

317.18 BLOCKADE OF AUDIOGENIC SEIZURES (AGS) IN GENETICALLY EPILEPSY-PRONE RATS (GEPR) BY THE MICROINJECTION INTO INFERIOR COLLICULUS (IC) OF BLOCKERS OF INHIBITORY AND EXCITANT AMINO ACID (EAA) METABOLISM. C.L. Faingold, C.A. Copley*, C.A. Boersma*, So. Illinois Univ. Sch. of Med., Dept. Pharmacology, Springfield, IL 62708

The neural basis for susceptibility of the GEPR to AGS is of considerable interest. The IC has been shown to be the most sensitive of 8 subcortical nuclei tested for induction or blockade of AGS (see Faingold et al., 1987). GABA may mediate sound-induced (binaural) inhibition in the IC. Greater than normal GAD-immunoreactivity is reported in the GEPR IC, and reduced effectiveness of binaural inhibition and of iontophoretically applied GABA is also observed. An EAA may be an excitatory neurotransmitter in IC, and increased release of EAA is also observed in GEPR IC during AGS. Microinjection of an EAA agonist, or a $GABA_A$ antagonist into the IC induces susceptibility to AGS in normal rats (Millan et al., 1986). An EAA receptor antagonist, and GABA agonists are effective anticonvulsants when injected into the IC of the GEPR. However, agents which affect GABA and EAA receptors could exert effects on neurons that are not modulated primarily by these amino acids under normal conditions, as suggested with EAAs in other brain sites.

The present study examined the effects on AGS susceptibility of agents which alter the amino acid metabolism but are not thought to act directly on receptors. GEPRs displaying maximal seizure severity (GEPR-9s) were chronically implanted with guide tubes above IC and tested for AGS sensitivity one week later. Bilateral microinjections (0.5-1.0 μl) of gabaculine (10-100 μg/side) or L-canaline (25-50 μg) were infused into IC. L-Canaline is reported to block the synthesis of EAAs, and gabaculine is reported to block the catabolism of GABA. AGS scores (to a bell at 122 dB SPL) were evaluated according to the scale of Jobe (1981). Seizures were completely blocked (median seizure score = 0) in most animals by 2 hr after gabaculine infusions (N=8, to date), and the animals rarely recovered susceptibility. Ataxia and loss of righting reflex were observed in most animals. Two hours after L-canaline infusions all tonic seizure components were abolished (median score = 2, N=12, to date), and recovery was rarely observed even up to a week later. Thus, inhibition of EAA synthesis by L-canaline produces a sustained block of AGS. This finding along with the previous reports of altered function of EAAs, cited above, strongly support a role for increased EAA action in the IC in initiation of AGS. Inhibition of GABA catabolism by gabaculine irreversibly blocks AGS. This finding along with previous reports on altered GABAergic function, cited above, also strongly support a role of reduced GABAergic function in the IC in initiation of AGS in the GEPR. This apparent imbalance in inhibitory and excitatory influences in the IC may be an important factor in initiation of AGS in the GEPR.

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318.1 STUDIES OF PROPAGATING EPILEPTIC EVENTS IN THE LONGITUDINAL HIPPOCAMPAL SLICE. R.D. Traub, R. Miles and R.K.S. Wong. IBM T.J. Watson Res. Ctr., Yorktown Heights NY 10598 and Dept. of Neurology, Columbia Univ., New York, NY 10032.

Factors which control the spread of epileptic activity are not clear. Do synchronous events propagate at the speed of axonal conduction? If not, what other factors are involved? We examined this question with experimental and simulation techniques.

In experimental studies, longitudinal slices were cut from the CA3 region of guinea pig hippocampus. They were up to 8 mm long, providing good spatial resolution of propagating events. Intra- and extracellular responses to electrical stimulation at one end of these slices did not spread more than 2-3 mm from the stimulus site while synaptic inhibition was functional. Responses propagated at 0.4-0.6 m/s. In contrast, when inhibition was blocked with picrotoxin (PTX), the same stimulus elicited a locally synchronized event which propagated the entire length of the slice. The speed of propagation was 0.1-0.25 m/s.

Thus, propagation of synchronous events does not simply depend on axonal conduction delay in this preparation. Other possible contributing factors include neuronal threshold, the strength of excitatory synapses and their spatial distribution. Effects of varying these parameters were examined using a model constructed with 9000 pyramidal cells (in a 40×225 array, representing a slice 4.5 mm long) and 900 inhibitory cells (450 producing fast, PTX-sensitive inhibition; 450 producing slow, PTX-resistant inhibition). The probability of an excitatory synapse between 2 pyramidal cells was assumed to decay exponentially with intercellular distance (space constant λ_r) and axon conduction delays were included (Traub, Knowles, Miles and Wong, *Neuroscience*, in press). With λ_r = 600 μ, and fast inhibition blocked, stimulation of a cell at one end of the array led to a local synchronized burst which propagated at 0.14 m/s, agreeing with experiment. When λ_r was smaller, propagation was slowed, and as it increased propagation became first faster and then ill-defined, with synchronized activity occurring everywhere at once. In the latter case, a small, spatially diffuse subpopulation was first excited, which in turn stimulated the entire population. Velocity also increased with synaptic strength. Both in simulation and experiment, velocity slowed if small degrees of inhibition were present. When a band of cells was strongly hyperpolarized, propagation was blocked in the hyperpolarized region but reappeared distally after a delay; the duration of the delay increased with the width of the hyperpolarized band. This agrees with experiments where GABA was applied focally to reduce neuronal excitability in a region of the slice. We conclude that the spread of epileptic synchrony depends critically on the spatial distribution and strength of excitatory synapses and neuronal threshold as well as axon conduction. In longitudinal slices, the average projection radius of excitatory connections is significantly less than the extent of the slice.

318.2 ANALYSIS OF FIELD POTENTIALS IN RAT NEOCORTICAL SLICES. A.J. Bean, B.W. Strowbridge* and G.M. Shepherd, Departments of Pharmacology and Neuroscience, Yale University School of Medicine, New Haven, CT 06510.

Extracellular field potentials provide a means for comparing intracellular responses of single neurons to the activity of populations of neurons. As part of a study of the basic circuit organization of cortical regions, we have analysed the field potentials generated in lamina 2 of neocortical slices by electrical stimulation of lamina 6 (cf. Chervin and Conners, Soc. Neurosci. Abst. 12:350, 1986)

Coronal slices of parietal cortex (400 um) were prepared from Sprague Dawley rats (250-350 g.) anesthetized with pentobarbital (50 mg/kg i.p.). Recording (REC) (NaCl or K-Acetate filled glass capillaries) and monopolar stimulating (STIM) electrodes were placed in layers 2 and 6 respectively, under visual guidance.

The response evoked by a weak stimulus when STIM and REC were radially aligned typically consisted of a negative wave with a latency of 4-5 ms. Increasing the stimulus intensity elicted a sharper negativity at a briefer latency, often followed by a positivity. As REC was displaced laterally, similar fields of smaller amplitude were evoked. Intracellular recording revealed an EPSP with a latency which varied with stimulus intensity and was similar to the peak of the negative field response.

The sequence of changes in the field response during the administration of bicuculline (BIC) were examined. Four stages are observed during the application of BIC: 1) the initial negativity increased in amplitude, 2) the positivity increased in amplitude, 3) multiple sharp negative waves were apparent, the positivity was noticably broadened, and a second long duration negativity emerged, 4) the initial negativity was followed by a prominent second negativity with a 700-900 ms duration. Intracellular recordings showed a long duration, large amplitude depolarizing shift with superimposed action potentials that followed the initial negativity of the field response. In the presence of increased Mg^{2+}(4 mM), a low threshold all-or-none large positivity following the initial sharp negativity could be elicited. Increasing the stimulus intensity revealed the higher threshold long duration second negativity.

Our observations are consistent with the initial negativity being associated in part with local synaptic potentials, since its latency resembles that of the EPSP seen in intracellular records, and at low stimulus intensities it is broadened. Thus, the positivity may reflect distant synaptic activity. The enlargement of the positivity during BIC may therefore represent an increased population of cells generating synchronized synaptic potentials. (Supported by NIGMS CA-09085, NIH NS-07609, and the Office of Naval Research)

318.3 DEVELOPMENT OF SYNCHRONIZED BURSTS AND ASSOCIATED NMDA RECEPTORS IN CA2/3 AND CA1 AREAS OF THE RAT HIPPOCAMPUS. S.M. Bawin, B.J. Vasquez, M.D. Mahoney*, C. Lowery* and W.R. Adey. Departments of Neurosurgery, Physiology and Pharmacology, Loma Linda University and VAMC, Loma Linda, CA 92357.

Our laboratory has previously reported that NMDA receptor systems were involved in the generation of delayed synchronized bursts (DSBs) in the CA2/3 region of disinhibited or electrically stimulated adult rat hippocampal slices. DSBs originate in CA2/3 and are synaptically transmitted to CA1 in the adult hippocampus.

We have now studied the development of the generation and propagation of DSBs in hippocampal slices from 6 to 19 days old (d) rats. Concurrent extracellular recordings were obtained in CA1 and CA2/3 (cell layers and dendrites). Test pulses in the Schaffer collaterals induced CA2/3 DSBs in all pup slices following disinhibition of the tissue by bath application of penicillin (1.5 mM). Slices from the younger animals (6-11 d) started to exhibit DSBs after 8-10 min of treatment while slices from the older pups required longer perfusion time (30-60 min). Moreover, spontaneous synchronized bursts (sp. bursts) similar to the evoked DSBs occurred much more frequently in the younger pups. The synaptic transmission to CA1 increased markedly with age. Small DSBs were initially transmitted to CA1 in 6-11 d slices but the responses faded rapidly. By contrast, robust CA1 bursts followed CA2/3 DSBs in slice from older pups. Similar results were obtained in slices perfused in the absence of magnesium, but CA2/3 sp. bursts were much more frequent. Addition of 2-amino-5-phosphonovalerate (APV, 50 uM) to either treatment solution, abolished both sp. bursts and the evoked DSBs.

Iontophoresis of NMDA (20 mM in 20 mM NaCl, -80 nA, 1-30 s) in the CA2/3 apical dendrites triggered the onset of sp. bursts in penicillin-treated slices. This sensitivity decreased with maturation and long lasting (15-30 s) NMDA injections were required in the 14-19 d pup slices. When DSBs were evoked at a rate of 0.2 Hz, NMDA reduced the amplitude of the delayed dendritic EPSP and the bursting stopped for 15-30 s following treatment. Injection of NMDA in the CA1 dendrites induced similar decrease in the transmitted DSBs. In parallel studies, NMDA receptor binding was measured in lysed and detergent treated P2 membrane fractions, using [3H]glutamate (0.1uM) and APV (100 uM). NMDA sensitive binding was reliably detected in both the CA1 and CA2/3 areas of 9, 14 and 19 d pups.

In conclusion, although the NMDA receptor system responsible for synchronized bursting in CA2/3 appears functional in 6-11 d pups, the mechanisms of transmission to CA1 are still poorly developed. Our findings could help explain why focal hippocampal seizures fail to propagate in the immature brain. (Supported by the Veterans Administration and Dept. of Pediatrics, LLU).

318.4 DISINHIBITORY EFFECTS OF A NMDA (N-METHYL-D-ASPARTATE) RECEPTOR ANTAGONIST IN THE HIPPOCAMPAL SLICE PREPARATION. R. Čapek and B. Esplin, Dept. Pharmacology and Therapeutics, McGill University, Montreal, Quebec, H3G 1Y6, Canada.

We have previously reported that phencyclidine reduced inhibition in the hippocampal slice and suggested that this effect may be due to a depression of the inhibitory interneuron with resulting decrease in GABA release (Bourne et al., *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 323:168,1983). One of the actions of phencyclidine is to suppress the NMDA responses in a non-competitive manner (Honey et al., *Neurosci. Lett.*, 61:135,1985). If synaptic activation of the inhibitory interneuron by presumably glutamatergic terminals involves NMDA subtype of the glutamate receptor, then its block could result in disinhibition.

To test this possibility, the effects of a competitive NMDA receptor antagonist, 2-amino-5-phosphonovaleric acid (APV), on inhibition of the CA1 pyramidal cells were investigated using conventional recordings of the field potentials in the superfused hippocampal slice preparation of the rat.

Population spikes in the CA1 pyramidal cell body layer were evoked by orthodromic stimulation of the stratum radiatum. They were inhibited by conditioning stimulation delivered 10, 20, 40, 60 or 80 msec before the orthodromic stimulation either to the alveus (antidromic-orthodromic) or to the stratum radiatum (paired pulse).

APV, dissolved in the superfusion medium in concentrations which did not affect the unconditioned population spike, was perfused for 60 min. In 80 or 100 μM concentration, the drug reduced inhibition at the first three interstimulus intervals in the antidromic-orthodromic test. The effects were qualitatively similar in the paired pulse test. In some experiments, APV attenuated inhibition at the shortest interstimulus interval while producing facilitation at intervals of 20 msec and longer. Lower concentration (40 μM) of APV was without a clear effect. Within 60 min after discontinuation of the drug, the inhibition partly recovered.

These results suggest that NMDA receptors contribute to synaptic activation of the inhibitory interneurons by a single afferent volley, perhaps inducing repetitive firing. In contrast, the discharge of the pyramidal cells evoked by synaptic activation was not affected by a block of NMDA receptors, as repeatedly reported. The functional significance of this difference between the excitatory synapses on the inhibitory interneurons and those on the pyramidal cells remains to be established.

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318.5 **CHANGES IN SYNAPTIC TRANSMISSION EVOKED IN CA1 SUBFIELD OF THE HIPPOCAMPAL SLICES BY BRIEF APPLICATION OF Mg++-FREE RINGER: A CORRELATE FOR LONG TERM POTENTIATION (LTP)?** C Drapeau*, M Avoli and G Kostopoulos. MNI & Dept of Neurol & Neurosurg McGill Univ.

LTP, a mechanism possibly underlying learning and memory, is a phenomenon in which tetanic stimulation of a set of input fibers potentiates synaptic transmission in that pathway for hours or days. Initiation of LTP in the hippocampus depends upon the activation of N-methyl-D-aspartate (NMDA) receptors, a specific class of receptors for the excitatory amino acid glutamate which become functional only at depolarized membrane potential. This feature is due to a voltage-dependent Mg++ block upon the channels coupled with NMDA receptors. Here we studied in the CA1 subfield of rat hippocampal slices the changes in synaptic transmission which occur when [Mg++]o is brought to near zero. Extracellular field potentials were recorded with 4M NaCl filled micropipettes while stimuli (0.05-0.2Hz) were delivered through an electrode placed in stratum (s.) radiatum to activate the Shaffer collateral, commissural pathway. Perfusion of the slices (n:18) with Mg++-free Ringer for 20-30min evoked a potentiation of the response to s. radiatum stimulation consisting of: (i) an increase in the amplitude and a prolonged decay of the extracellular EPSP recorded in s. radiatum; (ii) an increased amplitude of the population spike recorded at the soma. At moderate intensity of stimulation the amplitude increase of the population spike was 210±18% (mean±SD; $p<0.005$). Furthermore this potentiation was normally observed up to 185min after replacement with control Ringer, the potentiation at 40min being 184±23% ($p<0.005$). In presence of the specific NMDA antagonist APV (50-100µM) the potentiation evoked by Mg++-free Ringer could be observed in only 8 of 13 experiments while no long lasting effects were seen in these experiments following reintroduction of control Ringer. However, once LTP was induced by perfusion with Mg++-free Ringer, APV could no longer affect this potentiation (n:5).

These data show that a brief exposure of the hippocampal slices to Mg++-free Ringer is sufficient to elicit LTP and provide further evidence for a role played by NMDA receptors in the induction of LTP, though not in its maintenance.

Supported by Canadian MRC (MA 8109)

318.6 **EPILEPTIFORM DISCHARGES EVOKED BY LOWERING [Mg++]o IN THE HUMAN EPILEPTOGENIC NEOCORTEX MAINTAINED"IN VITRO"** J Louvel*, R Pumain*, M Avoli and A Olivier* (SPON: P Gloor). MNI & Dept of Neurol & Neurosurg McGill Univ, Montréal and INSERM U97, Paris.

Recent experiments have shown that conductances activated by NMDA receptors underlie the bursts of action potentials induced by focal stimuli in human neurons in slices obtained from epileptogenic neocortex (Neurosci Abstr 12:675,1986). NMDA receptors are coupled to a ionophore which is permeable to Ca++, Na+ and K+ and is gated in a voltage-dependent manner by extracellular Mg++. In addition decreasing [Mg++]o in rat hippocampal slices uncovers conductances activated by NMDA receptors and causes epileptiform discharges to appear. Therefore, it appeared of interest to study the effects of perfusing slices of human epileptogenic neocortex with Mg++-free Ringer.

Human slices (1st or 2nd temporal gyrus) were obtained during resection of epileptogenic tissue. Extracellular field potententials and extracellular ionic concentrations (K+ and Ca++) were measured using double-barrelled ion selective microelectrodes. Focal stimulation was delivered through electrodes placed in the white matter or within the cortex. Switching from control to Mg++-free Ringer induced within 50-100min the appearance of spontaneous or stimulus-induced synchronous epileptiform discharges, which could last up to 80s and were characterized by a prolonged negative potential shifts with superimposed fast negative transients. Such discharges were accompanied by increases in [K+]o (maximally 6.2mM from a baseline level of 3.25mM) and decreases in [Ca++]o (maximally 0.23mM from a baseline level of 1.8mM). In any experiments the largest and fastest changes in [Ca++]o and [K+]o occurred at the same sites from which the largest negative field potentials were recorded. Bath application of the selective antagonist of NMDA receptors 2APV suppressed in a reversible manner both spontaneous and stimulus-induced epileptiform discharges. These findings demonstrate that in slices obtained from human epileptogenic neocortex, prolonged epileptiform discharges are generated when [Mg++]o is lowered. Since these discharges were blocked by 2APV, they appear to be related to the activation of NMDA receptors.

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318.7 **EPILEPTIFORM DISCHARGES EVOKED BY LOWERING [Mg++]o: A STUDY OF POST-SYNAPTIC INHIBITORY POTENTIALS IN THE CA1 SUBFIELD OF THE HIPPOCAMPAL SLICE.** V Tancredi & M Avoli. Dept Exp Med, Univ of Tor Vergata, Italy; MNI & Dept of Neurol & Neurosurg McGill Univ.

Synchronous epileptiform discharges can be recorded in hippocampal slices perfused with Mg++-free Ringer. Since this type of discharge is blocked by antagonists of N-methyl-D-aspartate receptors (NMDA), the underlying mechanism appears to be related to the uncovering of NMDA activated conductances. Here we studied in rat hippocampal slices whether inhibitory synaptic mechanisms are preserved in this "in vitro" model of epilepsy. Field potentials were recorded in the CA1 and CA3 subfields with 4M NaCl filled electrodes; CA1 hippocampal pyramidal neurons (HPNs) were impaled with 4M K-acetate filled pipettes. Stimuli were delivered at 0.2-0.02Hz in stratum (s.) radiatum and alveus.

Perfusion with Mg++ - free Ringer induced within 35-40min the appearance of epileptiform bursts (duration: 50-300ms) which occurred at intervals of 1.5-5s and were recorded in both CA1 and CA3 subfield. Disconnection of these two areas abolished spontaneous discharges in CA1 but not in CA3. However, following this procedure, HPNs in CA1 were still capable of generating synchronous discharges of 3-5 population spikes in response to weak stimulation of the s. radiatum. Intracellular recordings demonstrated that these stimulus-induced epileptiform bursts were associated to a prolonged depolarization with multiple discharges of fast action potentials while alvear, antidromic stimuli were capable of inducing a hyperpolarizing, recurrent IPSP. Bath application of the NMDA antagonist aminophosphonovalerate (APV,50µM) reduced the stimulus-induced epileptiform bursts evoked by Mg++-free Ringer in the CA1 subfield. At the same time APV decreased the amplitude and the duration of the recurrent IPSP.

These results show that as in models where GABA antagonists are used to induce epileptiform activity, spontaneous epileptiform discharges evoked by lowering [Mg++]o originate in the CA3 subfield. However, in this type of experimental condition GABAergic potentials are preserved. Furthermore the effects exerted by APV upon the IPSP suggest that NMDA receptors play a role in the recurrent circuit responsible for the somatic hyperpolarizing IPSP generated by CA1 HPNs.

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318.8 **EFFECTS OF 4-AMINOPYRIDINE ON CA3 AND DENTATE NEURONS OF RAT HIPPOCAMPAL SLICES.** P Perreault & M Avoli. MNI & Dept of Neurol & Neurosurg McGill Univ.

Previous work has shown that the convulsant 4-aminopyridine (4-AP), a blocker of K+ currents, can potentiate synaptic transmission at both excitatory and inhibitory synapses. Furthermore, in CA1 hippocampal pyramidal cells (HPCs) studied "in vitro", a GABA-mediated long-lasting depolarization (LLD) occurred spontaneously and following orthodromic stimulation in the presence of 4-AP (Brain Res 400: 191-195, 1987). Typical epileptiform events were not observed in CA1 cells. We noted, however, that spontaneous potentials also occurred synchronously in CA3 and dentate areas. This led us to investigate the effects of 4-AP (50µM) on these regions .

Standard rat hippocampal slices were used and cells were impaled with K-Acetate filled microelectrodes using conventional intracellular recording techniques.

As in CA1 HPCs, 4-AP potentiated both the EPSPs and the IPSPs evoked in CA3 neurons by stimulating the mossy fibers. LLD also developed progressively between the early and late IPSPs and partially obscured them. Two to five late spikes (latency varying between 20 and 120ms) of variable amplitude (10-120mV) and usually clustered on the peak of the early IPSP also appeared. Increasing the intensity of the stimulation triggered a burst followed by the LLD and a prolonged hyperpolarization. Bursts sometimes followed by LLD also occurred spontaneously. Focal application of bicuculline (20µM)in the dendritic area could block the LLD but NMDA receptor antagonists (APV and CPP) were without effects on the bursts. In dentate granule cells, GABA mediated LLD also occurred spontaneously and following stimulation of the perforant path. However, bursts were never recorded in this area.

These results suggest that epileptiform bursts can be induced even in the presence of enhanced inhibition. Differences observed among subfields might be related to differences in the intrinsic membrane properties of their cells or in their synaptic circuitry.

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318.9 THE 5-HT_{1A} RECEPTOR AGONIST 8-OH DPAT ATTENUATES LOW CALCIUM-INDUCED BURSTING IN THE HIPPOCAMPAL SLICE. A. Obenaus[1], J.M. Klancnik[1,2], J.J. Miller[1]. Dept. of Physiology[1] and Psychology[1,2], Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

Rhythmic synchronous bursting discharges are observed in the CA1 region of hippocampal slices perfused with artificial CSF containing low concentrations of Ca^{2+} (<0.1mM). These bursts are characterized by 2-5 second depolarizing shifts of 4-10 mV in the extracellular DC potential upon which multiple unit activity may be superimposed. This low Ca^{2+} bursting can be followed for 3-6 hours. This phenomenon provides a simple model to study cellular hyperexcitability which occurs in the absence of synaptic transmission. The CA1 region receives significant serotonergic projections from the medial and dorsal raphe nuclei, and is rich in serotonergic binding sites, primarily in the apical dendritic region. Although pharmacological activation of the 5-HT_{1A} receptor subtype appears to decrease neuronal excitability of CA1 pyramidal cells *in vitro* in normal artificial CSF, as measured by evoked population responses, other serotonergic actions in this brain region remain largely unknown. The purpose of the present experiments was to examine the possible modulation of burst discharges in the CA1 region of the hippocampal slice preparation by activation of 5-HT_{1A} receptors. Hippocampal slices (400um thick) were prepared from 200-300 gm male Wistar rats, and maintained in an *in vitro* chamber. After normal responses had been established, the normal artificial CSF was replaced by one low in Ca^{2+}. Burst activity was typically seen after 60-90 minutes after media replacement. A 10 minute perfusion of the selective 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH DPAT), resulted in a progressive and dose-dependent (1-30uM) attenuation of burst activity. Both burst amplitude and burst frequency decreased at a dose-dependent rate. The onset of this effect was seen within 10 minutes of perfusion, with the bursts irreversibly decreasing until they disappeared (e.g. at 10uM 8-OH DPAT this occurred within 80-120 min. after perfusion). At lower doses (<10uM.) some slices first exhibited a transient increase in burst rate (but not in amplitude) which was not seen at higher doses. These results indicate a possible role for 5-HT, and particularly the 5-HT_{1A} receptor subtype, in the attenuation of burst activity induced by low Ca^{2+} media. The mechanism underlying this effect remains to be elucidated.(Supported by a Canadian MRC Program Grant to J.J.M.)

318.10 THE EFFECTS OF THE 5-HT_{1A} RECEPTOR AGONIST 8-OH DPAT ON EVOKED RESPONSES IN THE *IN VITRO* HIPPOCAMPUS. J.M. Klancnik[1,2], A. Obenaus[1], J.J. Miller[1]. Depts. of Physiology[1] and Psychology[2], University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

The functional role of serotonin within the hippocampus is largely unknown. Previous studies employing the *in vitro* hippocampal slice preparation have shown that putative 5-HT_{1A} receptor subtype agonists, including the highly selective 5-HT_{1A} ligand, 8-hydroxy-2-[di-*n*-propylamino]-tetralin (8-OH DPAT), decrease population spike amplitude in the CA1 region of the hippocampus. Recent *in vivo* work from our laboratory has found 8-OH DPAT to increase population spike amplitude in the dentate gyrus (DG), a region with greater serotonergic innervation and receptor density than the CA1. The present experiment employed an *in vitro* preparation to investigate the effects of 8-OH DPAT on population responses in the CA1 and the DG.

Hippocampal slices (400 um thick) were prepared from 200-300 gm male Wistar rats and maintained in an *in vitro* chamber perfused with artificial CSF. Population responses evoked by perforant path stimulation were recorded in the DG, and responses evoked by Schaffer collateral stimulation were recorded in the CA1. Stimuli were 0.1 msec. cathodal square wave pulses, at a current which evoked population spikes of approximately one-half maximum amplitude. After collection of baseline data, preparations were perfused for ten minutes with 8-OH DPAT in concentrations from 1.0 to 30.0 uM. Population responses were evoked and recorded at the rate of one per minute over a period of three to five hours.

8-OH DPAT produced a dose-dependent decrease in population spike amplitude in both the CA1 and DG, with a more pronounced effect in the CA1. The drug action exhibited a similar time course in both regions, with a maximum effect occurring at a latency of 10-30 min. after perfusion onset, and a subsequent return to baseline values after one to three hours.

Although these data indicate a similarity of 8-OH DPAT action between CA1 and DG in the hippocampal slice, they show opposite actions between the *in vivo* and *in vitro* preparations. These results suggest that the functional effects in the whole animal may be due to pharmacological action on 5-HT_{1A} receptors at extrahippocampal loci.
(Supported by a Canadian MRC Program Grant to J.J.M.)

318.11 **THE** EFFECT OF LIPID PEROXIDATION ON GABA UPTAKE AND RELEASE IN SYNAPTOSOMES FROM RATS WITH FERROUS-INDUCED SEIZURES. Z.H.Zhang, C.H.Zuo*, X.R.Wu*, Y.Hua* and H.Wang*
Div. of Neurology, Dept. of Pediatrics, First Teaching Hospital of Beijing Medical University, Beijing, People's Republic of China

The deleterious consequences of lipid peroxidation (LP) to membrane function is well established. Both uptake and release of neurotransmitters depend on the intact membrane structure and function. To determine the effect of LP on GABA uptake and release, 10 Wistar rats of 30 days old were devided into 2 groups: C (control, n=5) and F (ferrous-treated, n=5). We observed seizure behavior for 2 hours after intraventricular injection of 10uL of either normal saline (C group) or 300mM $FeSO_4$ in saline (F group). Then, rats were decapitated and synaptosomes were prepared from cortex and hippocampus. The synaptosomes were assayed for LP (thiobarbituric acid test), GABA uptake and release (Ca^{++}-dependent and high K^+-evoked release, Gordon-Weeks PR: Neurosci Letters 1984, 52:205). In F group, LP greatly increased but both uptake and release significantly decreased relative to controls:

	F(n=5)	C(n=5)	F/C
LP (n mole/min/mg protein)	11.10±0.85**	1.26±0.18	881%
GABA uptake (p mole/min/mg protein)	15.82±1.46**	20.87±1.59	76%
GABA release (fractional release rate from 4 to 6 minute after K^+-stimulation, %)	4.92±0.31**	12.31±0.58	40%

(** $p < 0.01$)

Our previous studies showed that intraventricular injection of ferrous caused seizures as well as LP in brain tissue. The present data demonstrated that GABA release was more severely damaged than GABA uptake by LP. This imbalance between release and uptake might reduce the inhibition of GABA, which could contribute to the neurochemical pathogenesis of ferrous-induced seizures.

318.12 INVLOVEMENT OF SUBSTANTIA NIGRA GABA A RECEPTORS IN SEIZURES OF ADULT RATS. E.F. Sperber, D. Zhao* and S.L. Moshé. Albert Einstein College of Medicine, Bronx, NY 10461

Pharmacological activation of the GABA system in the substantai nigra (SN) has been observed to suppress a variety of experimental seizures in adult rats. In order to identify whether the GABA A receptor type is involved in the modification of seizures, we compared the effects of nigral infusions of a GABA agonist, muscimol and a GABA antagonist, bicuculline.

Adult male rats were implanted with bilateral cannulae in the SN reticulata. Following a two day recovery period, rats received one dose of muscimol (12.5-200ng/0.25ul) or bicuculline (25-200ng/0.25ul) at two day intervals. Each drug had its own saline-treated control group. Thirty minutes after the muscimol infusions and 5 minutes after the bicuculline infusions, rats were exposed to flurothyl (FE) convulsions. The latency to the onset of a generalized clonic seizure was considered the seizure threshold.

A series of one-way ANOVAs were used to compare the FE seizure latencies following each drug dose with its respective saline group. Results indicate that rats infused with the higher doses of muscimol, 25, 50, 100 and 200ng/0.25ul had significantly longer seizure latencies than the control rats ($p < .04$). In contrast, rats infused with the higher doses of bicuculline, 100 and 200ng/0.25ul had significantly lower seizure thresolds than the saline-infused rats ($p < .01$). Since both muscimol and bicuculline have a greater affinity for the GABA A receptor, our data suggest that the nigral GABA A receptor is involved in the modulation of seizures in adult rats.
(Supported by NIH grant NS-20253 from the NINCDS and R-36986 from the United Cerebral Palsy Association.)

318.13 **BACLOFEN HAS A PRO-EPILEPTIFORM EFFECT IN THE DENTATE GYRUS OF RATS.** D.D. Mott* and A.C. Bragdon. Depts. of Pharmacology and Medicine (Neurology), Duke Univ. and VA Medical Centers, Durham, NC 27710.

Baclofen is a GABA analog with a number of pre- and post-synaptic inhibitory actions on neurons. Baclofen has anticonvulsant activity against some models of epilepsy in vivo and epileptiform activity in vitro. However, baclofen has been shown to be proconvulsive in other models, baclofen has not proven useful against human epilepsy, and baclofen overdose has been reported to cause seizures in nonepileptics. Here we report a proepileptic effect of baclofen in the dentate gyrus alone with evidence for its underlying mechanism.

Hippocampal slices from 100-200 gm male Sprague-Dawley rats were studied in ACSF containing 3.3 mM K^+, 1.8 mM Ca^{++}, and 1.3 mM Mg^{++}. Perforant path (PP) stimulation evoked an EPSP and a population spike (PS) recorded extracellularly in the granule cell layer of the outer blade of the dentate gyrus. For quantitative studies, the PP stimulus intensity was set to produce a PS about 80% of maximal in control ACSF. Recurrent inhibition was induced by antidromic activation of mossy fibers (MF) from CA3c five msec before PP stimulation. Feed-forward inhibition was induced by stimulation of dentate commissural-associational (C-A) fibers in the alveus of CA3 coincident with PP stimulation (Bragdon and Wilson, Soc. Neurosci. Abstr. 10: 549, 1984). For both recurrent and feed-forward inhibition, the degree of inhibition was quantified as the % reduction in the PP-evoked PS. All observations were made first in control ACSF, next after at least 15 min in 10 uM baclofen, and last at least 15 min after washout with control ACSF. All baclofen effects were reversible.

Baclofen reduced the PP-evoked PS mildly, but converted the usual single-PS evoked response to an epileptiform response with two or more population spikes suggesting disinhibition of granule cells. Baclofen reduced recurrent inhibition by about 2/3 -- from 72% to 23% at maximal MF stimulus intensity. Feed-forward inhibition was unaffected by baclofen in 3 of 4 slices, and only mildy reduced (from 60% to 40%) in the fourth slice.

These results show that baclofen has a pro-epileptiform effect on dentate granule cell responses. This appears to result from suppression of recurrent inhibition, rather than from enhanced PP-granule cell excitatory transmission. These results also suggest that baclofen suppresses recurrent inhibition but not C-A feed-forward inhibition. If both forms of inhibition share the same final common pathway of inhibitory interneurons, this raises the possibility that baclofen preferentially suppresses transmitter release from mossy fibers, but not from dentate commissural-associational fibers.

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318.14 BACLOFEN INDUCES SPONTANEOUS, RHYTHMIC ACTIVITY IN HIPPOCAMPAL SLICE. D. V. Lewis and D. Mott* Depts. of Peds (Neurol.) and Pharmacology, Duke Univ., Durham, NC 27710.

The GABA-b agonist, baclofen, is known to be a powerful antagonist of spontaneous interictal burst activity in the hippocampal slice. However, we have recently observed an unexpected effect of baclofen. At low concentrations, baclofen induces spontaneous, rhythmic, low amplitude sharp waves in the hippocampal slice.

Hippocampal slices (625 uM) were prepared from male Sprague-Dawley rats, 23 to 34 days of age (55 to 130 gms). They were maintained in a submersion chamber with a stimulating electrode in the s. radiatum of the CA2-3 junction and extracellular recording electrodes in the s. radiatum and s. pyramidale of the CA3b region. Artificial cerebrospinal fluid (ACSF) composition was, in mM, NaCl 120, KCl 3.3, CaCl2 1.8, MgSO4 1.2, NaHCO3 25, NaH2PO4 1.23 and dextrose 10 at pH 7.4. Baclofen (+/-) was dissolved in the ACSF in concentrations of 0.1, 0.5, 1.5, 5.0, 20.0 and 100 uM.

At baclofen concentrations of 0.5 and 1.5 uM, rhythmic, spontaneous waves (RSW's) were observed in 8 out of 8 slices, each from a different animal. In several animals, RSW's appeared in 0.1 uM baclofen also, but were difficult to discern from the background noise. The voltage of the RSW's was maximum in the s. radiatum of CA3 and increased with increasing baclofen concentration to reach 0.3 to 0.6 mV in 1.5 uM. In the s. pyramidale, the RSW's were much less obvious and often were lost in the background noise. At 5 uM baclofen and above, the RSW's were suppressed. Washing off the baclofen entirly eliminated the RSW's. Usually, the RSW's were monophasic, negative deflections in s. radiatum from 100 to 200 msec in duration appearing as a sharp wave at slow recording speeds. They recurred rhythmically about every 2 sec in 1.5 uM baclofen, and tended to be more frequent in lower concentrations when they were of lower amplitude. Sampling the extracellular feild in other regions of the slice revealed synchronous RSW's in CA1 and CA2, but not in subiculum, entorhinal cortex or dentate. Preliminary experiments showed that after cutting the Schaffer collaterals in CA2, the RSW's were no longer seen in CA1 but continued in CA3. In one experiment, (-) baclofen was used and also elicited RSW's.

We think these RSW's are analogous to the rhythmic activity reported by Schneiderman (Brain Res.,398:231, 1986) in low levels of penicillin and might relate to a reduction of recurrent inhibition by low levels of baclofen (Ault and Nadler, Br. J. Pharmac.,78:701, 1983). Supported by NINCDS Grant NS22170.

318.15 POTENT CONVULSANT ACTION OF THE ADENOSINE RECEPTOR ANTAGONIST, XANTHINE AMINE CONGENER (XAC). Philip F. Morgan, Jurgen Deckert, Kenneth A. Jacobson*, John W. Daly and Paul J. Marangos, NIH, Bethesda, Maryland 20892, USA

A [^{3}H]Xanthine amine congener of 1,3-dipropyl-8-phenylxanthine ([^{3}H]-XAC) has recently been characterized as a potent adenosine receptor antagonist (Jacobson et al., P.N.A.S. 83:4089-4093, 1986). In the present study, convulsant properties of XAC have been assessed and compared to those of caffeine.

Naive male NIH stock Swiss Albino Mice (25-30g) were infused with convulsants through a lateral tail vein using a 25 gauge butterfly needle fed from a syringe infusion pump as previously described (Morgan and Stone, Br. J. Pharmacol. 77:525-529, 1982). Using this paradigm, convulsion thresholds (i.e. the amount of convulsants required to elicit convulsions) of 39.8+2.0 mg/kg (n=10) and 109.8+2.3 mg/kg (n=10) were calculated for XAC and caffeine respectively. This potent convulsant activity of XAC is to some extent remarkable since a recent study indicates that XAC penetrates poorly into the C.N.S. (Fredholm et al., J. Cardiovasc. Pharmacol. 9:396-400, 1987).

Pretreatment of animals with adenosine receptor agonists (1 mg/kg, i.p., 20 minutes prior to infusion, of either 2-chloroadenosine, cyclohexyladenosine or 5'-N-ethylcarboxamide adenosine) significantly decreased the seizure threshold of both XAC and caffeine (p's < 0.05). Pretreatment of animals with the adenosine uptake blockers, nitrobenzylthioinosine or dipyridamole (0.25 mg/kg, i.p., 20 minutes prior to infusion) did not significantly affect the seizure threshold to either XAC or caffeine. The benzodiazepine agonist diazepam (5 mg/kg, i.p., 20 minutes prior to infusion) significantly increased the seizure threshold to both XAC (p < 0.05) and caffeine (p < 0.01), whereas the benzodiazepine antagonist RO 15-1788 (10 mg/kg, i.p., 20 minutes prior to infusion) significantly increased the seizure threshold to caffeine (p < 0.01), but not XAC (p > 0.05). All vehicles employed were without significant effect on the seizure threshold of either XAC or caffeine (p's > 0.05). The results suggest that actions at benzodiazepine receptors may be a tenable hypothesis to explain the convulsant actions of caffeine, but not those of XAC.

318.16 COMPARISON OF PHYSOSTIGMINE PRETREATMENT, WITH OR WITHOUT ATROPINE SULFATE, AS PRETREATMENT FOR SOMAN-INDUCED SEIZURES IN GUINEA PIGS. N.K. Marshall, D.J. Hinman and J.F. Glenn. Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, APG, MD 21010-5425

Pretreatment of animals with physostigmine, a reversible inhibitor of acetylcholinesterase, reduces lethality resulting from exposure to soman (pinacolyl methylphosphonofluoridate, GD), an irreversible cholinesterase inhibitor. While it is important to increase survival following GD exposure, it is also desirable to ameliorate other signs of GD toxicity, e.g., EEG seizures and motor convulsions. In this study we tested the effects of pretreatment with physostigmine and atropine sulfate alone, and combined, in freely-moving guinea pigs.

The EEG was recorded from indwelling cortical electrodes (stainless steel screws). Following a 30 min. baseline recording, the subjects were given one of the following pretreatment(s): (I) saline; (II) saline and physostigmine (0.26 mg/kg); (III) saline and atropine sulfate (4mg/kg); or, (IV) atropine sulfate (4mg/kg) plus physostigmine (0.26 mg/kg). The dose of physostigmine produced 70-80% inhibition of whole blood cholinesterase. The animals were dosed with $2LD_{50}$ GD (56ug/kg, SC) thirty minutes following pretreatment. [Group II also received atropine methylnitrate (4mg/kg, IM) and pralidoxime (2PAM; 25mg/kg, IM) therapy 30 seconds after GD. Atropine methylnitrate was given to block peripheral muscarinic sites and 2PAM to reactivate GD-inhibited AChE peripherally.] The EEG and motor behavior of all animals were monitored for 2 hours following GD administration. In some cases, the animals were re-tested at 24 hours and one week post-GD. The results are summarized in the table below:

Group	(n)	SEIZURE Incidence (%)	SEIZURE $\bar{X}$ Onset (min)	LETHALITY Incidence (%)	LETHALITY $\bar{X}$ Onset (min)	24 Hour Survival (%)
I	(8)	75#	7.0	100	13.5	0
II	(9)	100	5.8	0	-----	N/A
III	(5)	100	8.0	80	25.0	0
IV	(5)	0	----	0	-----	100

Group	(n)	CONVULSIONS Stage 1 (min/%)	Stage 2 (min/%)	Stage 3 (min/%)	MOTOR RECOVERY (min)	(%)
I	(8)	6.4; 100	7.0; 100	8.6; 100	-----	0
II	(9)	3.8; 100	4.5; 100	8.8; 44	N/A*	100
III	(5)	6.8; 100	8.4; 100	11.8; 100	-----	0
IV	(5)	6.2; 100	8.8; 100	----- 0	50.4	100

#2 animals died without seizing; N/A: not available; *observed but not quantified.

The results indicate that pretreatment with physostigmine in conjunction with atropine sulfate was the most effective pretreatment for soman poisoning.

318.17 EFFECTS OF PRENATAL PHENYTOIN AND VALPROIC ACID TREATMENT ON REPRODUCTIVE OUTCOME, DEVELOPMENT AND BEHAVIOR IN RATS. William J. Pizzi and Robert M. Jersey*. Dept. of Psych., Northeastern Illinois University, Chicago, IL 60625.

Recent experimental work in animals has confirmed clinical observations in children of a Fetal Anticonvulsant Therapy Syndrome (FACTS). There are three main symptom clusters associated with FACTS: 1) Congenital Malformations, 2) Growth Retardation, and 3) Behavioral Abnormalities. This report presents the results of two studies examining the toxic effects of two commonly used anticonvulsants, phenytoin (PHT) and valproic acid (VPA).

Pregnant rats were dosed orally from gestational days (GD) 9-18 and allowed to proceed to term. Treatment groups consisted of normal controls (NC), vehicle controls (VC), 100 and 200 mg/kg PHT, 200 and 300 mg/kg VPA, and a VPA group dosed via an ALZET osmotic minipump which delivered approximately 190 mg/kg on GD 9.

Due to space limitations only selected results from Experiments 1 & 2 are presented here. PHT dams showed a significantly reduced weight gain throughout pregnancy with the PHT-200 group actually losing weight over the course of treatment. Both PHT-100 and -200 groups had an increased mortality in offspring followed to PND 30 (41 & 61%, respectively). In light of this high mortality it is important to note that PHT blood levels taken approximately 90 min. after the the last treatment on GD 18 were in the human therapeutic range (11.5 and 26.2mcg/ml, respectively). Birth weights of PHT pups were significantly reduced with a dose-response relationship present. These reduced weights continued to be present at PND 30.

The VPA results are somewhat more complex in that the VPA-pump and VPA-200 groups failed to show differences in birth weights but in several of the PND 30 comparisons they were significantly heavier than controls. The VPA-300 group showed reduced body weights at PND 30.

Convulsive threshold testing failed to show protection from a 50mg/kg pentylenetetrazol challenge dose in any VPA group as recently reported by Sobrian and Nandedkar (1986). The PHT-100 male animals showed an increased duration of maximal seizure activity to the PTZ challenge.

318.18 CNS TRANSPLANTATION IN ANIMAL MODELS OF EPILEPTIFORM ACTIVITY. P.A.Schwartzkroin, O.Lindvall*, P.Brundin* and A.Björklund. Dept. Histology, Lund University, S-22362 Lund, Sweden.

Transplantation of CNS neurons, as an approach to modifying and understanding central nervous system pathologies, has received wide attention. Studies have been carried out with animal models of, for example, Parkinson's, Huntington's and Alzheimer's Diseases -- neuropathologies in which specific cell types or CNS regions are predominantly affected. Relatively little work has been reported in which grafting techniques have been applied to models of epilepsy. This absence perhaps reflects a confusion about which cells are abnormal or lost in epileptic brain, as well as about the multiplicity of factors which can control neural excitability.

We have initiated grafting studies with two models of epileptogenesis: 1) spontaneous interictal spiking produced by intraventricular (icv) injections of kainic acid (KA); and 2) seizure activity produced by kindling. We reasoned that insertion of a population of neuromodulatory neurons might affect the excitability of these systems. Thus, we grafted CNS regions rich in GABAergic and noradrenergic neurons into hippocampus made epileptogenic by KA injections, and into substantia nigra (SN) and amygdala of animals receiving amygdala kindling stimulations. The source of GABA-rich transplants was fetal striatum and ventral mesencephalon (including SN-pars reticulata); NA-rich grafts were derived from fetal locus coeruleus.

Grafting procedures followed the general protocols for cell suspensions. For the KA animals, KA was first injected bilaterally followed at 1 week by cell suspension injections into the CA3 region of dorsal hippocampus and implantation of recording electrodes. Occurrence of interictal spiking was monitored for at least 2 months. The kindling animals receiving amygdala grafts were first transplanted; two to three months later they were implanted with amygdala electrodes and kindling commenced. In these animals, threshold for afterdischarge and time course to stage 5 seizures were assessed. In the kindling group to receive grafts into SN, each rat was first kindled to stage 5 criterion, and cell suspensions were then introduced into nigra through implanted cannulae. Threshold for triggering stage 5 seizures was monitored for two to three months.

Anatomically, we found that grafted neurons introduced into these relatively intact systems survive well. Histofluorescence techniques showed noradrenergic neurons of graft origin in hippocampus of transplanted KA animals; a fiber plexus from these cells was clear in much of dorsal hippocampus. GAD immunocytochemistry demonstrated cells, fibers and terminals of apparent graft origin in both KA-affected hippocampi and in SN of kindled rats. The functional importance of these grafts is not yet clear.

HYPOTHALAMUS

319.1 HYPOTHALAMIC SITES WHERE ELECTRICAL STIMULATION INCREASES METABOLIC RATE IN THE RAT. Peter A. Pawson, E. Preston*, N.Haas* and D.O. Foster*, Division of Biological Sciences, National Research Council Canada, Ottawa, Canada, K1A OR6.

The hypothalamus plays a key role in food intake regulation and thus in the regulation of energy balance. We are interested in the possibility that its role in energy balance also includes modulation of energy expenditure. Male rats (300g) were stereotaxically implanted with monopolar stimulating electrodes. After 1-2 weeks recovery, they were placed in a temperature-controlled chamber (28°C) for measurement of metabolic rate (MR as $\dot{V}O_2$) before and after stimulating various hypothalamic sites for 60 s. Two types of stimulation were used: (a) a set frequency (50 Hz) with current varied stepwise from 100 to 500 microamperes (μA); this method examines the effect of expanding the field of stimulation around the tip of the electrode; (b) a constant current (250 μA) with the frequency of stimulation varied from 10 to 300 Hz to generate a response curve for a focal part of the hypothalamus, providing a finer and more quantitative comparison of different hypothalamic regions. Results from the 2 techniques have, however, yielded similar conclusions. 47 sites have been studied, covering these hypothalamic regions: anterior hypothalamus (AH), paraventricular nucleus (PVN), lateral hypothalamic area (LH), ventral medial hypothalamic nucleus (VMH), dorsomedial hypothalamic nucleus (DM), posterior hypothalamus (PH), and an area between the LH and VMH (mid VMH-LH). The responses in fully conscious rats were as follows: A) 0-40% increases in MR: sites in the PH, LH, PVN and the mid VMH-LH; B) 41-90% increases: DM, the middle and posterior VMH; C) >90% increases: anterior VMH and the AH. In animals that were mildly sedated with Rompun ®, which eliminated any apparent contribution of arousal and body movement to increases in MR, only the stimulation of two contiguous sites, the anterior VMH and the posterior AH, effected appreciable increases in MR (40-60%). These areas thus appear to be the best candidates for further study of hypothalamic regulation of energy expenditure.

319.2 LOCAL ANESTHETICS PROCAINE AND LIDOCAINE STIMULATE CORTICOTROPIN RELEASING HORMONE SECRETION IN VITRO: CLINICAL IMPLICATIONS. A.E. Calogero*, M.A. Kling†, W.T. Gallucci*†, C.Saoutis*, R.Post†, G.P.Chrousos, P.W.Gold*†. DEB, NICHD, NIH and †BPB, NIMH, Bethesda

Local anesthetics like procaine and lidocaine can kindle limbic seizures in animals and produce psychosensory and mood changes, and stimulate the hypothalamic-pituitary-adrenal (HPA) axis in man. Corticotropin releasing hormone (CRH), given i.c.v. to animals, reproduces some of these effects. It causes limbic seizures, behavioral changes characteristic of the stress response, and activation of the HPA axis. CRH hypersecretion and altered limbic responsiveness have been postulated in primary affective disorder. In this regard carbamazepine (CBZ), an anticonvulsant which is particularly effective against limbic seizures, has been employed successfully in the treatment of affective disorders.

To test the hypothesis that CRH participates in some of the central nervous system (CNS) effects of local anesthetics, we examined the ability of procaine and lidocaine to stimulate hypothalamic CRH secretion in vitro and the ability of CBZ to inhibit it.

Single explanted rat hypothalami were incubated in plain medium for 20 min in 3 sequential wells to evaluate basal immunoreactive rat CRH (IR-rCRH) secretion. This was 36±2 pg/well/20 min (n=32). In 2 subsequent 20 min incubation periods, the hypothalami were exposed to graded concentrations of procaine or lidocaine or for comparison to a known CRH secretagogue (serotonin 10^{-9} M). At the end of the experiment each hypothalamus was exposed to 60 mM KCl to test tissue viability. Hypothalami that failed to respond to KCl by a 90% increase of IR-rCRH (5 interassay CV) above baseline were excluded from the analysis. IR-rCRH responses to procaine and lidocaine, expressed as percent IR-rCRH increase above above secretion (after logarithmic transformation) are shown below:

	0	10^{-10} M	10^{-8} M	10^{-6} M	10^{-4} M	Serotonin
Procaine	3.4±1.3 (25)	33±13* (11)	117±31* (7)	168±29* (9)	112±35* (8)	200±52* (17)
Lidocaine	3.4±1.3 (25)	71±14* (7)	95±41* (10)	155±39* (10)	152±31* (10)	200±52* (17)

*p<0.001 ANOVA, followed by Duncan Multiple Range Test

Procaine and lidocaine stimulated IR-rCRH secretion in a concentration-dependent fashion. These responses were inhibited by CBZ (procaine or lidocaine 10^{-6} M alone or with 10^{-6} M CBZ were, respectively, 168±29% vs 38±10%, p<0.001, and 155±39% vs 12±7%, p<0.001).

These findings suggest that CRH may participate in the CNS effects of local anesthetics and that carbamazepine's efficacy in affective illness could be in part related to effects on CRH secretion.

319.3 VASOPRESSIN INNERVATION OF SEXUALLY DIMORPHIC STRUCTURES IN RAT, GERBIL AND HUMAN BRAINS. Geert J. De Vries and Pauline Yahr. Department of Psychobiology, University of California, Irvine CA 92717.

The medial preoptic and anterior hypothalamic areas (MPOA-AH) regulate reproductive functions, such as gonadotropin release and male sexual behavior. Structural sex differences may underlie the sexually dimorphic nature of these functions. Sex differences exist in cellgroup size, synaptic organization, dendritic branching, steroid uptake and neurotransmitter distribution in the MPOA-AH of birds, rodents, carnivores and primates. It is not clear to what extent these sex differences are homologous. One sex difference for which this question may be answered concerns a cellgroup known in gerbils as the sexually dimorphic area pars compacta (SDApc) and in rats as the central part of the medial preoptic nucleus (MPNc). Both lie within larger sexually dimorphic cellgroups, i.e., the SDA and MPN, respectively[1,2], midway between the optic chiasm and the anterior commissure. Both contrast with the surround in that they receive denser inputs from the bed nucleus stria terminalis and lack acetylcholinesterase activity. Moreover, both the SDApc and MPNc are larger in males than in females, although this difference is more extreme in gerbils. The SDApc is often absent in females.

To study whether neurotransmitter input is similar in the SDApc and MPNc, we stained gerbil and rat brains immunocytochemically for vasopressin. We also stained sections of human hypothalamus containing the sexually dimorphic nucleus, which is larger in males than in females[3]. In gerbils, vasopressin fibers densely innervated the medial part of the SDA, but a particularly dense innervation was present in the SDApc. The lateral part of the SDA was lightly innervated by vasopressin fibers. In rats, vasopressin fibers were present throughout the MPN and, as in gerbils, were particularly dense in the MPNc. In the human, vasopressin fibers were more concentrated in the sexually dimorphic nucleus than in its surround. This innervation differed from that seen in the SDApc or MPNc. It was less uniformly distributed over the sexually dimorphic nucleus, and, in some places, was just as dense as in the surround. These data further support a homology between the SDApc and MPNc. They also suggest homology among all three cell groups, the human sexually dimorphic nucleus, the SDApc and the MPNc.

[1]Commins and Yahr, J. Comp. Neurol. 224: 132 (1984).
[2]Simerly, Swanson, and Gorski, J. Comp. Neurol. 225: 151 (1984).
[3]Swaab and Fliers, Science 228: 1112 (1985).

319.4 PROJECTIONS TO THE PROGESTERONE-SENSITIVE SITE OF THE VTA IN HAMSTERS: AN HRP STUDY. C.A. Lisciotto* and J.F. DeBold. Dept. of Psychology, Tufts Univ., Medford, MA 02155.

The ventral tegmental area (VTA), along with the ventromedial nucleus of the hypothalamus (VMH), is an important site for progesterone's effects on sexual receptivity in female hamsters. We have demonstrated with a number of different techniques that both of these brain sites are essential for this response. The critical importance of both the VTA and VMH for receptivity in hamsters is different from the control of this behavior in rats. In rats the VTA seems to be much less important than the VMH for receptivity. Studies on the neural circuitry mediating hormonal effects on receptivity in rats have focused on connections between the VMH and the central gray. The minimal neural circuitry necessary for the induction of receptivity in hamsters has not been worked out but must include the VTA. This study investigates how the progesterone responsive site within the VTA may be connected with other areas of the brain which have already been established as important for sexual receptivity.

HRP (0.02µl, 50% Sigma IV w/v in dH_2O) was stereotaxically injected into the VTA. The stereotaxic coordinates used were the same as those which were effective at inducing receptivity with progesterone implants. Following a 48 hr survival period the hamsters were sacrificed and perfused with paraformaldehyde-glutaraldehyde fixative. Coronal sections of 40 um were cut and processed using tetramethylbenzidine. The number and location of HRP-filled cells, rostral to the injection site were assessed.

Labeled cells were seen in several sites in the diencephalon and mesencephalon. One of the more interesting sites with labelled cells was the ipsilateral VMH indicating that the two critical progesterone sensitive sites in hamsters are directly interconnected. HRP filled cells were also seen in other sites which behavioral studies have implicated in the mediation of receptivity. For example, dense labeling was seen bilaterally in the medial habenula and ipsilateral lateral habenula. Much of the lateral hypothalamus and lateral preoptic area also contained HRP labeled cells. In addition, occasional but consistent labeling was found in the ipsilateral diagonal band, dorsal hypothalamus, zona incerta, peripeduncular nucleus and central gray. Many of the labelled areas have previously been shown to also receive projections from the VMH.

These observations suggest possible routes by which the critical progesterone-sensitive sites for sexual receptivity, the VTA and VMH, may communicate. This is an initial step in establishing the neural circuitry for the hormonal control of receptivity in this species.

319.5 DIFFERENTIAL DISTRIBUTION OF ESTROGEN AND ANDROGEN CONCENTRATING CELLS IN THE RAT MEDIAL PREOPTIC AREA. P.E. Micevych and T.R. Akesson. Dept. of Anatomy and Lab. of Neuroendocrinology, Brain Res. Inst., UCLA School of Medicine, Los Angeles, CA 90024.

The role of the rat medial preoptic area (MPOA) in mediating steroid-sensitive reproductive events is highly sexually differentiated. The exact neural substrates mediating these events have not been elucidated, however the MPOA contains sexually dimorphic structures and the entire region is enriched in steroid concentrating cells. Although testosterone (T) is metabolized to both dihydrotestosterone (DHT) and estradiol (E_2), only androgens masculinize the anatomy of this region during a critical postnatal period. This organizational influence that androgen exerts during development may be reflected in preferential accumulation of androgens in sexually dimorphic regions of the adult. To test this hypothesis, we studied the distribution of T, E_2 and DHT in the preoptic area of male rats. Adult male rats (n = 7) were castrated, two weeks later infused with ^{3}H-T, ^{3}H-E_2 or ^{3}H-DHT and processed for thaw-mount steroid autoradiography. After exposure periods of 4 months (^{3}H-E_2), 14 months (^{3}H-T) and 20 months (^{3}H-DHT), the distribution of steroid concentrating cells was defined as containing 3X the background concentration of reduced silver grains.

At the rostral extent of the MPOA, the anteroventral periventricular nucleus (AVPv) contained approximately four times as many ^{3}H-E_2 as ^{3}H-DHT labeled cells. Similarly, the preoptic periventricular nucleus (PvPO) was observed to be enriched in ^{3}H-E_2 labeled cells with a relative paucity of both ^{3}H-T or ^{3}H-DHT labeled cells. Within the medial preoptic nucleus (MPN) itself, distributions of steroid concentrating cells appeared to correspond to cytoarchitectonic divisions which consist of central (MPNc), anteroventral (MPNav; Bloch and Gorski, Neurosci. Abstr. 13, '87), medial (MPNm) and lateral (MPNl) parts. The vast majority (84%) of cells labeled with ^{3}H-T, ^{3}H-DHT or ^{3}H-E_2 were localized in the MPNm with comparatively few cells present in the MPNl. Within the MPNm, ^{3}H-E_2 labeled cells were uniformly distributed and were not preferentially localized in the MPNc or MPNav in contrast with the distributions of ^{3}H-T and ^{3}H-DHT. Although there were androgen-labeled cells scattered throughout the MPNm, the majority of these cells were localized in the MPNc and MPNav. These two highly sexually dimorphic subnuclei contained approximately 73% of the ^{3}H-T and ^{3}H-DHT labeled cells in the MPN. The present results support the hypothesis that the AVPv and PvPO are preferentially activated by estrogen. Significantly, the MPNc, which is larger in males compared to females, and the MPNav, which is present only in males, appear to be preferential androgen targets. These findings are consistent with the possibility that estrogens and androgens have specific roles in the activation of sexually differentiated processes in the adult male rodent. Supported by NS 21220.

319.6 ESTRADIOL CONCENTRATION BY HYPOTHALAMIC AND LIMBIC NEURONS WHICH PROJECT TO THE MEDIAL PREOPTIC NUCLEUS. T.R. Akesson,[1] R.B. Simerly[2] and P.E. Micevych.[1] [1]Dept. of Anatomy and the Brain Res. Inst., UCLA School of Medicine, Los Angeles, CA 90024 and [2]Howard Hughes Medical Inst. and Salk Inst., La Jolla, CA 92138.

While connectivities of the steroid hormone-accumulating regions of the rat hypothalamus and limbic system have been extensively studied, reports identifying projections of steroid-concentrating cells within these regions are scarce. The medial preoptic nucleus (MPN), itself a major target of gonadal steroids, receives its strongest inputs from other steroid-accumulating regions including the encapsulated (medial) bed nucleus of the stria terminalis (BSTenc), medial nucleus of the amygdala (MeA), ventrolateral part of the hypothalamic ventromedial nucleus (VMHvl), arcuate nucleus (ARC), and amygdalohippocampal zone (AHZ), suggesting pathways which may be involved in the integration of steroid-activated events. In the male hypothalamus, a substantial portion of testosterone is aromatized and subsequently retained as estradiol (E). To determine the distribution and numbers of estrogen-concentrating neurons which are afferent to the MPN, we have combined the techniques of steroid autoradiography and retrograde tract-tracing (see Arnold, Brain Res., 194:210, '80).

Adult male Sprague-Dawley rats received iontophoretic injections of fluorogold (FG) centered in the medial part of the MPN. One week later they were castrated, and one week following castration, 150 µCi hexalabeled estradiol (New England Nuclear)/100 g body weight was infused i.v. over a 50 min. period. Rats were perfused transcardially with 4% paraformaldehyde and brains were processed using standard autoradiographic techniques. Every 4th section capturing the anterior-posterior extent of each nucleus in 3 animals was analyzed by counting all FG, E and double-labeled cells contained in a 220 µm² area/nucleus/section. A 3X background criterion was used to distinguish steroid concentration.

Nucleus	No. of sections	No. of E cells sampled	% labeled by FG ($\bar{X}$ ± SEM)
BSTenc	34	724	31.7 ± 3.7
MeA	46	621	31.6 ± 5.1
VMHvl	30	563	40.9 ± 10.8
ARC	57	722	13.3 ± 1.4
AHZ	30	609	52.8 ± 3.8

Thus, the medial MPN, in addition to having a high capacity to concentrate estrogen, is also a locus of converging projections from estrogen-binding cells distributed in widely divergent regions of the hypothalamus and limbic system. These results provide morphological support for the possibility of multiple sites of estrogenic regulation of a circuitry which mediates autonomic and reproductive functions ascribed to the MPN. (Supported by NS21220 to P.E.M.)

319.7 ESTROGEN/PROGESTERONE TREATMENT IN ADULTHOOD AFFECTS THE SIZE OF MEDIAL PREOPTIC (MPOA) STRUCTURES IN THE MALE RAT. G.J. Bloch* and R.A. Gorski (SPON: B.M. Wenzel). Dept. of Anatomy and Lab. of Neuroendocrinology, Brain Res. Inst., UCLA Sch. of Med. L.A., CA 90024.

The volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) is greater in male than in female rats. Previous results suggested that males gonadectomized (Gx) for 2 weeks as adults and sacrificed after a single series of estradiol benzoate (EB) and progesterone (P) injections, had reduced SDN-POA volumes compared to those of rats Gx for 4 weeks, but not compared to rats Gx for 2 weeks (Gorski et al., Brain Res., 148, 333, 1978). Because we initially observed a significantly smaller SDN-POA in males Gx as adults, then treated with multiple injections of EB and P to assess lordosis behavior and LH release (positive feedback), we designed a study in which males were given the same regimen of multiple injections. 26 adult male Long-Evans rats were divided into 4 groups: intact,oil (n=6); Gx,oil (7); intact EB+P (6); and Gx,E+P (2 µg/day x 3) +P (500 µg 24 hours later) was started 35 days after Gx and was repeated 10 and 20 days later. After an additional 30 days, EB (30 µg) +P (2mg 73 hours later) was injected, and all animals sacrificed 3.5 to 4.5 hours after P. Gx EB+P males had significantly smaller SDN-POA volumes ($mm^3 x 10^{-3}$) than the other, normal sized groups (16.2±1.5 vs. 23.1±2.4 to 24.7±2.4). In order to characterize further the change in the MPOA, cytoarchitectonic analysis (Simerly et al., J. Comp. Neurol., 225, 151, 1984) was undertaken and revealed that Gx EB+P males had significantly smaller volumes of the medial division of the medial preoptic nucleus (MPNm): 148.7±6.1 vs. 177.8±6.7 to 197.4±6.0; and significantly larger volumes of the anteroventral preoptic nucleus (AVPv): 23.2± 1.8 vs. 16.2±0.7 to 16.6±1.2. The EB+P injections had no effect on the size of the central division of the medial preoptic nucleus (MPNc) or the suprachiasmatic nucleus. In addition, we drew a distinct, cell-dense region within the anteroventral MPNm, just posterior to the AVPv, which we have named the MPNav. This small group of cells was also significantly smaller in the Gx EB+P males (1.7±0.4 vs. 2.7±0.2 to 3.0±0.3) and was found to be virtually absent in females. For comparison, a group of 6 adult, intact Long-Evans females had the following volumes: SDN-POA, 11.3±1.3; MPNm, 90.2±3.5; MPNav, 0.2±0.2; and AVPv, 27.5±2.3. We conclude that treatment of adult Gx male rats with EB+P can cause significant changes in the size of MPOA structures. These changes are all in the female direction: smaller SDN-POA, MPNm and MPNav volumes although not as small as those of females, and a larger AVPv volume which is comparable to that of females. Because intact EB+P males were not different from intact or Gx oil groups, testicular hormones appear to "protect" these structures from the effects of EB+P. (Supported by HD-01182 and HD-7228.)

319.8 DISTRIBUTION OF NEUROPEPTIDES IN THE HYPOTHALAMUS OF GROUND SQUIRRELS. S.Reuss*, J.C. Speh*, E.C. Hurlbut*, R.Y. Moore (Spon: J.D. White). Depts. of Neurology and Neurobiology and Behavior, SUNY, Stony Brook, NY 11794 and Dept. of Biological Sciences, Mesa College, Grand Junction, CO 81501.

We report the immunohistochemical identification of several neuropeptides in the neurosecretory hypothalamic nuclei in the brain of two species of ground squirrels, Spermophilus tridecemlineatus and Sp. richardsonii. Adult animals, originally wild caught, were housed under LD for several months. At time of sacrifice, animals had been in induced hibernation for two months. Thirteen ground squirrels of both sexes (b.wt. 300-500 g) were perfused with periodate-lysine-paraformaldehyde fixative. Brain regions between the optic chiasm and the median eminence were sectioned at 40 um in the coronal plane and either stained with cresyl violet or incubated for immunohistochemical localization of oxytocin (OXY), vasopressin (VP), vasoactive intestinal polypeptide (VIP), corticotropin releasing factor (CRF) or neuropeptide Y (NPY). Immunoreactivity was visualized using the avidin-biotin-peroxidase method.

The light microscopical evaluation revealed no differences in the organization of immunoreactive cells and fibers between the species and, in addition, no sex-specific differences were noted.

The supraoptic nuclei (SON) contain a dense distribution of cells and fibers displaying VP-like immunoreactivity (VP-LI) as well as OXY-LI. In addition, NPY-LI fibers with varicosities and terminals are seen in the SON region. The nucleus supraopticus diffusus, the medially situated continuation of the bilateral SON, exhibit VP-LI and OXY-LI cells and fibers.

The paraventricular nuclei (PVN) exhibit heavily stained cells and fibers displaying VP- and OXY-LI. In addition, weak CRF-LI in cells is observed.

In the suprachiasmatic nuclei (SCN), a few cells (mainly in the ventral third of the nuclei) exhibit VIP-LI. Fibers showing VIP-LI and NPY-LI are found throughout the SCN region, while VP-LI fibers are present mainly in the dorsomedial part, extending caudally into the dorsal projection of the nucleus. VP-LI cells were not detected.

The lateral hypothalamic area (LHA) displays VP-LI and OXY-LI cells as well as fibers reaching the PVN and the SON. NPY-LI cells were seen only in the arcuate nucleus. Furthermore, fibers in the median eminence exhibit strong VP-, CRF- and OXY-LI.

This study was supported by NIH NS16304 to RYM.

319.9 THE DISTRIBUTION PATTERN OF NADPH DIAPHORASE REACTIVE NEURONS IN THE HYPOTHALAMUS: A STUDY IN CATS AND RHESUS MONKEYS. Haitao Hu*, Jayashree K. Rao*, and A. Jayaraman. Depts of Neurology and Pediatrics, Louisiana State University School of Medicine, New Orleans, LA 70112.

The enzyme nicotinamide adenine dinucleotide phosphate diaphorase (NADPH diaphorase) is found in specific subpopulation of cells of the central nervous system. In many areas of the CNS, these neurons also contain neuropeptide Y and somatostatin. Cells with NADPH disphorase are specifically spared in Huntington's disease and are resistant to destruction by several neurotoxins. A study was initiated to map in detail, the pattern of distribution of cells with NADPH diphorase in the CNS of cats and monkeys. This report discusses the distribution pattern of these neurons in the hypothalami of cats and monkeys. Five adult cats and four rhesus monkeys were sacrificed with a solution of 4% paraformaldehyde in phosphate buffer. The brains were cut into 40u thick sections and these sections were processed for NADPH diaphorase histochemistry (Sandell et al., '86). The results show that cells with NADPH diaphorase activity were most prominent in the anterior, middle and caudal levels of the lateral hypothalamus, dorsolateral hypothalamus in an area adjacent to zona incerta, the paraventricular nucleus and in the rostral areas of the anterior hypothalamus. Caudally NADPH diaphorase reactive cells were numerous in the posterior hypothalamus, supramamillary decussation and tuberomamillary nucleus. The dorsomedial and ventromedial nuclei and the anterior supraoptic nucleus also showed moderate number of cells with NADPH diaphorase activity. Only scattered cells with NADPH diaphorase activity were noted in the medial and lateral preoptic region. The infundibular, the suprachiasmatic and the mamillary nuclei were free of NADPH diaphorase reactive neurons. The pattern of distribution of these cells were similar in cats and monkeys. Further details of the colocalization pattern and the mechanisms underlying the survival of cells with NADPH diaphorase activity may contribute to our understanding of the pathogenesis of progressive neurologic deficits and/or the potential contribution to the clinical syndrome by the surviving neurons which contain NADPH diaphorase activity. Supported by the Scottish Rite Schizophrenia Research Program.

319.10 THE MORPHOLOGY OF PARAVENTRICULAR NEURONS CHARACTERIZED BY INTRACELLULAR FILLING OF NEURONS IN FIXED TISSUE SLICES. J.-H. Rho and L.W. Swanson. The Salk Institute, La Jolla, CA 92037.

The paraventricular nucleus of the hypothalamus (PVH) coordinates a wide range of neuroendocrine and autonomic functions. Anatomical studies using immunohistochemical and retrograde axonal transport methods have previously divided this nuclei into several distinct compartments, showing that largely separate populations of neurons project to the median eminence, posterior pituitary, and brainstem/spinal cord. To characterize morphologically different cell types contained within various subdivisions of the PVH, we have utilized a novel intracellular dye-filling method to first retrogradely label distinct subpopulations of neurons and then to iontophoretically inject lucifer yellow (LY) into labeled neurons. Using two different fluorescent tracers, fast blue (FB) injected into the blood, and fluorogold (FG) placed in the spinal cord, neuroendocrine cells, which are localized mostly in the dorsal medial parvocellular (PVHmpd) and magnocellular parts, were distinguished from autonomic neurons, which are localized mostly in the dorsal cap and ventral medial parvocellular parts (PVHmpv). After neurons were injected with LY, their morphological features were drawn with the aid of a computer-based graphics systems.

Neuroendocrine cells, some of which were immunostained with anti-CRF, were usually bipolar with thick proximal dendrites that tapered distally. Dendritic spines were sparse along their dendrites, which usually branched once near the soma and once again near their distal tips. The dendrites of parvocellular neurons in the PVHmpd appeared to be mostly restricted to this part while the dendrites of neuroendocrine neurons in the magnocellular subdivision often extended medially and ventrally beyond its boundaries. Local terminal boutons or short appendages were seen on the axons of neuroendocrine CRF cells in the region just outside the PVH where many GAD-positive neurons are found. In contrast, autonomic neurons were usually multipolar with 3 to 5 dendrites extending from the soma, and were of either the spiny and aspiny type. The spiny cell type was less frequent and several were found in areas ectopic to the dorsal cap and PVHmpv. Many long spines were distributed along the length of its major dendrites, which gradually tapered and extended for 300-400 microns from the cell body. The dendrites of aspiny neurons, on the other hand, were often highly branched, and of uniform thickness; they extended for considerable distances, and in many cases, crossed beyond the boundaries of their cytoarchitectonically-defined cell group. Further mapping of dendritic territories, and a correlation with the localized distribution of the many afferent inputs to the PVH will be necessary to gain an accurate understanding of neuronal information flow through the PVH.

319.11 DISTRIBUTION OF CHOLECYSTOKININ AND METHIONINE-ENKEPHALIN IN THE PREOPTIC AREA AND ANTERIOR HYPOTHALAMUS OF THE BRAZILIAN GRAY SHORT-TAILED OPOSSUM. G.E. Hoffman, C.A. Fox*, T. Larsen*, R.E. Watson, Jr. and C.D. Jacobson. Dept of Neurobiology and Anatomy, Univ. of Rochester School of Medicine, Rochester, N. Y. 14642 and Dept. of Veterinary Anatomy, Iowa State Univ., Ames, Iowa 50011.

The Brazilian gray short-tailed opossum, Monodelphis domestica, is a small, pouchless, marsupial which breeds well under lab conditions. The young are born after a 14 day period of gestation in a sexually undifferentiated immature state and are well suited for developmental studies. Prior to characterizing the developmental patterns associated with the localization of peptide neurotransmitters in the medial preoptic area (MPOA) and anterior hypothalamus (AH), the patterns of the adult animal must be identified. In this study, brains of adult male and female opossums were processed for localization of methionine-enkephalin (m-ENK) and cholecystokinin (CCK) like immunoreactivity. Animals were ether anesthetized and perfused intracardially with saline followed by Zamboni's fixative. Each brain was removed and processed for the immunocytochemical localization of m-ENK and CCK using the avidin-biotin with nickel intensification technique (Watson et al., Peptides 7, 1986; Hancock, M., J. Histochem. Cytochem. 32, 1984). Sections of the MPOA and AH were obtained using a freezing microtome. Antiserum to rabbit CCK and rabbit m-ENK were obtained from Immunonuclear. The results indicate that CCK like immunoreactivity is present in the MPOA and AH. The MPOA contains a light to moderately staining CCK fiber plexus which lies adjacent to the third ventricle. In addition, CCK containing fibers appear to be distributed throughout the paraventricular nucleus (PVN) and the supraoptic nucleus (SON). There is also a dense plexus of CCK positive fibers within the suprachiasmatic nucleus (SCN). M-ENK is distributed throughout the MPOA and AH. In the MPOA there is a wide band of m-ENK positive fibers adjacent to the third ventricle. This dense plexus of fibers follows the boundaries of the MPOA. In the AH m-ENK like immunoreactivity is located adjacent to the third ventricle. There is a dense fiber plexus located within the PVN. However, there is an apparent lack of m-ENK containing fibers within the SON and SCN. These results indicate that the localization patterns of CCK and m-ENK are similar to those observed in the rat and further support the use of this animal in studies investigating development and differentiation of the MPOA and hypothalamus. Supported by NIH grants HD 16148 and 18418 and NS 93625.

319.12 THE ORGANIZATION OF MESENCEPHALIC AND PONTINE AFFERENTS TO THE PARAVENTRICULAR AND SUPRAOPTIC NUCLEI IN THE RAT. M.C. Levin*, E.T. Cunningham, Jr. and P.E. Sawchenko (SPON: S.P. Duckles), The Salk Institute, and Dept. of Neurosci., Univ. Cal. San Diego, La Jolla, CA 92037.

Retrograde and anterograde tracing techniques were used to characterize the distribution and cells of origin of projections from the midbrain and pons to the paraventricular (PVH) and supraoptic (SO) nuclei of the hypothalamus in the rat. After discrete crystalline implants of the retrogradely transported fluorescent dye, True Blue, into the PVH, one group of labeled neurons comprised a continuous thin sheet of cells corresponding to descriptions of a so-called parvocellular part of the subparafascicular nucleus. In addition, labeled neurons were found throughout the longitudinal extent of the central gray, and were especially prominent in its dorsal and ventrolateral aspects. More discrete clusters of retrogradely labeled cells were found in the peripeduncular nucleus, the pedunculopontine nucleus, the lateral dorsal tegmental nucleus, the lateral parabrachial nucleus, the nucleus incertus, the locus coeruleus, the mesencephalic raphe nuclei, and in discrete aspects of the mesencephalic and pontine central tegmental fields. With the exception of the aminergic cell groups, whose projections have been characterized previously, deposits of the anterogradely transported plant lectin, Phaseolus vulgaris-leucoagglutinin (PHA-L), were placed in each of the regions enumerated above. The results confirmed the existence of projections from each region to the PVH, where each of the inputs ramified and gave rise to terminal specializations primarily within the parvocellular division of the nucleus. Only the pedunculopontine and lateral dorsal tegmental nuclei were found to provide projections that ended decisively in the magnocellular division of the PVH and in the SO, though these inputs to the magnocellular neurosecretory system were of only sparse to moderate density. The PHA-L material also revealed a surprising number of pathways interconnecting many of the mesencephalic and pontine cell groups that were found to provide afferent projections to the PVH and SO. The results provide new information concerning potential routes by which visceral sensory information conveyed initially to the nucleus of the solitary tract may reach, and be distributed to, autonomic- and neuroendocrine-related effector neuron populations in the PVH and SO. By contrast, no discrete cell group was identified that might serve as a relay through which somatic sensory information subserving the milk ejection reflex might be conveyed to oxytocin-containing magnocellular neurosecretory neurons.

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319.13 MAMILLARY, SUPRAMAMILLARY AND TUBEROMAMILLARY NUCLEI: CONNECTIONS WITH INFRALIMBIC CORTEX IN RAT. G.V. ALLEN* and D.A. HOPKINS. Dept. of Anatomy, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

The mamillary, supramamillary and tuberomamillary nuclei form a complex of nuclei in the posterior hypothalamus. Although these nuclei are usually considered to be components of distinct and separate neural systems, there is evidence that they may have some connections in common with the infralimbic cortex and brain stem tegmentum.

In order to investigate further the afferent and efferent connections of these nuclei with the cortex, injections of 0.01-0.02 µL of WGA-HRP were made into the infralimbic cortex and posterior hypothalamus. Following 1-2 day survival periods, the animals were perfused for light and electron microscopy with a buffered aldehyde fixative. For light microscopy, frozen sections were incubated for HRP reaction product with tetramethylbenzidine (TMB). Vibratome sections were incubated in TMB and diaminobenzidine prior to processing for electron microscopy.

At the light microscopic level, infralimbic cortical injections of WGA-HRP produced retrograde and anterograde labeling in the supramamillary and tuberomamillary nuclei, predominantly ipsilaterally. Dense anterograde labeling was also present in the dorsal parts of the medial mamillary nucleus, especially the medially located pars medianus. Following injections of WGA-HRP into the mamillary region, retrograde labeling was seen in the infralimbic cortex. At the electron microscopic level, labeled axon terminals were observed in the mamillary, supramamillary and tuberomamillary nuclei. Labeled terminals contained round synaptic vesicles and formed mainly asymmetric synaptic junctions with dendritic profiles. Labeled terminals were not observed in synaptic contact with neuronal somata. Label was present in somata and dendrites of the tuberomamillary and supramamillary nuclei.

The results demonstrate that the mamillary, supramamillary and tuberomamillary nuclei have complex synaptic relations with the infralimbic cortex. In particular, parts of the medial mamillary nuclei receive selective inputs to certain subdivisions from cortex (infralimbic and subicular) and brain stem tegmentum. In addition, the supramamillary and tuberomamillary nuclei appear to be reciprocally connected with the infralimbic cortex.

Supported by MRC of Canada.

319.14 **MAGNOCELLULAR TUBEROMAMMILLARY (TM) NEURONS PROJECT TO BOTH PARTS OF THE RAT SUPRAOPTIC NUCLEUS.** G.I. Hatton, Q.Z. Yang, and M.L. Weiss. Neuroscience Program, Michigan State Univ., E. Lansing, MI 48824-1117.

Supraoptic (SON) neurons are known to be affected by histamine stimulation, both in vivo (Brain Res. 78:151, '74) and in vitro (Neuroscience 16:307, '85) but the origin of an endogenous histaminergic input is unknown. The TM neurons are one possible source of histaminergic input (Handbook of Chemical Neuroanatomy, vol. 3: 126, '84) and retrograde tracing data suggest that TM may project to SON (Neurosci. 15:135, '85). As part of our studies of SON inputs, we made micropressure injections of fluorescent tracers (Fluoro-gold, 1-2% in distilled water, supplied by L.Schmued; or rhodamine-labeled microspheres, Tracer Technology, Bardonia, NY) into the SON. We noted that injections of tracer into the anterior (SONa) or the tuberal SON (SONt) labeled areas known to project to the SON (JCN 218: 121, '83; Neurosci. 15:135, '85), and resulted in retrogradely labeled cells within TM both ipsilateral and contralateral to injection site. Light fiber labeling was sometimes seen in the supraoptic decussation. Injections into SONt resulted in notably more cells labeling in the contralateral TM than after injections into SONa. Since the SONt contains a higher percentage of vasopressin producing neurons than the anterior portion, this suggests that TM neurons may show specificity. To confirm this projection, we have begun to investigate this pathway electrophysiologically in the in vitro horizontal slice preparation, which contains the TM and both parts of the SON. Intracellular recordings from cells in SONt (n=1) and SONa (n=2) show short latency excitatory responses (2-4 ms for SONa and shorter latency for SONt) to constant current stimulation (bipolar stimulating electrode, evoked by currents as low as 50 nA) of the area containing TM ipsilaterally. These cells were subsequently injected with Lucifer Yellow and will be immunocytochemically identified for peptide content. Further work is needed to confirm the amine content of TM neurons projecting to SON and to examine the contralaterally projecting TM cells. Supported by NIH grant NS 16942 and NS 09140 and NRSA Postdoctoral grant NS 08125 to MLW.

319.15 **FLUORESCENT TRACER INJECTIONS INTO PARAVENTRICULAR (PVN) AND SUPRAOPTIC (SON) NUCLEI DOUBLE LABELS NEURONS WITHIN SUBFORNICAL ORGAN (SFO) OF THE RAT.** M.L. Weiss and G.I. Hatton. Neuroscience Program, Michigan State University, E. Lansing, MI 48824-1117.

We hypothesized that single neurons in the SFO, nucleus medianus (NM) and organum vasculosum lamina terminalis (OVLT) would terminate on both the PVN and SON nuclei via collateral innervation, since these nuclei are midline structures and project to both PVN and SON. Thus, we injected anesthetized rats (Equithesin) with one of the two tracers: Fluoro-gold (1-2% in water; supplied by L. Schmued) or rhodamine-labeled microspheres into the PVN and the other tracer into SON of male rats. These tracers are not taken up by fibers of passage, they resist fading and they do not leach out of retrogradely filled neurons to label surrounding glia. Survival after injection ranged from 3-7 days, at which time animals were anesthetized and perfused with normal saline then 4% neutral buffered paraformaldehyde. Brains were frozen sectioned at 50 um and arranged in three adjacent sets consisting of every third section. One set was counterstained, a second was cleared in xylene and coverslipped for epifluorescent observation, the third set was reserved as backup. To date we have injected 27 rats, 2 animals had injections which were well-confined to the anterior SON but the PVN injections had slightly spread to include the zona incerta and reuniens nucleus of the thalamus. These two cases had retrogradely filled neurons within areas known to project to PVN and SON (JCN 218:121,'83; Brain Res.Bull.14:143,'85). We found many singly labeled cells within SFO, NM and a few within the core of OVLT. Doubly labeled cells (doubles) were found predominantely within the anterior ventral portion of the SFO within cells which were not brightly labeled. Brightly labeled cells tended to contain a single label, but they could be found adjacent to doubles. From the most anterior ventral portion of SFO through its middle (approx. 150 um caudad), the doubles tended to be clustered together along the edges of the nucleus. We plan to further confine our injections and to provide a more complete mapping of the distribution of doubles throughout the brain, especially the caudal brainstem. Supported by NRSA Postdoctoral award NS 08125 to MLW and NIH grant NS 09140.

319.16 MICROINJECTION OF KAINIC ACID INTO THE VENTRAL MEDIAL NUCLEUS OF THE HYPOTHALAMUS PREVENTS MURICIDAL BEHAVIOR IN RATS. D.M. Meenan* and C.F. FERRIS. Department of Physiology, University of Massachusetts Medical Center, Worcester MA. 01655.

It has been shown by numerous investigators that large electrolytic lesions to the posterior hypothalamus can prevent muricidal behavior in rats. The purpose of the present study was to examine mouse killing in rats following placement of discrete lesions in the hypothalamus by the microinjection of small volumes (20 nanoliters) of the neurotoxin kainic acid. Long Evans rats (n=26) were anesthetized with pentobarbital and stereotaxically implanted with 26-gauge guide cannulae aimed at the ventromedial nucleus (VMN) or the paraventricular nucleus (PVN). All rats were individually housed and maintained on a 14:10 L:D cycle for 18 days. Muricidal behavior was tested by placing a 6-8 week old mouse in the home cage of a rat for 60 min. Rats were considered to be spontaneous mouse killers if, over three consecutive days, they killed the mouse within 10 min after being placed in their cage. After the muricidal behavior was determined rats were unilaterally microinjected with 0.1 µg of kainic acid in a volume of 20 nanoliters. Four days later rats were sacrificed, the brains fixed, histologically processed and examined for the location of the lesions using the stereotaxic atlas of Konig and Klippel. Out of the total 26 rats only 5 displayed altered behavior in response to the kainic acid lesions. Four rats were prevented from killing while one rat which had not shown muricidal behavior killed its mouse. The four rats prevented from mouse killing had lesions in the VMN and/or the ventral portion of the the dorsal medial hypothalamus. The one rat that displayed muricidal behavior in repsonse to the microinjection of kainic acid had a lesion that was medial to the DMH and dorsal to the VMH. The remaining 21 rats had lesions in the posterior hypothalamus (n=3), PVN (n=5), fornix (n=3), basal stria terminalis (n=2), nucleus reuniens thalami (n=4), anterior hypothalamus (n=1), and stria terminals (n=2). These data suggest that discrete chemical lesions in the area of the VMH can alter muricidal behavior. (Supported by NIH grant NS-23557)

319.17 Hypothalamic tanycytes and brainstem neurons identified by monoclonal antibody 3D10. S.A. Tobet, D. Blank*, and T.O. Fox, Dept. Biochemistry, E.K. Shriver Center, Waltham, MA 02254 and Prgm. Neuroscience, Harvard Medical School, Boston, MA 02115

To examine the regulation of hypothalamic development, we are using monoclonal antibodies obtained after immunizations with neonatal rat brain. Some of the antibodies obtained recognize particular neural elements that are distributed widely throughout the neuraxis while others are restricted to certain cell groups. One especially selective antibody, 3D10, binds to one cell type in the hypothalamus (HYP) and selected cell groups in the brainstem.

For immunizing BALB/c mice, the HYP and temporal lobe (TL) were removed from neonatal rats (postnatal day 4: P4), homogenized in saline, and injected i.p. Three days after the second immunization spleens were removed and fused with NS-1 myeloma cells. Supernatants from hybridoma cells were screened immunocytochemically on 20 micron sagittal sections from P4 rat brains. Further characterization of 3D10 immunoreactivity was carried out using free-floating 50 micron tissue sections from neonatal rats that were perfused with saline followed by 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). Using HRP-conjugated secondary antibodies (anti-IgM), 3D10 antigen(s) were visualized as 3,3'-diaminobenzidine reaction product.

The 3D10 immunoreactivity appeared intracellularly in restricted populations of cells. In the HYP, reaction product was seen in subsets of tanycytes (TYs), with the greatest number around the organum vasculosum of the lamina terminalis (OVLT) and in the region of the median eminence. Immunoreactive product was apparent throughout the entire length of individual TYs. In brainstem intense staining occurred in a subset of locus ceruleus (LC) perikarya and in cells that appear to be part of the A5 noradrenergic cell group. Lower levels of immunuoreactivity were also seen in neurons of the raphe nuclei.

The selectivity of 3D10 for TYs in the third ventricle and for LC raises the possibility that the coincident immunoreactivity in these cells results from transport of molecules secreted into the cerebrospinal fluid (CSF). It has been proposed that tanycytes transport substances from the CSF; in addition, the LC lies directly adjacent to the fourth ventricle, with some processes reaching the ventricular surface. Alternately, these selected cells might synthesize a common epitope. Experiments are underway to characterize the 3D10 antigen(s) biochemically and to test these hypotheses. Supported by NIH Research Grant HD-20327, Training Grant HD-07251, and MR Core Grant HD-04147.

319.18 **INTRACELLULAR RECORDINGS OF THERMOSENSITIVE NEURONS IN RAT HYPOTHALAMIC TISSUE SLICES.** M.C. Curras, S.R. Kelso and J. A. Boulant. Department of Physiology, Ohio State University, Columbus, OH 43210; and Department of Biological Sciences, University of Illinois, Chicago, IL 60680

Rostral hypothalamic neurons are considered important in thermoregulation. About 40% of these neurons are sensitive to hypothalamic temperature, and many of these thermosensitive neurons receive afferent input from peripheral thermoreceptors. To understand the basis of neuronal thermosensitivity, 3 M KCl or K acetate microelectrodes were used to study the effect of temperature on intracellular activity recorded from neurons in frontal tissue slices of the rat hypothalamus. This study only includes neurons showing stable membrane potentials of at least -45 mV and action potentials in response to depolarizing pulses. Determinations were made of the effects of temperature on firing rates, resting membrane potentials, input resistances, and postsynaptic potentials. Most neuronal activity was recorded in the preoptic area and anterior hypothalamus while tissue temperature was changed 3-5°C above or below 37°C. Neurons were classified as warm-sensitive, cold-sensitive or temperature-insensitive based on their firing rate responses to tissue temperature. A subpopulation of the temperature-insensitive neurons included "silent" neurons which produced no spontaneous action potentials, regardless of temperature.

At neutral temperatures (35.8°-37.6°C), hypothalamic neurons had a mean resting membrane potential of -65 mV and a mean input resistance of 167 megohms. Regardless of thermosensitivity, most neurons showed an increase in input resistance during decreases in tissue temperature. Some warm-sensitive and cold-sensitive neurons displayed ramp-like depolarizations preceding action potentials. In both types of thermosensitive neurons, these ramp-like depolarizations and the frequency of action potential generation were greatly affected by inhibitory and excitatory postsynaptic potentials, suggesting that synaptic networks are important determinants of hypothalamic neuronal thermosensitivity.

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319.19 OSMOSENSITIVITY IN BRAIN SLICES: HYPOTHALAMUS AND HIPPOCAMPUS. R.D. Andrew, B.A. Ballyk and M. Fagan. Anatomy Dept., Queen's University, Kingston, Ontario K7L 3N6

In the intact rat, a 5 - 50 milliosmole (mOsm) increase in plasma osmolarity increases the firing rate of magnocellular neuroendocrine cells (MNCs). These cells synthesize oxytocin or vasopressin in the paraventricular nucleus and supraoptic nucleus (SON) of the hypothalamus and release them from axon terminals in the neurohypophysis. Osmosensitivity is apparently retained but reduced in coronal hypothalamic slices. It has been suggested that increased excitatory synaptic traffic plus an intrinsic depolarization of the MNC itself accounts for MNC osmosensitivity (Leng et al., 1985, Vasopressin p. 333).

Our extracellular recordings from SON revealed that 60% of rat MNCs (n=12) and 42% of cat MNCs (n=36) became more excitable during a 65 mOsm increase of the superfusing solution. Paradoxically, 7 of 7 MNCs tested also displayed an increase in the orthodromic spike threshold evoked 0.5 mm dorsal to SON during this large increase in osmolarity. This unexpected reduction in excitability was further examined in the hippocampal slice preparation.

Population potentials recorded in the stratum pyramidale of CA1 were evoked antidromically from alveus or orthodromically from CA3. Over 5 min, a 35 mOsm increase by addition of NaCl reduced the antidromic population spike by 31% and the orthodromic spike amplitude by 29% (n=8). With a 65 mOsm increase, the reduction was 40% and 66%, respectively (n=7). With an intermediate 52 mOsm increase using mannitol, the reduction was 35% and 36%, respectively (n=9). All of these responses were reversible. Intracellular recordings from CA1 neurons revealed that, although the action potential threshold usually did not increase, the amplitude of the compound EPSP (evoked at a level straddling spike threshold) was reduced in 10 of 11 cells. Each of these changes in hippocampus elicited by raising the osmolarity may be explained by an increase in threshold of the conducted action potential along CA1 and CA3 axons. Whether synaptic transmission itself is also affected requires further study.

In conclusion, when the osmolarity of the superfusate increases, there is an apparent increase in the threshold of the conducted action potential as gauged from evoked antidromic and orthodromic field potentials of CA1 cells. In hypothalamic slices, a similar effect on neurons presynaptic to MNCs could explain why evoked orthodromic input to MNCs is reduced as the osmolarity increases.

319.20 ELECTROPHYSIOLOGICAL EXAMINATION OF NEURONS IN THE BED NUCLEUS OF THE STRIA TERMINALIS: CHARACTERISTIC PROPERTIES AND RESPONSES TO AMYGDALOID AND HYPOTHALAMIC STIMULATION. M. Dalsass and A. Siegel. Neurology, VA Medical Center, East Orange, NJ 07019, and Dept. of Neurosciences, New Jersey Medical School, Newark, NJ 07103.

The bed nucleus of the stria terminalis (BNST) can powerfully modulate such emotional forms of behavior as quiet biting attack, affective defense and flight behavior. Several of the possible anatomical pathways associated with these BNST elicited behaviors have been described. The BNST receives major inputs from the amygdala and hypothalamus and in turn, projects its axons, in part, to the hypothalamus, midbrain periaqueductal gray matter and the nucleus of the solitary tract, target structures that play key roles in the regulation of emotional behavior and autonomic nervous system function. Little is presently known about the electrophysiological properties of the BNST or of the possible role it may play as an integrative structure for amygdaloid and hypothalamic inputs.

Rats were anesthetized with chloral hydrate and stimulating electrodes were placed in the medial amygdala, lateral amygdala and hypothalamus. Glass micropipettes (5-40 mohms), filled with 2M K citrate and fast green dye, were used for extracellular recording in the BNST.

BNST neurons typically showed slow (2.8±2.4 HZ; mean ± SEM) spontaneous firing rates and relatively long duration (2.2±0.37 msec; mean ± SEM) action potentials. They could be orthodromically activated from one or more stimulating sites with latencies of 7 to 14 msec. In addition, the BNST appears to be organized so that the amygdaloid and hypothalamic inputs show increasing convergence along its dorsal to ventral extent. Some neurons were also antidromically activated from sites in the hypothalamus and basolateral amygdala with latencies of 4 to 7 msec. Their slow conduction velocity (0.65 m/sec) indicates that these neurons are unmyelinated. The results show that: 1) most BNST neurons have characteristic neuronal features with regard to spontaneous discharge rate and spike duration, 2) BNST neurons can be antidromically activated from sites in the amygdala and hypothalamus, and 3) the majority of BNST neurons respond orthodromically to stimulation from these sites, with the greatest convergence occurring near the ventral border. The results suggest that the ventral aspect of the BNST may play an important role in integrating inputs from the amygdala, hypothalamus and brainstem.

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FEEDING AND DRINKING VI

320.1 THE FAILURE OF PERIPHERAL BUT NOT CENTRAL ANGIOTENSIN II RECEPTOR BLOCKADE TO REDUCE SALT APPETITE IN THE RAT. A.N. Epstein, S.Y. Chow*, R.R. Sakai* and D.A. Wilson*. Univ. of Pennsylvania, Dept. of Biology and Psychology, Phila. Pa. 19104 USA

The salt appetite of the sodium deplete rat is completely suppressed by concurrent central blockade of angiotensin II (ANG II) and aldosterone (ALDO) receptors (Sakai et al, AJP 1986). Central blockade of only one of these hormones causes only a partial reduction of the salt appetite, supporting our earlier idea that the hormones of sodium conservation act synergistically to arouse salt appetite in the rat. Here we examine whether ANG II of renal or of central origin is the important synergist.

Rats were fitted with either an intracerebroventricular (ICV) cannula (n=6) or an intravenous (IV) jugular catheter (n=6) and were sodium depleted by combining natriuresis (furosemide, 10 mg/rat, SC) with removal of ambient sodium. At least 18 hours after the beginning of the depletion 3% NaCl was returned for 2 hours and the rats were free to express a salt appetite.

Peripheral ANG II receptors were blocked in sodium depleted rats by continuous IV (cIV) infusion of the ANG II receptor antagonist SARILE (SAR-1, ILE-8 ANG II) at a dose of 10 ug/min beginning two hours before and continuing during the two hour access to 3% NaCl. There was no suppression of 3% NaCl intake by the end of the 2 hour access period (veh infusion 9.6 ± 2.9 ml; SARILE 10 ug/min 9.8 ± 2.6 ml) despite the blockade of peripheral ANG II receptors as shown by the failure of cIV ANG II (64 ng/min for 15 minutes) to evoke water drinking at the end of the appetite test.

In contrast, central blockade of ANG II receptors (continuous ICV SARILE, 25 ug/hr, 2 hrs before and during the 2 hr appetite test) reduced salt appetite (veh vs SARILE; 6.3 ± 0.8 ml vs 4.3 ± 0.7 ml at 30 min; veh vs SARILE; 8.0 ± 0.7 ml vs 6.3 ± 0.5 ml at 120 min), and blocked water drinking to a pulse ICV injection of ANG II (6 ng) at the end of the two hour appetite test.

The failure of peripheral but not central blockade of ANG II receptors to reduce depletion-induced salt appetite suggests 1) the activation of peripheral ANG II receptors is not necessary for the expression of depletion-induced salt appetite whereas activation of central ANG II receptors is important for expression of the behavior, and 2) the role of peripheral ANG II may be to evoke the release of aldosterone which then a) promotes renal sodium conservation and b) participates in the synergy with ANG that leads to arousal of salt appetite.

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320.2 **SALT APPETITE AND EXCRETION TO VENTRICULAR ANGIOTENSIN: FOREBRAIN OR HINDBRAIN?** D.A. Fitts and D.B. Masson* (SPON: G. Clark). Univ. of Wash., Seattle, WA, 98195.

Angiotensin II (ANG) infusion into the anterior cerebral ventricles causes drinking of both water and NaCl solutions in rats. The site(s) of action for the effect on water intake is near the anterior ventricles, because large doses of ANG injected into the fourth ventricle (4V) have no effect. However, chronic infusions of ANG into the area postrema (AP) or 4V reportedly increase daily intake of 0.15 M NaCl, suggesting that forebrain infusions of ANG might cause NaCl intake by ANG traveling to the 4V.

The present experiments tested infusions of 0, 10, 100, or 1000 pmol/h ANG in the 4V for 3 h in an effort to stimulate salt drinking. Rats received one 4V infusion at 4.6 µl/h in the presence of both water and 0.3 M NaCl for drinking. Urine was analyzed for Na, K, and osmolality. Other rats with cannulas in the lateral ventricles (LV) received an infusion of 0 or 100 pmol/h ANG at the same volume under the same conditions.

Nine vehicle-infused rats with 4V or LV cannulas drank 0 to 2.5 ml of water or NaCl in 3 h. Nine rats with verified 4V cannulas all drank similar amounts of water and NaCl to these controls regardless of the dose of ANG. By contrast, 5 rats receiving 100 pmol/h ANG in the LV rapidly drank both water and NaCl, averaging 23 ml water and 11 ml NaCl in 3 h. No rat given any dose of ANG into the 4V drank as much water or NaCl as the least rat infused into the LV.

Excretion of Na, K, and total solutes were unaffected by infusions of ANG into the 4V, even at the very large dose of 1000 pmol/h. Infusions of 100 pmol/h ANG into the LV of animals not allowed to drink Na more than doubled Na excretion over 2-4 hr.

Na ingestion and excretion were unaffected by ANG infusions into the 4V, but ANG in the LV dramatically increased both. Thus, these effects of short term ANG infusions into the anterior ventricles are not caused by posterior travel of the infusate. Our results do not necessarily refute findings of increased NaCl drinking by 4V and AP infusions because of large differences in the methodologies of the two studies.

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320.3 THE CENTRAL VASOPRESSIN AND/OR OXYTOCIN SYSTEMS MAY BE NEURAL SUBSTRATES FOR THE REGULATION OF SODIUM APPETITE. K.A. Gruber*, S.L. Eskridge*, and M.F. Callahan (SPON. M. Levitt). Wake Forest University Medical Center, Winston-Salem, North Carolina 27103.

Sodium (Na) appetite can be produced by Na depletion (e.g. diuretics), by other treatments which disrupt fluid/electrolyte balance, or by stress. Angiotensin II (A-II) and aldosterone (ALDO), a mineralocorticoid, are two hormones whose circulating levels are increased by Na depletion. Exogenous administration of A-II or ALDO produces Na appetite, and simultaneous administration results in a potentiated effect. Attenuation of diuretic-induced Na appetite can be produced by A-II or mineralocorticoid receptor antagonists. Since intracerebroventricular (ICV) administration of A-II or ALDO receptor antagonists are more effective in attenuating Na appetite than peripheral administration, a central site-of-action is indicated (Sakai et al A.J.P. 251:R762, 1986). Recently, atrial natriuretic peptides (ANP) have also been shown to attenuate Na appetite. Despite the demonstration of numerous substances or states which regulate Na appetite, a common neural substrate(s) responsible for these effects has not been identified.

We have recently shown that activation of the central vasopressin (VP) or oxytocin (OX) systems are common pathways for centrally acting factors to increase sympathetic drive (e.g. Gruber and Eskridge A.J.P. 251:R476, 1986). Activators of VP or OX neurons include A-II, ALDO, hypertonic saline, and stress; while A-II receptor antagonists and ANP are inhibitors. Thus, there appear to be parallels between VP/OX neuron regulation, and regulation of Na appetite. To address the question of the role of the central VP and/or OX sytem in Na appetite, we measured diuretic-induced Na appetite in rats after blockade of CNS V_1 VP/OX receptors.

Rats were given access to water and 3% saline for 48 hours. The saline was removed, each rat given 2 X 15 mg injections of furosemide 3 hours apart, and a low sodium chow was provided. Twenty hours later food and water were removed, and the rats given ICV infusions of Earles balanced salts solution (EBSS) or a V_1/OX receptor antagonist (100 ng/0.5 ul/min for 30 min). No behavioral effects of the antagonist infusion were noted. The infusion was stopped, and solutions of fresh water and 3% saline were again offered. Each rat was appraised of the availability of saline by touching the drinking spout to their lips. Rats infused with EBSS drank 6-12 mls of saline in 2 hours. In contrast, adminisration of a V_1/OX receptor antagonist significantly reduced saline consumption by ~50%.

The results provide prelminary evidence that activation of central VP and/or OX systems may induce Na appetite.

320.4 EFFECTS OF CHORDA TYMPANI NERVE SECTION ON THE IMMEDIATE AND LONG-TERM EXPRESSION OF SODIUM APPETITE IN RATS. G.J. Schwartz*, Monell Chemical Senses Center, & H.J.Grill (SPON: P. Glimcher), Dept. of Psychology, Univ. of PA, Phila., PA 19104.

To determine the contribution of chorda tympani afferent input to the expression of sodium appetite in the rat, rats with bilateral chorda tympani (CT) nerve section received 24-hr. two-bottle preference tests and taste reactivity tests when sodium replete and sodium deplete. In preference tests, sodium deplete CT rats demonstrated a significant increase in the absolute intake of and preference for 0.5 M NaCl, as did sham-denervated controls. In taste reactivity tests, sham-denervated rats shifted their response to 1 min., 1 ml intraoral infusions of 0.5 M NaCl from a pattern of mixed ingestive and aversive responses to a pattern of exclusively ingestive responses when sodium deplete. CT rats, however, failed to show this shift from mixed aversive and ingestive responses to a pattern of solely ingestive responses. In addition, sham-denervated rats showed an increased in the latency to reject a 0.5 M NaCl infusion when sodium deplete, while CT rats failed to show this increase. Neither CT nor sham-denervated rats altered their taste reactivity responses to 1 min., 1 ml intraoral infusions of 0.1 M sucrose or 0.0001 M Quinine hydrochloride as a function of sodium depletion. The results suggest that CT afferent input is necessary for the increase in the ingestive consummatory response to NaCl in sodium deplete rats. In addition, it appears that gustatory input remaining following bilateral CT nerve section is sufficient to mediate an increase in the absolute intake of and preference for NaCl in sodium deplete rats.

320.5 SUBFORNICAL ORGAN (SFO) LESIONS IMPAIR DIURESIS-INDUCED WATER AND SALT INTAKES. R.L. Thunhorst, K.A. Ehrlich* & J.B. Simpson. Dept. of Psychology, Univ. of Washington, Seattle, WA 98193.

The SFO participates in thirst generated by angiotensin II (AII), but involvement of the SFO in salt appetite generated by AII, or other stimuli, has not been demonstrated. In the present experiment, we determined effects of SFO lesions on water and salt intakes in response to furosemide diuresis and low sodium diet, a procedure which elevates plasma AII. Rats on ad libitum water and 0.3 M NaCl received SFO lesions (n=8), control lesions (n=6) or sham surgery (n=7). Testing occurred 8 days after surgery. Prior to testing, food, water and 0.3 M NaCl were removed from the cages and funnels were positioned for urine collection. Fluid intakes from glass burettes were recorded for 2 hr in two separate measurement periods. The first 2-hr measurement period began 1 hr after furosemide diuresis (20 mg/kg i.p.) and included water as the sole drinking fluid. This period was followed by 20 hrs access to low sodium diet and water. The second 2-hr measurement period began with the removal of the low sodium diet and included water and 0.3 M NaCl in choice. This period was followed by 20 hrs access to regular chow, water and 0.3 M NaCl. Initial body weights did not differ between groups. In the first 2-hr period, SFO lesions significantly reduced drinking rate (ml/30 min) and cumulative 2-hr intakes. However, the cumulative 22-hr intakes, UV, water balance, UNaV, and weight loss after diuresis were not different between groups (all F's (2,18) $\leq$1.45). Thus, the groups achieved equivalent hydrational status by 22 hr post-furosemide with low sodium diet. In the second 2-hr period, SFO lesions significantly reduced 2-hr cumulative 0.3 M NaCl intakes, but not rate of intake, compared with the other groups, and significantly reduced water balance compared to sham rats. There were no effects of lesion on 2-hr water intake, UV, UNaV, or Na balance (all F's (2,18)$\leq$3.13). Effects of lesion on cumulative Na balance (24 hr post-furosemide) did not achieve statistical significance. Lesion condition did not affect intakes recorded after the second 2-hr period. We conclude that SFO lesions impair the initial ingestional responses to fluid and electrolyte loss via diuresis and low sodium diet, but these animals show complete compensation for these deficits by 24 hr.

	1st 2-hr period		2nd 2-hr period		
Lesion group	2-hr H2O (ml)	22-hr UNaV (umol)	2-hr H2O (ml)	2-hr NaCl (ml)	24-hr Na bal (umol)
SFO	1.1±1.7*	1789±175	0.1±0.4	4.4±4.5*	-555±1264
Control	3.0±1.6	1721±259	2.4±2.7	7.9±3.9	527± 743
Sham	4.2±1.1	1841±410	1.8±2.1	9.9±3.4	750± 886

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320.6 THE SALT INTAKE OF RAT DAMS INFLUENCES THE SALT INTAKE AND BRAIN ANGIOTENSIN RECEPTORS OF THEIR ADULT OFFSPRING. Karen E. Moe. Dept. of Psychology, Washington State University, Pullman, WA 99164-4830

Sodium-replete rats consume salt. When sodium-depleted, they consume even more. The renin-angiotensin system is an important component of the regulationof this salt appetite. The appetite appears very early in life, but little is known about the contribution of influences during development to its expression in adults. Contreras and his colleagues have shown that manipulation of maternal salt intake can influence the salt intake of weanling-aged rats (Dev. Psychobiol. 20:111, 1987) and the ratio of water-to-salt intake of adult offspring (by affecting water but not salt consumption; J. Nutr. 113:1051, 1983). The work reported here shows that the salt intake of adult offspring is positively related to maternal salt intake. Moreover, changes in maternal salt intake also result in long-term modification of brain angiotensin receptors.

Female Sprague-Dawley rats were given a sodium-deficient food to which NaCl had been added (0.1, 1., 4% by weight). A week later, they were mated. The dams and their litters remained on the diets until postpartum day 25-27, when Purina rat chow replaced them.

At 70 days postpartum, the now-adult offspring were individually housed with ad lib access to Purina chow and distilled water. A few days later, 0.15 M saline was also provided. 24-hr intakes of both water and saline were recorded. There was a positive relationship between the level of maternal sodium intake and the amount of saline consumed during adulthood. Mean 24-hr saline intakes for males: 20 $\pm$ 4, 24 $\pm$ 7, 43 $\pm$ 10 (0.1%, 1%, 4% diet group, respectively). Mean 24-hr saline intake for females: 20 $\pm$ 4, 33 $\pm$ 4, 37 $\pm$ 10 ml (0.1%, 1%, 4% diet group, respectively).

Later, binding to brain angiotensin receptor sites was assessed in the female offspring, using 125-I-angiotensin II (8 concentrations) and a standard tissue homogenate technique. Binding was assessed in two regions important for the regulation of blood pressure and fluid balance: (1) tissue containing the area postrema & nucleus of the solitary tract (AP/NTS); (2) tissue from the anterior dorsal region of the third ventricle, containing the septum, subfornical organ and portions of the median preoptic area (SFO/SEP). Maternal sodium intake was positively related to receptor density in the SFO/SEP but negatively related to receptor density in the AP/NTS. Receptor affinity was negatively related to maternal sodium intake in the SFO/SEP and positively related in the AP/NTS. It is not clear fromthese experiments whether there is a causal relationship between the receptor changes and the saline intake changes. However, regardless of the nature of the relationship, these results suggest that changes in maternal salt intake may have long-term consequences for the regulation of blood pressure and fluid balance (including salt appetite).

320.7 THE ABILITY OF ANGIOTENSIN II VS. ANGIOTENSIN III TO AROUSE A SALT APPETITE IN RATS. Charles R. Bredl and Karen E. Moe. Dept. of Psychology, Washington State University, Pullman, WA 99164-4830.

Angiotensin acts with aldosterone to elicit salt appetite in rats (Fluharty & Epstein, Behav. Neurosci., 97:746, 1983). Angiotensin II (A II) has generally been considered the active ligand for most phenomena regulated by angiotensin, while angiotensin III (A III) is described as a metabolite with weaker activity. However, the electrical properties of A III cause it to "stick" to untreated glassware more than A II does, and the purity of commercially-supplied A II and A III differ. Moreover, metabolism of A III is much quicker than that of A II, both in blood and brain (Harding et al., J. Neurochem., in press). Previous studies comparing the relative efficacy of A II vs. A III at behavioral actions did not control for these factors. When they are taken into account, A III and A II are equipotent at producing pressor and dipsogenic responses (Wright et al., Am. J. Physiol. 249:R514, 1985). In addition, Wright et al's work with the aminopeptidase inhibitors amastatin & bestatin (which block the conversion of A II to A III, and of A III to smaller fragments, respectively) suggests that A III, and not A II, is the centrally active form of angiotensin. With regard to salt appetite Mutter et al (Br. Res., 322:347, 1984) reported that a salt appetite could be elicited in pigs by both A II and A III, but they did not control for the factors described above. The work presented here assessed the relative potency of A II vs. A III at eliciting salt appetite in rats, controlling for those factors

Male Sprague-Dawley rats were given subcutaneous injections of DOCA (synthetic aldosterone precursor; 1 mg/day) or its oil vehicle for 6 days. On days 5 & 6, the rats received intracerebroventricular injections of cerebrospinal fluid (CSF), A II & A III (angiotensin doses: 10, 100 pmoles/2 µl CSF) in counterbalanced order. Water and 0.3 M saline consumption were recorded at 5, 10, 15, 20, 30 & 60 min after ICV injection. As shown in the table below, A III and A II were equipotent at arousing a salt appetite in DOCA-treated rats. These results agree with recent work comparing the ability of the two angioensins to elicit pressor & dipsogenic actions (above).

The effect of pretreatment with aminopeptidase inhibitors (amastatin & bestatin) on A II- and A III-induced salt appetite will also be presented. Together, these results may help define which form of angiotensin is biologically active & the critical mechanisms involved in salt appetite.

Angiotensin II		Angiotensin III	
10 pmoles	100 pmoles	10 pmoles	100 pmoles
2.4 ± 1.3	5.7 ± 0.8	2.1 ± 0.9	5.1 ± 1.3

60 min. intake (ml; mean ± SEM) of 0.3 M NaCl; n = 10 (10 pmoles) and n = 8 (100 pmoles)

320.8 ANGIOTENSIN II MAY MEDIATE A HISTAMINERGIC MECHANISM FOR FOOD-RELATED DRINKING IN THE RAT. F.S. Kraly and R. Corneilson*. Dept. of Psychology, Colgate Univ., Hamilton, NY 13346.

Adult male Sprague-Dawley rats (n=23) were tested for 1 hr for eating and drinking after 24-hr deprivation from food. Tests were begun by offering pelleted chow 10 min after injections of drugs or 0.9% NaCl. Blockade of central and peripheral angiotensin converting enzyme (ACE) activity using 100 mg/kg s.c. Captopril (CA) inhibited water intake by 33% (p(.01) and water to food ratio (W:F) by 36% (p(.01). Whereas i.c.v.t. 25 ug CA failed to affect food-related drinking (p's).10), 25 ug i.c.v.t. CA combined with .5 mg/kg s.c. CA (a dose that blocks only peripheral ACE) inhibited water intake by 31% (p(.01) and W:F by 20%. These results suggest that the renal renin-angiotensin system plays a role in drinking elicited by eating in the rat.

Subsequent work revealed that 100 mg/kg s.c. CA inhibited water intake by 23% (p(.05) in 12 rats in which combined antagonism of H_1 and H_2 receptors for histamine, using i.p. dexbrompheniramine (DXB) and cimetidine (C), inhibited water intake by 22% (p(.01) following 1 mg/kg DXB plus 16 mg/kg C and by 36% (p(.001) following 2 mg/kg DXB plus 32 mg/kg C. When 100 mg/kg s.c. CA was given simultaneously with i.p. DXB and C, the water intake was inhibited by 23% (p(.05) after CA plus 1 mg/kg DXB and 16 mg/kg C and by 38% (p(.001) after CA plus 2 mg/kg DXB and 32 mg/kg C. Thus, the effects of CA and DXB plus C were not additive for inhibition of food-related drinking.

Finally, 100 mg/kg s.c. CA inhibited (p's(.001) drinking elicited by 2.5 or 20 mg/kg s.c. histamine. In contrast, i.p. 1 mg/kg DXB plus 16 mg/kg C (treatment sufficient to abolish drinking elicited by s.c. histamine) failed to inhibit drinking elicited by 0.12 mg/kg s.c. angiotensin II.

In summary, (1) blockade of peripheral and central ACE inhibits food-related drinking and drinking elicited by s.c. histamine, and (2) such blockade of ACE and blockade of H_1 and H_2 histamine receptors are not additive in their effects on inhibition of food-related drinking. These findings together support the hypothesis of renal renin-angiotensin mediation of a histaminergic mechanism for drinking elicited by eating in the rat.

320.9 EFFECTS OF IMMUNONEUTRALIZATION OF BRAIN PEPTIDES ON WATER INTAKE IN THE RAT. C.R. Franci* and S.M. McCann. Department of Physiology, UTHSCD, Dallas, TX 75235.

Water intake elicited by overnight water deprivation was analyzed in different groups of male rats bearing indwelling cannulae in the third cerebral ventricle (3V) following intraventricular (icv) microinjection of 2 µl of normal rabbit serum (NRS), angiotensin II antiserum (AB-AII), atrial natriuretic peptide antiserum (AB-ANP), vasopressin antiserum (AB-VP) or oxytocin antiserum (AB-OT). Animals were used twice with the first injection of normal rabbit serum or the experimental antiserum followed by reversal of the treatment one week later. Water was offered immediately, one or three hours after icv injection. There was no difference in water intake between the control session (NRS) and the experimental session (AB-AII, AB-ANP, AB-VP or AB-OT) when water was offered immediately after icv injection. Water intake was highly significantly reduced at all times measured, (i.e. from 15 minutes to 4 hours) when the water was offered 1 hr after icv injection of AB-AII; however, the other antisera did not significantly impair drinking with this protocol. When water was offered three hours after icv injection, it occurred normally following injection of NRS, was almost completely inhibited by antisera against AII but was also significantly inhibited, although less so, by antisera against ANP, VP and OT. To further examine the specificity of the antisera effects, AII was injected icv in normally hydrated rats and the effect on water intake observed. Three hours after icv injection of NRS and antibodies against ANP, VP or OT there was equivalent water intake induced by AII; however, AB-AII dramatically reduced the dipsogenic effect of AII. In these hydrated rats no animal drank water following icv injection of isotonic sodium chloride, NRS or AB-AII three hours before. Thus 1 hr following injection of antisera, only AB-AII reduced water intake elicited by overnight deprivation, but 3 hours after injection AB-VP and AB-OT and AB-ANP were partially effective. The specificity of the AII antiserum effect was shown by the fact that it was the only antiserum which could alter AII-induced drinking. The results suggest that although AII is primarily responsible for dehydration-induced drinking, the other peptides may play a permissive role, since their antisera were partially effective, at least at fairly long times after antiserum injection, which would allow the diffusion of all antiseras to the effective sites within the hypothalamus.

320.10 THE EFFECTS OF IBOTENATE LESIONS OF THE MEDIAN PREOPTIC NUCLEUS ON DRINKING BEHAVIOR IN THE RAT. J. T. Cunningham*, M. J. Sullivan, G. L. Edwards, R. Farinpour*, and A. K. Johnson. Departments of Psychology and Pharmacology, and the Cardiovascular Center, University of Iowa, Iowa City, IA 52242

Electrolytic lesions of the anteroventral region of the third ventricle produce permanent response deficits to challenges (e.g., Angiotensin II [ANG II]; hypertonic saline [HTS]) that produce drinking responses and vasopressin release in normal animals. More recently, similar response deficits in rats have been demonstrated with electrolytic lesions of the median preoptic nucleus (MnPO) (Mangiapane et al., Neuroendo., 37: 73-77, 1983; Gardner and Stricker, AJP, 248:R224-R230, 1985). In the present study, the excitotoxin, ibotenic acid, was used in an attempt to determine if these drinking response deficits were due to the destruction of either fibers of passage or cells contained in the MnPO.

Adult male rats were injected with either vehicle (1 ul) or ibotenic acid (5 ug/1 ul) in the ventral MnPO. All subjects were then tested for drinking responses to ANG II (1.5 and 3 mg/kg sc) and HTS (3% and 12% 1 ml/100 gm b w). Rats with ibotenic acid injections into the MnPO drank significantly less water to both the 3% and the 12% doses of HTS than did the vehicle-injected controls. However, the ibotenic lesioned rats did not show a drinking response deficit to either dose of ANG II. This differential effect of ibotenic acid on drinking behavior suggests that its effects are not due to mechanical damage or a motor performance deficit but that the drinking response to HTS is mediated by a population of MnPO neurons that are sensitive to the neurotoxic effects of ibotenic acid.

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320.11 **SUBFORNICAL ORGAN CONNECTIVITY AND DRINKING TO CAPTOPRIL OR CARBACHOL IN RATS.** D.B. Masson* and D.A. Fitts. Univ. of Wash., Seattle, WA. 98195

Transection of the efferent and afferent connections of the subfornical organ (SFO) at its rostral stalk decreases drinking to peripheral angiotensin II (ANG) similar to ablation of the SFO itself. Other drinking responses, such as those to captopril (CAP) or carbachol (CBC), are reduced by SFO lesions. This study investigates whether CAP and CBC might also act through the well established neural connections of the rostral pole of the SFO.

Captopril studies. Lesions were placed with a rotating wire knife through the wall of the third ventricle between the rostral pole of the SFO and the anterior commissure. Control rats had either sham lesions (knife not extended) or histologically identified missed cuts. In acute drinking studies, control rats of both types drank 3.5±2 ml of water in 90 min following ip injection of 4 mg/kg CAP. Rats with complete transections drank significantly less, 1.3±1 ml. Other rats received CAP for 5 days at 0.1 mg/ml in the water with 0.3 M NaCl also available. All groups, regardless of the lesion condition, increased NaCl intake from baseline levels. Both studies affirm the importance of the SFO and its rostral connections in CAP-related water, but not saline, intake.

Carbachol studies. Groups of rats with knife cuts or control lesions received a 4-μl lateral ventricular (ICV) injections of either 1.2 or 4 nmol CBC. All rats drank water rapidly within the first 15 min, and rats at the higher dose continued drinking during the second half of the 30-min test. The lesion made no difference in the response, with data for transected rats falling between sham and missed cut rats at both doses. The transected rats showed deficits in drinking to ip CAP.

Conclusion. Our data support the hypothesis that CAP causes drinking via an angiotensinergic mechanism acting at the SFO, because the rostral connections of the SFO are critical for drinking to occur to both CAP and ANG. However, the data do not support the hypothesis that the SFO is a unique receptor site for ICV carbachol.

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320.12 EFFECTIVE THYROXINE ON DIPSOGENIC RESPONSIVENESS TO ISOPROTERENOL IN FOOD-DEPRIVED RATS. Michael J. Katovich, LI-Fen Yeh and Stephen P. Baker (SPON: J. Poulakos), Dept. of Pharmacodynamics College of Pharmacy, University of Florida, Gainesville, FL 32610.

The effect of thyroxine (T4) replacement on the increased beta-adrenergic dipsogenic responsiveness of fasted rats was studied in male Sprague-Dawley rats. Food-deprivation significantly decreased serum thyroxine (T4) and triiodothyronine (T3) levels, increased the dipsogenic response to isoproterenol and elevated renal beta-adrenergic receptor concentration. Daily administration of T4 (40 ug/kg) to food-deprived rats restored serum thyroid levels to normal. Thyroxine replacement also reduced the increased beta-adrenergic dipsogenic responsiveness in the food-deprived rats to control levels. In addition, daily administration of thyroxine reduced the beta-adrenergic receptor concentration in renal cortices to that observed in controls. Thyroid treatment tended to decrease the isoproterenol-induced renin release in food-deprived rats and increase the response in the control rats. These results suggest that the relative hypothyroid state observed in the food-deprived rat may be responsible for the increased concentration of renal beta receptors and the enhanced activation of the renin-angiotensin system resulting in an increased dipsogenic response induced by isoproterenol. Collectively, the data reaffirm the interaction of thyroid hormone and beta-adrenergic responsiveness, although it is of interest that in regards to renal beta-receptors the concentrations are decreased to normal whereas previous studies in hypothyroid rats demonstrate an increase to normal of cardiac beta-receptors. This would suggest thyroid hormone may normalize a response in an opposite direction depending on the direction of the disturbance. (Sponsored by NIH grants HD 18133, HD 19742, HL 32099 and GM 34905).

320.13 AN INCREASE IN MEAN ARTERIAL PRESSURE (MAP) BY IV INFUSION OF PHENYLEPHRINE (PE) INHIBITS THE DRINKING RESPONSE TO SC INJECTIONS OF HYPERTONIC SALINE IN THE RAT. M.M. Robinson. University of Western Ontario, London, Ontario, Canada, N6A 5C1.

It has been previously shown that the pressor response to IV infusion of angiotensin II inhibits the drinking response (Robinson & Evered, 1987). The present study investigated whether an acute rise in MAP is also inhibitory to the drinking response to SC injection of hypertonic saline. Rats (350-450 g) were tested in pairs and were infused IV with either PE (250 ug/ml) or with isotonic saline as control. The rate of infusion was identical in the paired animals and was varied (0.5-1.0 ml/h) to raise and maintain the MAP of the PE treated rat 25-35 mmHg above preinfusion values for the duration of the experiment. Fifteen minutes after the start of the IV infusion, each pair of rats was injected SC with either isotonic saline, 1.5 or 4 M NaCl (0.5 ml/100 g b.w.). The SC injection of hypertonic saline caused a dose dependent increase in the 60 min water intake of control rats and of rats whose MAP was raised with PE. However, the drinking response of the PE treated rats was significantly lower at both doses of hypertonic saline (1.5 M: 3.5 $\pm$ 0.9 vs 5.8 $\pm$ 0.5 ml; 4 M: 6.1 $\pm$ 1.7 vs 11.2 $\pm$ 1.2 ml, $p < .05$). PE had no effect on the drinking response to S.C. isotonic saline (2.1 $\pm$ 0.3 vs 2.4 $\pm$ 0.5 ml). PE increased urine output in rats given either 1.5 M NaCl (13.6 $\pm$ 1.0 vs 7.0 $\pm$ 1.1 ml, $p < .05$), or 4 M NaCl (15.1 $\pm$ 1.4 vs 11.7 $\pm$ 1.3 ml, $p < .05$) Total solute and Na^+ excretion were greater in rats given 4 M NaCl than 1.5 M NaCl (7.7 $\pm$ 0.6 vs 4.5 $\pm$ 0.5 osmols; 2.7 $\pm$ 0.3 vs 1.2 $\pm$ 0.1 equivalents Na^+, $p < .05$). Raising MAP in rats given 1.5 M NaCl also increased solute and Na^+ excretion (6.3 $\pm$ 0.7 vs 1.2 $\pm$ 0.1 osmols; 2.1 $\pm$ 0.1 equivalents Na^+, $p < .05$), but had no further effect on solute or Na^+ excretion in the rats given the higher dose of hypertonic saline. In rats treated similarly, but not allowed to drink, hypertonic saline caused a dose dependent increase in plasma osmolality and Na^+ concentration which was not affected by increasing MAP with IV infusion of PE. In summary, an acute rise in MAP inhibits the drinking response to SC injections of hypertonic saline. Generally, this effect cannot be attributed to a decrease in plasma osmolality or Na^+ concentration, nor to a pressure natriuresis, although with the 1.5 M NaCl dose the latter may have been a contributing factor. (Robinson, M.M. & Evered, M.D. 1987, Am. J. Physiol., 252:R754-R759.)

320.14 NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY WITHIN THE AVIAN BRAIN REVEALS CENTRAL LOCI THAT MAY BE SENSITIVE SITES FOR STIMULATING FEEDING BEHAVIOR. W. J. Kuenzel. Dept. of Poultry Sci., Univ. of Maryland, College Park, MD 20742.

Neuropeptide Y (NPY) has been shown to be a potent stimulator of food intake in mammals following intracerebronventricular (ICV) administration to rats (Levine, A. S. and J. D. Morley. Peptides 5:1025(1984); Clark, J. T. et al. Endocrinology 115:427(1984)) or when directed to the paraventricular nucleus (Stanley, B. G. and S. F. Leibowitz. Life Sci. 35:2635(1984)). The neuropeptide likewise effects feeding in birds. Five µg of NPY administered ICV in chicks consistently produces a doubling of food intake during the first hour compared to saline injected controls. Experiments were conducted to ascertain the distribution of NPY-like immunoreactivity within neuronal cell bodies and fibers throughout the chick brain. Avian pancreatic polypeptide and NPY antisera were used as primary antibodies in two immunocytochemical procedures. The first was an indirect immunofluorescence technique using a secondary antibody conjugated to fluorescein isothiocyanate. The second was the avidinbiotin complex (ABC) method utilizing peroxidase. Chicks were perfused with physiological saline followed by 4% paraformaldehyde.

The most densely stained fibers and terminals with NPY-like immunoreactivity occurred within and about the paraventricular nucleus and the region immediately surrounding the ventral and anterior region of the third ventricle. Structures around the third ventricle that had densely stained fibers included the periventricular hypothalamic nucleus, medial portion of the suprachiasmatic nucleus, paraventricular organ, inferior hypothalamic nucleus, infundibular nucleus and median eminence. The hippocampus had numerous densely stained neuronal cell bodies.

A group of structures showing clear NPY-like immunoreactivity included the lobus parolfactorius, organum vasculosum of the lamina terminalis, bed nucleus of the stria terminalis, a fiber network that extended from the lateral ventricle of the forebrain through the lateral and medial septal nuclei to the third ventricle at the level of the sub-septal organ (sub-fornical organ), the ventromedial hypothalamic nucleus and the nucleus tractus solitarius.

Structures showing some immunoreactivity included portions of the olfactory bulb, hyperstriatum accessorium, subcommissural organ, nucleus of the basal optic root, area ventralis of Tsai, interpeduncular nucleus and stratum griseum et fibrosum superficiale.

In light of the dramatic effect of NPY on food intake in mammals and birds, it would be of value to examine structures showing high NPY-like immunoreactivity to determine their possible role in affecting appetite.

320.15 NEUROPEPTIDE Y (NPY) FACILITATES RECOVERY OF FEEDING FOLLOWING LATERAL HYPOTHALAMIC LESIONS IN RATS. B. Roland, C.V. Grijalva, M.W. Gunion, L. Goehler, and J.E. Morley. Dept. of Psych. and Med., UCLA, Los Angeles, CA 90024; and V.A. Med. Center, Sepulveda, CA 91343.

It has been demonstrated that intracerebroventricular (ICV) and hypothalamic injections of neuropeptide Y (NPY) elicits feeding and drinking behavior in rats (Clark et al, Endocrinology, 115:427, 1984; Gray & Morley, Life Sci, 38:389, 1986; Stanley, Chin & Leibowitz, Br. Res. Bull., 14:521, 1985). To discern whether the lateral hypothalamus (LH) is a predominant site of action of NPY, the LH was lesioned in order to test whether NPY-induced feeding would be abolished. Two groups of albino male rats were matched for body weight, were given anodal electrolytic lesions of the LH (1.2 ma for 10s) and then immediately were implanted with cannulae in the lateral ventricle. All rats were deprived of food overnight and were tested for feeding behavior the next morning. Testing consisted of presenting the rats with chow pellets, chocolate chip cookies or cookie mash (cookie and water slurry) and the acceptance of the various foods was recorded. On Day 1 postoperatively, all rats were injected ICV with 2µl BSA saline. Pre- and post-injection, the rats were tested to see if they demonstrated the typical symptoms of aphagia and adipsia, which are associated with the LH syndrome. On Day 2, one group (SAL, N=6) was injected ICV with 2µl saline and the other group (NPY, N=8) was injected with 10µg/2µl NPY. The 2 groups were tested pre- and post-injection for feeding on the various diets. Injections and testing continued up to Day 7.

The results indicated that NPY significantly facilitated the recovery of feeding behavior. In the postinjection food tests, NPY enhanced feeding behavior (acceptance of the cookie mash or cookies) from Day 2 ($p<0.02$) up until Day 6 ($p<0.02$). The SAL group did not show this effect. Once feeding was promoted by NPY, feeding was sustained independent of NPY. The NPY group significantly increased the preinjection acceptance of food by Day 3 ($p<0.018$). It was not until Day 7 that the SAL group exhibited significant recovery of ingestive behavior. Despite the fact that NPY infusions promoted eating in LH rats, the NPY group continued to lose weight over the first 5 days and were not significantly different from the SAL group until Day 6 ($p<0.05$). By Day 6, the NPY rats were at 81% of their preoperative body weight, while the SAL rats were at 75%. These results indicate that although the LH is involved in ingestive behavior, it may not be the predominant site of action of NPY. NPY may therefore facilitate recovery of function by stimulating areas outside of the LH.

320.16 EFFECTS OF BILATERAL PARTIAL MIDBRAIN TRANSECTIONS ON FEEDING AND DRINKING INDUCED BY NEUROPEPTIDE Y (NPY) IN RATS. JL Steinman, MW Gunion & JE Morley. GRECC, VAMC, Sepulveda, CA 91343.

NPY robustly increases both food and water intake (FI,WI) when infused into the lateral or 3rd ventricle (3V) or directly into hypothalamic regions in rats. In addition to forebrain modulation of FI & WI, we recently found that brainstem (4V) infusion of NPY is equally effective in elevating FI & WI. The present study asked whether rostro-caudal fibers connecting 4V areas to feeding-related forebrain areas mediate the effects of 4V-NPY on FI & WI.

Rats were implanted with 3V or 4V cannulae 7 days after receiving bilateral midbrain knife cuts (KC) or sham treatment. Groups are abbreviated as follows: 3V+KC = 3K; 3V+Sham = 3S; 4V+KC = 4K; 4V+Sham = 4S. One week after cannulation, testing began with 0, 2.5, 5 or 10 ug NPY/3 ul saline-BSA given in a counterbalanced design. FI & WI were measured 1 & 2 hr post infusion. Partial midbrain transections reduced the effect of 4V infusions of NPY on both FI & WI and of 3V infusions of NPY on WI only.

NPY significantly elevated 2-hr cumulative FI in a dose-dependent fashion in all groups of rats. Difference scores (DS) were calculated, subtracting FI after 0 dose infusions from FI after NPY (2.5, 5 or 10 ug doses) infusions. When comparisons were made of the DS, the effect of NPY on FI was reduced significantly in 4K as compared to 4S rats (see below). KC did not alter the increase in FI after 3V infusions of NPY. KCs also affected NPY-induced drinking. While NPY increased WI in both 3S & 4S groups, this increase was attenuated significantly in 3K & 4K groups (see below).

Increased FI after brainstem stimulation by NPY probably requires fiber connections to the forebrain which were severed in this experiment, while forebrain stimulation by NPY does not require these or adjacent fibers. By contrast, the dipsogenic effects of NPY, whether induced at sites near 3V or 4V, appear to depend on fibers traversing through the midbrain.

NPY INCREASES FI & WI ABOVE 0 (CONTROL) LEVELS

	group	DS (2.5 - 0)	DS (5.0 - 0)	DS (10.0 - 0)
FI (g/hr) X ± sem	3S	1.1±.7	2.1±.9	3.7±.7
	3K	1.9±.5	2.6±.6	2.2±.7
	4S	2.1±.9	2.8±.8	4.1±.9
	4K	0.6±.7	0.4±.8	1.7±.8*
WI (ml/hr) X ± sem	3S	1.7±.7	3.5±.7	3.8±.9
	3K	2.1±.5	1.0±.6*	-0.8±.9**
	4S	3.5±.8	3.4±.6	3.5±.9
	4K	0.8±.8*	0.5±.8**	0.9±.8*

*: $p<.05$; **: $p<.01$, as compared to sham controls at each dose.

320.17 EFFECT OF NEUROPEPTIDE Y ON INGESTIVE BEHAVIORS OF RATS FOLLOWING AREA POSTREMA/NUCLEUS TRACTUS SOLITARIUS ABLATION. C. Shawn Kreig*, Jon N. Kott, Nancy J. Kenney and Stephen C. Woods. Dept. Psychology Univ. Washington Seattle, WA 98195.

Following ablation of the area postrema and subjacent caudal-medial aspect of the nucleus tractus solitarius (AP/cmNTS), rats decrease food intake and body weight. Cell bodies of the NTS contain neuropeptide Y (NPY), a brain-gut peptide which acts centrally to increase food and water intake. This study examines the effect of central administration of NPY on food and water intake of AP/cmNTS-lesioned rats.

Seventeen male Long-Evans rats were fitted with lateral ventricle (LV) cannulae under pentobarbital anesthesia. One week later, 8 rats received thermal AP/cmNTS ablations and 9, sham lesions under pentobarbital anesthesia. Rats were maintained on ad lib pelleted chow and water throughout the study Testing began 5 days after the second surgery. On test day 3, each rat received 5 ug NPY in 1 ul isotonic saline. On test days 1, 2 and 4 (control days), each rat received an equivolume injection of isotonic saline. Food and water intake were measured .5, 1, 2, 4 and 24 hr following the LV injections.

Food Intake: During the first 30 min following injection, food intakes of both sham- and AP/cmNTS-lesioned rats were greater on the NPY test day than on control days ($p<.01$ in each case). Four hours after NPY injection, cumulative food intakes of both groups remained elevated ($p<.01$ for each comparison). Total 4-hr intakes of lesioned rats did not differ from those of sham-lesioned animals. Rats in both groups compensated for the initial increase of feeding on the NPY test day by decreasing food intake between 4 and 24 hr after the injection ($p<.01$). Total 24-hr food intake on the NPY test day did not differ from that on control days for either group. AP/cmNTS-lesioned rats did eat less than controls over the entire 24-hr following NPY injection as is typical of the total daily intake of rats during the first 2 weeks following AP/cmNTS ablation ($p<.01$).

Water Intake: NPY treatment resulted in increased water intakes by both lesioned and sham-lesioned rats during the first 4 hr after injection ($p<.05$ for each comparison). Water intake was decreased from 4-24 hr following NPY administration such that total 24-hr water intakes of both lesioned and sham-lesioned rats on the NPY test day did not differ from those on saline control days. Cumulative water intakes of lesioned rats did not differ from those of sham-lesioned rats at either 4 or 24 hr on either the NPY test day or on control days.

These data suggest that AP/cmNTS ablation does not destroy responsiveness to the ingestive effects of LV NPY.

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320.18 BRAIN SEROTONIN AND CARBOHYDRATE APPETITE: DOES THIS PHYSIOLOGICAL CORRELATION OCCUR IN INSECTS? R.W. Cohen, S. Friedman and G.P. Waldbauer. Dept. of Entomology, University of Illinois, Urbana, IL 61801.

The neurotransmitter serotonin (5-hydroxytryptamine) has been implicated in the control of carbohydrate appetite in rats and humans. It is well known that insects produce serotonin, and research has shown that serotonin has functions that generally parallel those in mammals. Thus, it may be asked whether serotonin is involved in determining the level of carbohydrate intake by insects.

One possible candidate for the study is the corn earworm, Heliothis zea (Lepidoptera: Noctuidae), a major pest of field crops in the Western Hemisphere. A previous study (Waldbauer et al., Physiol. Zool. 57: 590, 1984) has shown that final instar corn earworm larvae, like laboratory rats, have the ability to self-select a balanced diet from a choice of two diet cubes which are identical except one lacks only protein and the other lacks a digestible carbohydrate. These larvae consume a ratio by weight of about 80% protein and 20% carbohydrate. Direct measurements of brain serotonin, using an HPLC assay, show that an increase in brain serotonin levels is correlated with increased carbohydrate consumption by the caterpillars. Brain serotonin levels are diminished in larvae that fed on protein diets. To assess the impact of brain serotonin levels on actual carbohydrate intake, we performed two pharmacological experiments involving the incorporation of p-chlorophenylalanine (PCPA), a known mammalian inhibitor of serotonin, and tryptophan (serotonin precursor) into diet cubes of self-selecting larvae. Larvae feeding on PCPA-treated diets had reduced brain serotonin levels and increased their carbohydrate consumption, whereas those feeding on tryptophan-treated diets decreased their carbohydrate consumption accompanied by a relatively large increase in brain serotonin levels. The data suggest that this correlation between serotonin and carbohydrate appetite may be prevalent throughout the animal kingdom. The correlation also suggests that nutrient self-selection is mediated by internal feedback involving neurotransmitters which monitors nutrient intake.

320.19 SEROTONIN ANTAGONISTS AND FEEDING. Paul J. Fletcher* (SPON: T. Wishart). Psychiatric Research Div., Saskatchewan Health, CMR Bldg., Univ. of Saskatchewan, Saskatoon, Sask., S7N OWO, Canada.

It has been proposed that 5-HT systems are involved in the control of satiety. However, in contrast to the well-documented anorectic effects of direct and indirect 5-HT agonists only a small number of reports have described increased food intake following treatment with 5-HT antagonists. The present experiments examined the effects of two 5-HT antagonists, methysergide and cyproheptadine, in three different paradigms.

In the first paradigm non-deprived male Sprague-Dawley rats were given daily access to a 35% sucrose solution. Methysergide (1.25, 2.5 and 5 mg/kg) did not alter sucrose intake over the first 1/2 h of testing, but at 1 h and 2 h intake was reduced by 5 mg/kg methysergide. Cyproheptadine (2.5, 5, 10 mg/kg) also failed to alter sucrose intake at 1/2 h but a dose-dependent decrease was observed at 2 h. In a second paradigm rats were given daily access to a sweetened wet mash diet. Methysergide did not alter food intake over the first 1/2 h, but a dose of 5 mg/kg reduced intake at 1 and 2 h. Cyproheptadine induced a dose-dependent decrease in intake at 1/2 h, but this effect was not apparent at 2 h. The failure to observe an increased intake was not due to an insensitivity of the tests employed since chlordiazepoxide (5 and 10 mg/kg) markedly increased the consumption of sucrose and the sweetened mash diet. In a third paradigm methysergide and cyproheptadine were administered after 1/2 h access to a sweetened mash diet, a procedure which induced satiety in vehicle treated animals. Methysergide significantly enhanced subsequent food intake. Cyproheptadine induced a modest increase in food intake in this test. Chlordiazepoxide markedly increased food intake in satiated animals.

These results show that 5-HT antagonists can induce decreases, increases or no change in food intake depending upon the feeding paradigm employed, and the time period examined. This indicates a complex relationship between 5-HT antagonism and feeding. However, since increased food intake following treatment with 5-HT antagonists was observed only in fully satiated animals this may support the hypothesis that 5-HT systems are involved in the process of satiety.

(Supported by Saskatchewan Health and the Saskatchewan Health Research Board).

320.20 ZINC DEFICIENCY CONDITIONS TASTE AVERSIONS IN RATS. D. S. Cannon*, L. E. Carrell* and I. L. Crawford. Departments of Psychiatry and Neurology, University of Texas Health Science Center at Dallas and Veterans Administration Medical Center, Dallas, TX 75216.

Decreased food consumption by zinc deficient rats is usually assumed to be the result of an anorexigenic pathophysiological state (e.g., Essatara, M. et al., Physiol. & Behav., 32:475, 1984). The present study investigated the possibility this decreased food intake is due to learned aversion to the deficient diet rather than to anorexia. Similar learned aversions have been demonstrated following other dietary deficiencies (Rozin, P. & Kalat, J.W., Psychol. Rev., 78:459, 1971). Three groups of Sprague-Dawley rats were studied. One group (ZD) was fed ad libitum a biotin-enriched Zn deficient diet containing 20% spray dried egg white and <1 ppm ZN (Wallwork, J.C. & Crawford, I.L., Fed. Proc., 46:885, 1987). Two control groups were fed a similar diet containing 25 ppm Zn. One control group was fed ad libitum (AL) and the second group was pair fed (PF) the mean consumption of ZD rats the previous day. After 3 weeks, an assay of plasma Zn from tail blood documented metal deficiency in Group ZD. The means of Groups ZD, PF, and AL, respectively, were 0.46, 1.14, and 1.18 ug/ml, $F(2,25)=46.2$, $p<.001$ All animals were then offered a zinc-normal lab chow with which they were familiar. The means of Groups ZD, PF and AL, respectively, during the first hour the lab chow was presented were 6.8, 9.2 and 1.8 g, $F(2.27)=46.2$, $p<.001$; and during the first day, 16.8, 26.0, and 21.8 g, $F(2.27)=13.9$, $p<.001$. To control for the effect of group differences in body weight, first day consumption was reanalyzed using analysis of covariance with body weight as the covariate. The residual means for the three groups were 24.1, 28.4 and 12.1g, $F(2.26)=14.9$, $p<.001$. The 1 hour and weight-adjusted 24 hour data suggest there was little general anorexia in Group ZD. After 2 weeks on the zinc-normal diet, plasma Zn in Group ZD had returned to normal levels. All groups then were offered the Zn-deficient diet, and Group ZD consumed significantly less than either of the other groups. Means of Groups ZD, PF and AL, respectively, were 5.3, 19.2, and 20.7 g, $F(2.27)=47.3$, $p<.001$. Even with weight as the covariate, Group ZD consumed significantly less than either other group. These results indicate Zn deficiency conditions aversion to associated foods.

CONTROL OF POSTURE AND MOVEMENT VII

321.1 ON A NEW ANALYTICAL APPROACH TO THE STABILITY AND PROGRESSION OF A BIPED. Y. Hurmuzlu* and G.D. Moskowitz* (SPON: W. Freedman) Drexel University, Department of Mechanical Engineering and Mechanics, Philadelphia, PA 19104.

The missing factor in all previous locomotion studies is a complete understanding of the role of the impact of the limbs with the ground and switching of the body weight from one limb to the other in the overall locomotion picture. In this work a numerical code was developed for modelling the dynamics of a general multibody chain system, which is based on the kinetostatic approach (Vukobratovic, M. and Potkonjak, V., Springer Verlag, 1982). The impact and switching effects were included in the model. The stability of the nonlinear system was studied by utilizing phase plane portraits (i.e. a plot of the joint velocities versus the corresponding joint angles). Impact and switching play the most important role in achieving a stable gait. The dynamics of an inverted pendulum is modified by the occurrence of impact and switching, which results in asymptotically stable limit cycles on the phase plane portrait. The proposed ideas and principles have already been verified for three and four element, three dimensional models. To demonstrate the ideas clearly, without getting lost in the details of a very complicated model, a three element, two dimensional example was used. The system proves to be asymptotically stable for a wide range of perturbations in joint velocities and rotations. The regions of stability and instability are presented on an overall phase plane portrait.

321.2 SCALING CHARACTERISTICS OF MUSCLE ANTAGONISTS IN ACCURATE, STEP ISOMETRIC CONTRACTIONS. D. M. Corcos, G. L. Gottlieb, G. C. Agarwal and B. Flaherty*, Dept. of Physical Education, University of Illinois at Chicago and Depts. of Neurosurgery and Physiology, Rush Medical College, Chicago, IL 60612.

We have previously shown that agonist EMG quantity increases as movement distance and target width increase. This is because greater forces can be generated for longer movements and for movements to larger targets. The findings for antagonist muscles are less clear since larger EMG quantities are not associated with longer movements in all subjects. We have also shown that agonist and antagonist EMG increases as inertial load increases. The increases in muscle activation are related to the increased inertial forces involved in the movement. Since both these paradigms are complicated by the fact that muscle length is changing, we conducted a set of isometric experiments to ascertain the relative scaling of muscle groups when force and force precision are manipulated.

Subjects generated isometric step flexion contractions at 18, 36, 54 and 72% of their maximal voluntary contraction (MVC) at four levels of force precision (3, 6, 9 and 12% of MVC). This paradigm is the isometric equivalent of a Fitts' task in which different distance movements are made to different size targets. Four surface EMGs were recorded from biceps, brachioradialis, lateral head of triceps and long head of triceps. EMG quantity increased in both agonist muscles as force increased. In some subjects, as force increased, both antagonist muscles decreased in quantity. Least antagonist EMG occurred for MVC. In other subjects, agonist and antagonist EMG both increased as greater force was generated with most EMG for MVC. We are currently investigating the extent to which these different "strategies" for antagonist muscle activation are modifiable by changes in how the task is performed.

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321.3 HOW DO HUMANS MODULATE THE STRIDELENGTH DURING LOCOMOTION? Aftab F. Patla, J.M. Silviera*, Neural Control Lab, Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1

One of the most common adaptation that we have to make during locomotion is the alteration of the stridelength to meet the demands imposed by the terrain. Based on kinematic measures Warren et al. (J. Exp. Psych., 12:259, 1986) proposed that stridelength is controlled by modulating the vertical impulse applied during the stance phase. Power analysis during locomotion (Winter, D., J. Motor Behav., 15:302, 1983) suggests three possible mechanisms for the control of stridelength: 1) "push off" during late stance; 2) "pull off" during early swing; 3) "braking" during late swing. The purpose of this study was to examine the modulation of the muscle activity patterns when subjects are asked to voluntarily lengthen their stridelength.

Subjects (n=6) walked on a treadmill at their normal self paced speed. They were instructed to increase their ipsilateral stridelength and break a photocell switch placed at the end of the treadmill when a buzzer, triggered by the ipsilateral heel switch sounded. The buzzer came on unexpectedly and randomly at heel contact (HC) or toe off (TO). EMG signals from the tibialis anterior (TA), medial gastrocnemius (MG), biceps femoris (BF), vastus lateralis (VL), rectus femoris (RF), along with footswitch signals and the stimulus plus photo cell signal were collected. For each condition (HC & TO) the strides during which the subject altered the stridelength (P) as indicated by the photocell signal, and normal strides (N) were rubberbanded (to 100%) and ensemble averaged. Following this, average EMG (AEMG) values were calculated for the following durations in the stride: 30-50%, 50-70% and 80-100% representing push off, pull off and braking action. Ratios of AEMG in "P" over AEMG in "N" were calculated for each of the three durations and compared to ratios of two separate "N" strides.

The most consistent (at least 4 subjects) changes in the EMG for the HC condition were: 1) increase in the push off action (↑MG, ↑BF, ↑VL - extensor activity at the ankle, knee and hip); 2) increase in the pull off action (↑TA, ↑MG, ↑BF, ↑RF - flexor activity at the ankle, knee and hip), and 3) reduced braking action (↓BF) and increased knee extension (↑VL). These results suggest that we can and do alter more than the vertical impulse to alter stridelength. The interesting aspect of the results in the TO condition was the fact that 4 subjects were unable to change their stridelength in the cycle when the buzzer sounded. These subjects showed minimal changes in the stride when the buzzer sounded. The TO condition presents subjects with only one option to alter their stridelength: reduced braking action. Thus these results suggest that at least two of the three options (↑push off, ↑pull off, ↓braking) are required to lengthen the stridelength.

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321.4 AGONIST AND ANTAGONIST ACTIONS DURING THE DYNAMIC PHASE OF HUMAN STEPPING. L. Carrière and A. Beuter. Dept. de Kinanthropologie, Université du Québec à Montréal, Montréal, P.Q., H3C 3P8.

In order to grasp the relationship between electrophysiological (EMG) activation patterns and the corresponding Kinematic properties it is important to take into account the conditions under which muscles act on the bones. The purpose of this study was to examine the relationships between EMG activation patterns and kinematics of the dynamic phase of stepping performed at natural cadence. Kinematics and EMG parameters were analyzed on six subjects stepping over an obstacle of three different heights (25, 50 and 75% of shank length). A videomotion analysis system was used to track the displacement of reflective markers on the lower limb joints. Surface bipolar electrodes were secured on the rectus (RF) biceps (BF) femoris and semitendinosus (ST) muscles. Electrokinematic phase plots generated with EMG and kinematic data revealed a generally consistent pattern across subjects although cocontraction became larger in the landing phase and the diagrams were scaled up as obstacle height increased. The duration of the flexion phase was not affected by obstacle height. The bursts generally appear in the following order: BF-ST (simultaneously), RF during the flexion phase, and RF and BF-ST (simultaneously) in the extension phase. In most subjects there was very little EMG activity during the transition from flexion to extension. While the activation patterns were similar across subjects, the amplitude and duration of the different EMG episodes varied and influenced the shapes of the electrokinematic phase plots. In most subjects, there seemed to be a consistent timing in reaching knee flexion velocity across height conditions but there was a shift toward earlier knee extension velocity. This pattern is reversed in one subject and seems to be due to BF activity after the transition phase. Results indicate that in general during the dynamic phase of stepping the activated biarticular muscle acts as an agonist on the distal joint whereas it acts as an antagonist on the proximal joint, hence the CNS seems to favor the control of the distal joint actions. From the results it appears that the subjects used either a continuous feedback control mode or a more ballistic control mode in which feedback was used mostly in the final phase of motion across all conditions.

321.5 RELATIONSHIP BETWEEN INTERNAL AND EXTERNAL FORCES ACTING ON THE CAT HINDLIMB DURING OVERGROUND LOCOMOTION. E.G. Fowler*, R.J. Gregor, R.R. Roy, J. Hodgson* and J. Broker*. Kinesiology Department and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Three components of the ground reaction force (GRF) have been reported by Manter for a single cat (3.3 kg) during overground locomotion (J. Exp. Biol. 15:522-540, 1938). Recently, several investigators have reported tendon forces from selected hindlimb muscles during treadmill locomotion (J. Neurophysiol. 41:1203-1216, 1978; J. Biomech. 17:685-694, 1984).

The purpose of this investigation was to evaluate hindlimb kinetics by measuring both GRFs and muscle forces during overground locomotion in the cat. The right achilles, left plantaris, and both tibialis anterior tendons of a 4.2 kg cat were implanted with tendon buckle transducers. The achilles tendon was implanted with a modified oval buckle (J. Biomech. 16:691-701, 1983) while all other tendons were implanted with an E shaped buckle (J. Neurophysiol. 41:1203-1216, 1978). The floor of a plexiglass enclosed walkway was instrumented with a specially designed force platform. Two piezoelectric elements (Kistler 9251A) capable of measuring vertical (Fz) and horizontal (Fy) components of the GRF were secured between two metal plates. The force platform was isolated from the surrounding walkway and a piece of wood identical to the floor surface was attached to the top plate for concealment. The cat then stood and walked over the platform while high speed films (100 fps) were taken and synchronized to tendon forces and GRF records. Separate trials were then recorded for right and left hindlimb contact with the force platform during the support phase of slow walking.

Peak Fz during stance was 17.3N for the right and 23.1N for the left hindlimb. Peak achilles tendon force was 20.7N while peak plantaris forces was 13.6N. Peak tibialis anterior forces were 3.9N on the right and 3.6N on the left side.

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321.6 STRIDE LENGTH VARIABILITY AND THE AVERAGE VELOCITY OF HUMAN GAIT. N.H.Mayer, M.Ridenour* and L.Kent*. Temple University and Moss Rehabilitation Hospital, Philadelphia, PA 19141

Movement whose endpoint is a target with explicit visual boundaries was described by Fitts as having a loglinear relationship between movement time (MT), movement amplitude (A), and accuracy (W, target width), ($MT=a+b\log_2[2A/W]$). For movements where endpoint targets are not guided by explicit visual boundaries, Schmidt proposes that accuracy is proportional to the average velocity of the subject's movements ($W_e=a+b[A/MT]$), where W_e is the effective target width. This study examines the Schmidt model with respect to a human locomotion paradigm, where visual guidance of endpoint accuracy is usually absent. Using analogous measures of locomotion, we applied Schmidt's model in the following hypothesis: If stride time and stride length are controlled by the experimenter as independent variables but the accuracy of the stride is not visually monitored by the subject, then stride length variability will be proportional to the average gait velocity.

Fifteen healthy adult female subjects participated in this study. Each test consisted of 16 random combinations of stride length and stride time repeated three times, for a total of 48 trials. Stride length ranged from .8 to 1.6m, stride time ranged from 8 to .7s, with a projected velocity ranging from .1 to 2.2m/s. Subjects paced on an electronic walkway by the auditory cue of a metronome, which indicated stride time, and a visual cue (a .125m width floor marker), which indicated stride length. Subjects were instructed to be as accurate as possible to both the marker and the beat.

Both stride time and stride length cues had significant effects on the variability of actual stride length. A linear relationship was observed between stride length variability and the velocity of walking (r=.9), i.e. the ratio of stride length and stride time.

Schmidt's model is supported by the results of this study, namely that stride length variability is proportional to average gait velocity when endpoint accuracy is not guided by explicit visual boundaries. However, compared to the upper limb tasks that led to the development of Fitts' and Schmidt's models, interpretation of endpoint variability may be more complicated in lower limb tasks such as gait.

321.7 SPEED-RELATED CHANGES IN HINDLIMB INTERSEGMENTAL DYNAMICS DURING THE SWING PHASE OF LOCOMOTION. D. Wisleder*, J. L. Smith, and R. F. Zernicke. Laboratory of Neuromotor Control, Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

Although motion-dependent feedback from the hindlimb can affect the stereotypic output of lumbosacral central pattern generators during locomotion, basic synergies and many details of the neuromuscular pattern remain after limb deafferentation (*e.g.,* Grillner, S. & Wallen, P., *Ann. Rev. Neurosci.* 8: 233-261, 1985). Nevertheless, some elements of the deafferented pattern show variations that may be related to speed-dependent modulations in hindlimb intersegmental dynamics during the swing phase of locomotion. To examine possible reasons for these variations, rigid-body dynamics can be used to quantify how the trajectories of limb segments can be influenced by muscle contractions, gravitational forces, and interactive forces that are related to angular and linear velocities and accelerations of limb segments (*e.g.,* Smith, J.L. & Zernicke, R.F., *Trends NeuroSci.*, 10: 123-128, 1987). Preliminary data for normal cats suggest that significant interactions occur between motion-dependent torques and muscle torques during the swing phase of locomotion (Hoy, M.G. & Zernicke, R.F., *J. Biomechanics* 18: 49-60, 1985). These initial data, however, were collected for a limited range of speeds, and our present study subtantially extends those initial data by examining the coordination of muscular and interactive hindlimb torques for a wide range of locomotor speeds and gait modes.

Normal adult cats (2.4-3.0 kg) were trained to locomote on a motorized treadmill at speeds (0.4 to 3.0 m s^{-1}) that elicited walking, trotting, and galloping gaits. Hindlimb trajectories were recorded, using high-speed ciné film (100 frames s^{-1}), for at least three different speeds within each of the three gait modes. Joint centers were digitized, and angular and linear kinematic data were smoothed and differentiated (Hatze, H. *J. Biomechanics* 14: 13-18, 1981). A planar, three-segment (thigh, leg, and paw) rigid-body model of the cat hindlimb (Hoy, M.G. & Zernicke, R.F., *J. Biomechanics* 19: 867-877, 1986) was used to examine intersegmental dynamics during the swing phase of locomotion.

With increases in speed of locomotion, the intralimb interactive torques and muscular torques increased significantly. In particular, at the knee joint during the second half of swing when the paw is lowered for contact by ankle extension (E-1 phase), torques arising from leg angular acceleration tended to extend the knee, while a flexor muscle torque at the knee counteracted that tendency. With slow walking, interactive knee joint torques may have been sufficient to decelerate knee extension without requiring contractions of posterior thigh muscles, such as the semitendinosus. At higher speeds of locomotion, however, the knee torques related to leg angular acceleration increased significantly, and substantially elevated knee flexor torques were needed to counteract the tendency of the knee to extend. Supported by NIH grant NS 19864.

321.8 ROLE OF CENTRAL AND PERIPHERAL MECHANISMS IN THE PAW-SHAKE RESPONSE. G.F. Koshland and J.L. Smith. Department of Kinesiology, UCLA, Los Angeles, CA 90024

Our previous work demonstrated that paw-shake responses could be elicited in chronic spinal cats after extensive deafferentation (DEAFF) of the hindlimb (Koshland *et al., Neurosci. Abst.* 1983). In this study, we examined details of the neuromuscular patterns preserved or altered after DEAFF. For the control condition, responses were evaluated in five adult cats recorded three months after spinalization (T-12) and then three to five months after unilateral ganglionectomy (L3-S1) for the experimental condition. Responses were elicited in spinal cats by wrapping tape around the paw, but after DEAFF, the paw was insentient, but noxious stimuli applied to an area of the upper thigh consistent with the S2 receptive field evoked similar multicycle responses.

After DEAFF, responses were difficult to evoke, and data from one cat were excluded because only single-burst reponses were elicited. On average, the number of cycles per response in the DEAFF limb was 5 ±2 cycles, or five cycles less than that during control responses (10 ±2 cycles). Cycle periods, defined as the interval between successive lateral gastrocnemius (LG) onsets, were more variable and longer (DEAFF 125 ±32 ms; control 98 ±15 ms). After DEAFF, cycle periods within a single response remained relatively constant over successive cycles, while under control conditions, the duration of successive cycle periods increased linearly from 68 ms to 112 ms.

With DEAFF, LG burst duration was unchanged as brief bursts of 40 ms occurred during each cycle. In contrast, activity of the tibialis anterior (TA) and vastus lateralis (VL) was altered by DEAFF. With feedback, TA and VL burst durations increased with increasing cycle periods, but after DEAFF, burst durations of both muscles were brief (35-45 ms) and unaffected by cycle period. These results suggest that spinal mechanisms in the DEAFF cat set muscle activation at a minimal duration, but with motion-dependent feedback, TA and VL burst durations are modulated by and related to the limb dynamics as well as the cycle period.

Some aspects of the paw-shake synergy were also altered. In the DEAFF limb, TA onset occurred later in the cycle and preceded the LG burst by 26 ±10 ms, regardless of cycle period. Under control conditions, TA onset occurred earlier in the cycle, and the interval preceding the LG burst was correlated with TA burst duration ($r = 0.63$) and cycle period ($r = 0.81$). Under both conditions, LG onset occurred 15 ms before TA offset, and the resulting cocontraction was an immutable feature of the pattern. In most DEAFF responses, VL and TA were coactive, similar to the mixed synergy described for control conditions (Smith *et al., J. Neurophysiol.* 54: 1271-1281, 1985). In 20% of the responses from three DEAFF cats, however, VL onset was delayed and coincidental with LG bursts, similar to the extensor synergy more typical of walking and scratching in the cat.

The results suggest that spinal networks responsible for the paw-shake response set minimal criteria, while hindlimb afference modifies the temporal structure of successive cycles, burst durations of the TA and VL, and possibly the intralimb synergy. Supported by a NIH grant, NS 19864.

321.9 **RHYTHMIC MODULATION OF CUTANEOUS AFFERENT MEMBRANE POTENTIAL DURING FICTIVE LOCOMOTION IN THE CAT.** J.P. Gossard *, J.-M. Cabelguen *, P.Saltiel * , T. Drew , S. Rossignol , Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec, Canada, H3C-3J7.

Our previous studies have shown that there is a rhythmic modulation of dorsal root potentials (DRPs) during fictive locomotion in the cat and that even individual primary afferent fibres may antidromically discharge rhythmically. The aim of the present project was to study the spontaneous and evoked presynaptic events in single, identified, cutaneous primary afferents during fictive locomotion.

Nineteen cats were decorticated under steroid anaesthesia and paralysed. Locomotion-related activity was recorded from selected hindlimb muscle nerves (Sartorius and Vastus Lateralis) as well as from the proximal stump of the cut dorsal root (L6) using Ag/AgCl electrodes. Intracellular DC recordings of primary afferent fibers were made with glass micropipettes filled with potassium citrate. Impaled afferent fibres whose membrane potential was greater than 45 mV were identified by high frequency (>700Hz) stimulation of Peroneal Superficialis and Tibialis Posterior nerves. Primary afferent depolarisation (PAD) was evoked in the identified fibre by stimulation of the other cutaneous nerve (single pulse, 200 μs, 60-150 μA).

Forty percent of the 140 units retained showed clear rhythmic fluctuations of their membrane potential at the periodicity of locomotion. The majority of them were not spontaneously discharging. Typically, there were two peaks of depolarisation per cycle. The first, and usually the largest, occurred near the maximum of flexor activity, the second, near the peak of extensor activity. The relative amplitude of each depolarisation could vary from one afferent to another in the same experiment although the extracellularly recorded DRPs did not change. Each peak of depolarisation was followed by a trough of hyperpolarisation. The first one was synchronous with the flexion-extension transition, the second one, with the extension-flexion transition. Some units showed only the flexion-related depolarisation and the subsequent hyperpolarisation. Furthermore, it was possible to evaluate the changes in the amplitude of evoked PADs throughout the locomotor cycle. PADs were minimal during the flexor activity, increased during the flexion-extension transition and reached their maximum amplitude during the period of extensor activity.

These results thus indicate that central networks responsible for the generation of locomotion cyclically modulate the membrane potential of cutaneous primary afferent fibres as part of the locomotor program. The maximal depolarisation of the cutaneous fibres during the period of flexor activity suggests that the efficacy of transmission in cutaneous pathways would be reduced at the presynaptic level in that phase. This might be reflected in the reduction of PADs evoked between cutaneous fibres in that period. The relative importance of these central mechanisms as well as movement-related mechanisms in the control of cutaneous reflexes during real locomotion is under study. (Funded by the MRC; J.P.G. and P.S. are supported by the MRC; J.-M.C. supported by CNRC-CNRS; T.D. is a FRSQ Scholar).

321.10 INTERLIMB COORDINATION OF REPETETIVE RHTYMIC MOVEMENTS. A.M. Gentile and R.A. States* Teachers College, Columbia University, NY,NY 10027.

Prior research has indicated potency of the walking cycle in determining upper limb movement cycle (Craik, R. et al. in R.M. Herman et al., Neural Control of Locomotion, 1976; Muzzi et al., Human Movement Sciences, 3:337-354, 1984). The present study examined coordinative patterns in a non-locomotor task involving all four limbs to elucidate conditions under which coupling strength varies.

Four variations of a task involving cotemporaneous rhythmic movement of the upper and lower limbs were tested using 22 Ss. Single- or multi-joint claps were combined with alternating single-joint foot taps or multi-joint "step-like" movements. All testing was done with Ss seated at a table. Timing of clapping and tapping movements was measured by recording contact of the Ss' hands with each other and contact of the feet with floor plates. For each S, time of hand contact was plotted against normalized lower limb cycle using foot contact as the central reference point. The standard deviation of that distribution was used as a measure of coordinative linkage.

Results indicated significant differences in coordinative linkage when the upper and lower limbs were engaged in movements of different extents. Large clapping movements combined with small foot taps yielded little linkage between the two cycles, whereas large "step-like" movements appeared to entrain clapping cycles. One explanation for these results is that unlike large arm movements, large multi-joint leg movements activate a locomotor synergy that promotes tightly coupled coordination of all four limbs.

An ancillary finding showed that coordinative linkage was a stable characteristic for individuals. Retesting of the small clapping and tapping task after a two month interval produced high correlations for coordinative linkage (.74), clap and tap periods (.94 & .88), and tap variability (.74).

321.11 **Cutaneous Reflexes Evoked by Mechanical Stimulation During Locomotion in the Same Chronically Implanted Cats Before and After Spinalisation.** M. Bélanger, T. Drew, J. Provencher* and S. Rossignol. Centre de Recherche en Sciences Neurologiques, Dépt de Physiol., Univ. de Montréal, Montréal, Québec, Canada, H3C-3J7.

Previous studies have shown that adult cats can walk with the hindlimbs following spinalisation at T13. To study the adaptability of this spinal locomotion, we examined the responses to mechanical perturbation of the hindlimb during locomotion in the same chronically implanted cats before and after spinalisation.

EMG activity was recorded from flexor and extensor muscles of the hindlimbs while cats walked on a treadmill at speeds of 0.3-0.4 m/s. A digital time code allowed the EMG signals to be synchronized with a simultaneous video recording. Mechanical stimulation to the dorsum of the paw was applied at random during the swing phase of locomotion with a rigid rod to which a microswitch was attached. After a period of 7-9 weeks of control experiments the cats were spinalised at either T10 or T13.

In the pre-spinalised condition, mechanical stimulation evoked an initial locking of the ankle which was followed by pronounced knee flexion. Subsequent flexion of the ankle joint further removed the foot from the stimulation. On the other hand, the hip angular movement was little affected by this stimulation. The EMG responses to mechanical perturbations were relatively similar to those observed in previous studies of intact cat (Wand et al. **Exp. Brain Res.**, 38: 109-114, 1980). Short latency excitatory responses in an ankle flexor (TA) (8 ms) and extensor (GL)(20 ms) muscles acted to stabilize the ankle joint. As importantly, there were also very large short latency excitatory responses in FDL which undoubtedly contribute to the locking of the ankle. The knee flexion is at least partly explained by the very large responses evoked in St (15 ms). Small excitatory responses were also observed in the hip flexor muscles (Srt & Ip) (12 ms). Secondary responses (>50 ms) were seen in several muscles. They were especially marked in the TA, as observed by Wand et al. (1980), and were probably responsible for the subsequent ankle flexion.

Kinematic analysis of the post-spinalised perturbed cycles showed that the joint angular patterns were very similar to those observed before spinalisation. However, the degree of knee flexion observed was much greater so that the ankle joint was raised above the knee joint. Overall the post-spinalised EMG responses were similar to those observed prior to spinalisation. However, secondary responses, especially in TA, were notably decreased.

In summary, the complexity and timing of joint angular movements and EMG responses to mechanical stimulation were preserved after spinalisation. This demonstrates that the spinal cord can not only generate the basic locomotor rhythm but is also capable of integrating the sensory inputs arising from a perturbation to produce a functional and well adapted compensatory response.(Funded by the Canadian MRC, T.D. is Funded by FRSQ and M.B. is supported by NSERC).

321.12 MAPPING BASAL FOREBRAIN SITES AT WHICH ELECTRICAL STIMULATION ELICITS LOCOMOTOR STEPPING IN THE ANESTHETIZED RAT. H.M. Sinnamon. Lab. of Neuropsych., Wesleyan University, Middletown, CT 06457.

The basal forebrain has been implicated in the initiation of locomotion. This study attempts to localize the critical regions by mapping with low current levels. Rats were initially anesthetized with a barbiturate (25 mg/kg) and mounted in a stereotaxic apparatus such that electrically-elicited stepping movements turned a wheel. Maintenance anesthesia was provided by periodic intraperitoneal injections of Nembutal (7 mg/kg) and injections of Xylocaine into the scalp incision. The basal forebrain was stimulated with 50 Hz, 0.5-duration pulses at currents of 25 and 50 uA in trains of 10-sec duration delivered through glass pipettes, 50-70-um tip diameter, filled with either 5M Sodium Acetate or 2M Sodium Chloride. Stimulation was applied at 200 um steps, first with three 10-sec trains of 50 uA and next with three 25 uA trains. If they were negative, the general ability of the rat to locomote was confirmed by stimulating the posterior hypothalamus through a fixed electrode. The pipette was fixed at the deepest point in the last penetration and it was the reference in constructing the map.

The best studied region to date is at the level of the posterior medial preoptic area; it is represented by 195 stimulation sites in 12 electrode penetrations. With 25 uA, locomotion was elicited by stimulation in a confined cluster of sites centered in the lateral hypothalamus. The lateral edge of the cluster merged only as far as the medial aspect of the substantia innominata/ventral pallidum. Increased currents (50 uA) increased the lateral and medial extent of the lateral hypothalamic cluster only slightly but did reveal another cluster around the nucleus reuniens of the anterior thalamus. Poststimulation locomotion was associated with sites in the bed nucleus of the stria terminalis. Bracing responses, characterized by pelvic flexion and forelimb extension was consistently elicited by stimulation of the internal capsule and the ventral pallidum. The general pattern of results is consistent with proposal that activation of axons originating in the lateral preoptic area can produce locomotion in this preparation.

321.13 DEVELOPMENT OF THE PEDUNCULOPONTINE NUCLEUS. II. IN VITRO. R. D. Skinner, D. L. Davies, C. Conrad*, V. Henderson* and E. Garcia-Rill (SPON. J. Johnson), Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Cholinergic neurons in the pedunculopontine nucleus (PPN) (and laterodorsal tegmental nucleus - LDT) are known to be selectively labeled using the NADPH diaphorase histochemical technique (Vincent et al. '83). NADPH diaphorase labeling of PPN and LDT neurons is evident at the time of birth (see accompanying abstract). The present study was undertaken to determine if PPN (and LDT) cells could be cultured and identified in vitro using this histochemical technique. Neuron-enriched cultures were prepared from the dissected midbrain tegmentum of embryonic Sprague-Dawley rats at gestational ages of 16 and 19 days. Cells were dissociated by trypsinization (0.2%) and trituration, seeded onto poly-L-lysine coated glass coverslips and cultivated in Dulbecco's Modified Eagle Medium fortified with fetal bovine serum (10%), penicillin and streptomycin. The nutrient medium was replenished 3 times a week; cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 and air. Cultures were serially harvested at 5-day intervals for 20 days. They were fixed in 4% paraformaldehyde, incubated (1 mM NADPH, 0.2 mM nitroblue tetrazolium, 5 mM sodium malate) at 37°C for 1-2 hr, rinsed and photographed. Cultures then were processed for neurofilament localization immunocytochemically in order to identify neurons. The antiserum to neurofilament was a gift of Dr. D. Dahl. Both the unlabeled peroxidase-antiperoxidase and indirect immunofluorescent (FITC) techniques were utilized.

The cultures consisted of dispersed neurons and some epithelioid cells presumed to be glia. During the first 5 days in culture the neurons elaborated extensive neuritic arbors and the glia proliferated to form a subconfluent cell carpet. NADPH diaphorase labeling first became evident at 10 days in culture but was maximal at 15 and 20 days. Large numbers of astrocyte-like cells were NADPH diaphorase positive, making it imperative to confirm the presence of neurofilaments in any NADPH diaphorase labeled cells. A small percentage of neurofilament-containing cells also was found to be NADPH diaphorase positive.

These findings suggest that a small population of midbrain tegmentum neurons are present in culture and may represent PPN and LDT neurons (based on NADPH diaphorase labeling). However, it has not been demonstrated that a separate or additional population of neurons from the midbrain tegmentum does not manifest NADPH diaphorase labeling in culture. NADPH diaphorase labeling in the PPN was not observed in intact gestation day 16 and 19 brains (but is evident at birth). Similarly, cultures did not begin to demonstrate NADPH diaphorase labeling until 10 days in culture.

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321.14 DEVELOPMENT OF THE PEDUNCULOPONTINE NUCLEUS. I. IN VIVO. C. Conrad*, V. Henderson*, R. D. Skinner, P. Abraham* and E. Garcia-Rill (SPON. J. Johnson), Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Previous findings from our labs suggest that the pedunculopontine nucleus (PPN) in the rat may be part of the mesencephalic locomotor region, an area known to modulate spinal locomotion oscillators (Skinner et al. '84). Developmentally, there appears to be a transition period in the nature of supraspinal control of locomotion at around two weeks of age. Spontaneous locomotor movements may occur in an adult animal in which the spinal cord was transected before 15 days of age, but transection after that time appears to permanently inhibit the occurrence of such movements. This study is part of a series of experiments tracing the postnatal development of the PPN in the rat brain. One aim is to determine if obvious morphological changes occur in development which may be related to the effects mentioned. Neonatal (0-24 days) rat brains (n=50) were processed using the NADPH diaphorase histochemical technique, which selectively labels PPN (and laterodorsal tegmental - LDT) cholinergic neurons (Vincent et al. '83). After fixation with 1% paraformaldehyde -1.2% glutaraldehyde, 50μ sagittal sections of the brainstem were cut on a vibratome, incubated at 40°C for 15-60 min, mounted, dried and coverslipped. Labeling was evident in the PPN and LDT at all ages studied. PPN cells with an evident nucleus were drawn and measured. The mean cell body area was constant for the first 8 days (0-2, 181±54μ^2; 3-5, 196±17μ^2; 6-8, 175±14μ^2), then increased markedly over the next 9 days (9-11, 284±52μ^2; 12-14, 394±96μ^2; 15-17, 498±58μ^2). At 18-20 days there was a decrease in mean cell area (370±129μ^2), followed by another increase at 21-24 days (440±55μ^2). Labeled adult PPN neurons have a mean area of 316±26μ^2. Distributions of cell body areas revealed that all cells appeared to grow significantly, peaking in size at 15-17 days. A loss of the larger cells appeared to occur at 18-20 days (perhaps accounting for the decrease in mean cell size at that age), while at 21-24 days an increase in the number of small cells suggests that shrinkage of the population at large had occurred. However, even at 21-24 days cell size was larger than in the adult.

These results suggest that a peak in cell size coincides with the 15 day transition period described. Reorganization after that time suggests the occurrence of some large cell loss with overall shrinkage of cell size over the next several days. Due to the variation in level of labeling across animals, exact cell counts were not considered reliable. These changes may reflect highlights in the development of locomotor control.

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321.15 RHYTHMIC AND TONIC ACTIVITY OF CELLS IN THE AREA OF THE PEDUNCULOPONTINE NUCLEUS IN RELATION TO FICTIVE LOCOMOTION. E. Garcia-Rill and R. D. Skinner, Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205-7199.

Previous studies have established the presence of low threshold locomotion-inducing stimulation sites in the posterior midbrain, by definition the mesencephalic locomotor region (MLR), in the area of the pedunculopontine nucleus (PPN) as well as in the cuneiform nucleus (CF) (Shik et al. '66, Garcia-Rill et al. '82). The present study was carried out in the fictive spontaneous locomotion preparation in order to determine the presence of rhythmic and/or tonic unit activity in the CF and PPN in relation to alternating or tonic neurographic activity of limb nerves in the absence of peripheral input. In addition, selective histochemical labeling of cholinergic PPN neurons using the NADPH-diaphorase technique (Vincent et al. '83) was carried out in order to localize architectonically those recording sites marked with Fast Green.

Of the 231 neurons studied in the posterior midbrain, 48% were related to fictive spontaneous locomotion. In the area dorsal (CF) to the NADPH-diaphorase positive region, 32% (14/44) of the cells studied showed related activity. Of these, 72% displayed bursting in relation to neurogram "bursts", while 28% fired tonically during ("on"), or stopped firing tonically during ("off") alternation. Within the NADPH-diaphorase positive region (PPN), 49% (71/145) of cells showed related activity, only 23% of these were "bursters" while 77% were "on" or "off" cells. In the area ventral to the NADPH-diaphorase positive area (pontine reticular formation) 69% (27/42) of the cells studied showed related activity, mostly of the "burster" type (70%) compared to the "on/off" type (30%). However, neurons in this area were found to respond to passive movement of the limbs and their activity appeared to reflect ascending spinal input. Cells studied in more dorsal locations (CF and PPN) were not modulated by peripheral input.

These results indicate a segregation of cells related to ongoing rhythmic activity compared to those related to the onset and termination of alternation. In general, the CF appears to be more related to the frequency of rhythmic activity while the PPN appears to be more related to the duration of rhythmic episodes. These findings may be related to the differential neurochemical control of locomotion evident in the MLR, in which injections of GABA antagonists in increasing amounts can induce stepping at higher and higher frequencies (i.e. walk-trot-gallop), whereas injections of Substance P in increasing amounts can induce longer and longer episodes of locomotion (at the same stepping frequency) (Garcia-Rill et al. '86,'87).

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321.16 ELECTRICAL ACTIVATION OF RAT LATERAL GASTROCNEMIUS-SOLEUS (LGS) AND TIBIALIS ANTERIOR (TA) MUSCLES BY SIMULATING TEMPORAL PATTERNS OBSERVED DURING TREADMILL LOCOMOTION: DOES CO-CONTRACTION INFLUENCE STEP CYCLE FREQUENCY? R.S. Hutton, R.R. Roy and V.R. Edgerton. Depts. of Psychology and Kinesiology, University of Washington, Seattle, WA 98195, and U.C.L.A., Los Angeles, CA 90024.

The frequency of cyclical limb movements may be limited by mechanical interactions of antagonistic muscles at higher cycle rates (Hutton & Enoka. Exp. Neurol. 93:369, 1986). This problem was investigated by electrically simulating activation patterns and monitoring muscle forces in LGS and TA muscles of 6 Sprague-Dawley rats (350-400 g). Based on EMG data recorded from freely moving rats (Roy, Hutchison & Edgerton, Med. Sci. Sport Ex. 18:S45, 1986), SOL mean cycle (c) durations (time between EMG onset of one cycle to EMG of next cycle), c/s, burst durations (BDs), and TA onset times (OTs) following SOL EMG onset were determined at 3 treadmill speeds (See Table). Muscle force was monitored from the detached tendons or

m/min	n	Cycle Duration (ms)	c/s	BDs (ms): SOL	TA	TA OTs (ms)*
40	7	294	3.4	149	80	140
54	6	246	4.1	106	71	125
67	5	219	4.6	94	66	110

* Taken from step cycles in one animal.

directly at the approximate insertion sites using a Statham transducer. To determine mechanical interactions attributed to the slow-twitch SOL muscle, forces were measured with the LGS intact or after the SOL was denervated.

With tendons detached, TA force temporally overlapped with LGS force over periods ranging between 20-50 ms at 3.4 c/s and 0-50 ms at 4.1 c/s. Overlap in forces at 4.6 c/s ranged between 0-10 ms. LGS force traces were asymmetrical, taking longer to return toward baseline than the time to peak force. Without SOL, force patterns were more symmetrical. In contrast, co-contractions were not observed when LGS contractions followed TA activation. At all cycle frequencies, above baseline residual forces (range: 50-400 g) in LGS were only seen during subsequent TA activations. Without SOL, LG forces approached baseline values (0-250 g). TA forces always reached baseline before LGS or LG activation. With tendons intact, phase relationships in onset forces were markedly improved and no co-contractions were evidenced across cycle velocities. All muscle forces reached baseline before subsequent activation. LGS or LG force patterns now appeared symmetrical. Therefore, co-contraction does not influence faster step cycle frequencies but at lower cycle frequencies may serve to stabilize the foot at toe-off (end of E_3 phase). Factors associated with mechanical coupling of LGS and TA through the foot (e.g., torque, viscoelastic properties) appear to improve phase relationships and force onset-offset symmetry at all cycle frequencies tested.

321.17 CUTANEOUS REFLEX RESPONSES IN CAT POSTERIOR THIGH MUSCLES DURING TREADMILL LOCOMOTION. C.A. Pratt, C.M. Chanaud and G.E. Loeb. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20892

Chronically implanted bipolar "patch" electrodes sutured onto the fascial surfaces of the posterior thigh muscles were used to record the electromyographic (EMG) responses evoked by stimulation of ipsilateral hindlimb cutaneous nerves during treadmill locomotion. Single biphasic pulses were delivered every 1500 ms via chronically implanted nerve cuff electrodes to the saphenous, plantar, superfical peroneal (SP) or sural nerves at stimulus strengths ranging from 1.2xT (times threshold) to 8xT. Thresholds for different afferent populations were determined on the basis of the shape of the evoked volleys recorded in tripolar nerve cuffs placed on either the femoral or sciatic nerves. The following muscles were implanted in various combinations in five cats (a=anterior, p=posterior, m=middle): biceps femoris (BFa,m,p); semitendinosus (ST); gracilis (GRa,m,p); semimembranosus (SMa and SMp); tensor fascia latae (TFLa and TFLp); tenuissimus (TEN); caudofemoralis (CF) and adductor femoris (AF).

Consistent reflex responses were produced at stimulus strengths of 2-4xT which activated fast conducting fibers (50-70 m/s); slower fibers (30-40 m/s) were stimulated at 8xT. The responses included short latency (8-10 ms) excitation, P1 (estimated central delay of 1.7-3.7 ms); a P2 excitation occurring at a latency of 30-34 ms, and inhibition at latencies of 14-16 ms.

All four nerves had similar excitatory effects on the knee flexors BFp, ST and TEN. Typically, low threshold stimulation produced gated P1 responses during the late stance (E3) and flexion (F) phases of the step cycle when these muscles are normally active. At higher stimulus strengths, an ungated P1 response appeared throughout all phases of the step cycle, and, at 8xT, a gated (late E3-F) P2 response was also produced. All nerves had similar inhibitory effects on the hip extensors CF and BFa.

The reflex responses in the remaining muscles were not as stereotyped. Some muscles (SMa, GRm, GRp, TFLa, TFLp, AF) responded differently to different nerves. For instance, SP produced P1 and P2 excitatory responses in GRm while plantar and sural produced inhibition. Interestingly, excitatory responses were evoked by some nerves in the extensors GR, SM and AF during stance (E2-E3). Different responses could also be produced by the same nerve in different regions of a single muscle (BF, see above, and GR; plantar stimulation produced P1 and P2 responses during E2-E3 in GRa, inhibition followed by excitation in GRm and no effect or inhibition in GRp).

The regionalized specificity of cutaneous reflexes in mechanically complex muscles, some of which are differentially activated during locomotion, suggests a high degree of specificity in the premotoneuronal circuitry for different task groups.

321.18 CUTANEOUS REFLEXES IN THE CAT DISTAL HINDLIMB AND THEIR GATING DURING NORMAL TREADMILL WALKING. G.E. Loeb. Lab. of Neural Control, NINCDS, National Institutes of Health, Bethesda, MD 20892

As a probe of functional connectivity in the spinal cord, cutaneous nerve stimuli were delivered via chronically implanted cuff electrodes on the plantar and superficial peroneal nerves. Sciatic nerve volleys were recorded to determine thresholds for various fiber classes and estimated arrival time at the spinal cord. Bipolar, epimysial "patch" electrodes were used to record EMG responses with little or no signs of cross-talk even during large reflexes in adjacent muscles. Three cats were implanted similarly to record from soleus (SOL), medial or lateral gastrocnemius (MG or LG), plantaris (PLA), flexor digitorum brevis (FDB), flexor hallucis longus (FHL), flexor digitorum longus (FDL), peroneus brevis (PB), peroneus tertius (PT), peroneus longus (PL), tibialis anterior (TA), extensor digitorum longus (EDL), and biceps posterior (BP). Central delays given below were calculated (+2 ms) by subtracting estimated sensory and motor transmission times based on distances.

Thresholds for both plantar and peroneal stimulation were 35-50uA (0.1ms) which activated 60 m/s fibers; EMG reflexes were noted but no visible change in gait was seen on videotape. At 2-4xT (times threshold), >20 m/s fibers were activated and swing phase trajectory changes were noted. At 7-10xT, >10 m/s fibers were activated and noticeable yielding during stance phase was noted, but the animals showed no break in stride or apparent discomfort.

Most EMG responses consisted of some combination of short latency excitation or inhibition (P1 or N1, 1-3 ms central delay) and/or long latency excitation (P2, 10-12 ms). Plantar stimulation at 1.2-1.5xT produced P1 responses in EDL, FDB, and PL; P2 responses in FDL and BP; and N1 responses in SOL, MG, PLA, PT, and PB. Peroneal stimulation at 1.2-1.5xT produced P1s in FDL and BP and N1s in SOL, LG, PLA, FHL, PB, and EDL. With increasing intensity (4-8xT), BP, TA, and FDL became dominated by P2 responses. FDL and PL were particularly variable among the three animals; neither was consistently recruited during the slow walking employed.

Some excitatory reflexes were phased completely differently than normal recruitment. The P1 in TA was largest during E2-3 (stance) phase for all plantar stimulation levels. EDL tended to be inhibited during its two bursts of locomotor activity in E1 and E3 phases and had a P1 response during F phase and P1+P2 during E2.

The general impression from examining 36,000 stimulus-response pairings was that there appears to be an extraordinary specificity of spinal connectivity associated with individual muscles, in terms of cutaneous fields, fiber classes, step-cycle phases, central path lengths, and in some cases, strategies specific to individual animals. Taken together with the specifically phased recruitment of each of these distal muscles during the step cycle, it would seem that the central pattern generator has much greater internal complexity than is suggested by most models now being considered.

321.19 COMPARISON OF TREADMILL AND OVERGROUND LOCOMOTION IN THE CAT. J.W. Blaszczyk and G.E. Loeb (SPON: C.J. Heckman). Lab. of Neural Control, NINCDS, National Institutes of Health, Bethesda, MD 20892.

For convenience, locomotion is usually studied on a treadmill instead of overground. Previous reports of differences in interlimb coordination between the two situations (e.g. Wetzel et al.,1975, J.Neurophys. 38: 492-501) have made only vague suggestions regarding psychological effects of the visual environment.

Five cats moved at their preferred gait along either a 1m long treadmill or a 3m long, similarly enclosed, stationary walkway. Limb placement patterns at speeds of 0.5-1.3 m/s were recorded on video (60 fields/s) and analyzed as still fields using a video disk. Duration of the swing and stance phases and horizontal amplitude of the swing phase (stride length) were calculated for all four limbs. The duty factor (DF) was defined as the mean fraction of the step cycle for which a foot was on the ground; because this was found to be the same for all limbs, the means are given below. Phase differences between limbs were defined by the time interval between the onsets of the stance phases divided by cycle duration; differences below are between ipsilateral fore and hind limbs.

Under both conditions, animals tended to walk with either of two stable gaits: walking gaits in which the DF was 0.6-0.75 (i.e. three limbs were on the ground at a time on average), and trotting gaits in which the DF was about 0.5 (i.e. only two limbs were on the ground at the same time). However, for overground locomotion, the animals almost always adopted the conventional diagonal gaits (phase differences of about 0.25 for walk and 0.5 for trot), whereas in the treadmill, the animals often adopted the "pacing" equivalent of both gaits (phase differences of 0.1-0.2 between ipsilateral limbs).

By adopting the pacing variants, the animals were able to maintain the more stable walking DF at higher effective ground speeds because they could use a longer stride length (mean increase of 18%) without having their front and hind feet collide. In fact, animals on the treadmill were often noted to start out with diagonal gaits and make transitions after such collisions, particularly when walking near the front of the belt. The transitions consisted of prolongations of the hindlimb stance phases for 1-2 step cycles until the ipsilateral phase shift approached zero.

On the treadmill, the animal can see that it is walking toward a non-moving barrier directly in front of it. By maintaining the highest possible DF, the animal presumably improves its stability and ability to stop abruptly (e.g. if the belt stops moving). As effective ground speed increases, DF can be maximized either by reducing swing phase duration (apparently inefficient; not seen) or by increasing stance duration, which increases stride length beyond that allowed by the interlimb-girdle spacing. The latter requires either placement of one set of feet lateral to the other (not seen at these speeds but known to occur in some forms of gallop) or the phase-shifted pacing gaits observed in this study.

321.20 AN ELECTROMYOGRAM-TO-FORCE PROCESSOR AND ITS TESTING IN CAT HINDLIMB MUSCLES. J.L.F. Weytjens and G.E. Loeb (SPON: F.J.R. Richmond). Lab. of Neural Control, NINCDS, National Institutes of Health, Bethesda, MD 20892

EMG signals are easily recorded from most muscles during unrestrained behavior, but they provide only limited and often misleading suggestions about the mechanical roles of individual muscles because of the many non-linear, time-varying, and muscle-specific factors involved in actual force production. Therefore, a general purpose electromyogram-to-force (EtoF) processor would be of great value to experimentalists seeking to understand quantitatively the biomechanics of normal behavior and the control problems that must be solved by the central nervous system.

Such an EtoF processor requires an adequate mathematical representation of muscle that is both complex enough to account for the effects of kinematic factors such as muscle length and velocity and general enough to be applicable to muscles of different fiber types and architectures, yet simple enough to be mathematically tractable. For this study we used a unipennate model consisting of a parallel arrangement of a contractile element, a spring and a linear dashpot, in series through an angle of pennation with an elastic tendon. The contractile element was described multiplicatively by a first order linear differential equation for the "active state" which used bin-integrated EMG as input, a Gordon-Huxley-Julian-type active force-length relationship, and a Hill-type force-velocity relationship, augmented to include negative (active lengthening) velocities. Both the parallel elasticity of muscle and the series elasticity of tendon were modeled as nonlinear springs, with exponential force-length relationships below a transition length and linear ones above it. For this model we derived a system of two ordinary differential equations, which used EMG and muscle length (as functions of time) as inputs and produced fiber length, fiber velocity, angle of pennation, and muscle force (as functions of time) as outputs. Using appropriate initial conditions, this system is solvable for both passive and active muscle.

To test the model, one medial gastrocnemius muscle in each of five cats was implanted chronically with EMG electrodes (bipolar, epimysial "patch"), a muscle path-length transducer (saline-filled silicone rubber tubing with electrodes, stretched from origin to insertion), and a tendon-strain transducer (E-shaped clip onto tendon). Following recovery from surgery, the animals were videotaped and the signals from the implanted devices were recorded during natural behaviors, including locomotion at various speeds, jumping, paw-shaking, and scratching. Preliminary results indicate that accurate predictions of the time course of muscle force under naturally occurring dynamic conditions are within the reach of this model.

CATECHOLAMINES: CELL CULTURE

322.1 IDENTIFICATION OF LIVING DOPAMINERGIC NEURONS IN CULTURES OF THE MESENCEPHALON BY UPTAKE OF THE FLUORESCENT SEROTONIN ANALOGUE 5,7 DIHYDROXYTRYPTAMINE A.P. Mariani*, N.L. Silva and J.L. Barker (Spon. R. Nelson) Laboratory of Neurophysiology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892

Recently, Vaney (1986) described the visualization of serotonin-accumulating neurons in the mammalian retina following uptake of the fluorescent serotonin analogue, 5,7 dihydroxytryptamine (5,7 DHT). Similar serotonin analogues have long been known to be accumulated by dopaminergic neurons in the retina and we reasoned that 5,7 DHT might also be used to label and identify living dopaminergic neurons derived from the mesencephalon. Such an approach would allow focused single cell analyses and cell biological studies of identified, living dopaminergic neurons. Without a method to identify the living dopaminergic neurons, these studies would be difficult as dopaminergic neurons constitute one of the rarest transmitter phenotypes in the CNS. The mesencephalons of embryonic day 13 rats were dissected, dissociated and grown in vitro for 3 weeks. The cultures were then incubated with 5,7 DHT ($10\text{-}50 \times 10^{-6}$ M) for up to 1 hr. and examined in a fluorescence microscope with a water immersion objective. A small proportion of the neurons displayed a weak, but well-defined deep blue-violet fluorescence in both their cell bodies and processes. Spectral analysis of the fluorescence revealed that the emission peaked at 420 nm when excited at 365 nm. These "blue cells" were then photographed and their positions marked. Following fixation in aldehydes, the cultures were processed for both aldehyde-induced (Faglu) fluorescence and tyrosine hydroxylase (TH) immunohistochemistry. All of the "blue cells" recovered showed Faglu-induced fluorescence and over 80% were immunoreactive for TH, the rate-limiting enzyme in the catecholamine synthetic pathway. Based on these experiments, we conclude that the uptake of 5,7 DHT can be used to identify living dopaminergic neurons derived from the mesencephalon.

N.L. Silva is a National Research Council Fellow.

322.2 ELECTROPHYSIOLOGICAL PROPERTIES OF CATECHOLAMINERGIC NEURONS CULTURED FROM THE RAT MESENCEPHALON

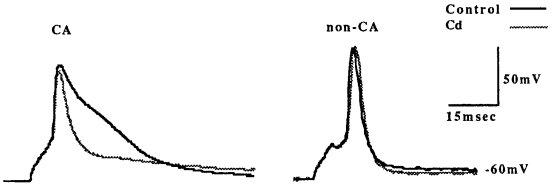
N. L. Silva, N.L. Harrison*, A.P. Mariani* and J.L. Barker, Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892

Catecholaminergic (CA) neurons from the embryonic rat mesencephalon can be maintained in culture and identified by fluorescence microscopy (adjoining poster, Mariani et al.). After 2-4 weeks in culture, recordings were made from both CA and non-CA neurons at room temperature using the whole-cell patch-clamp technique. Patch pipettes were filled with a standard 'intracellular' solution containing K gluconate sometimes supplemented with ATP. The external medium was a HEPES-buffered saline containing 1.5mM Ca and 1mM Mg.

All neurons had resting potentials between -50 and -60mV and many exhibited spontaneous action potentials (APs) and postsynaptic potentials. Depolarizing current injection elicited TTX-sensitive APs. CA neurons (n=20) had a mean input resistance of 420 Mohm; APs had an overshoot of 38mV, threshold of -33mV, and mean duration of 8.8ms (range 4.0-22.0). Non-CA neurons (n=9) had similar input resistance (425 Mohm), AP overshoot (40mV) and threshold (-31mV) but mean duration was 4.9ms (range 3.1-8.5). In some CA neurons, AP repolarization appeared biphasic, exhibiting a characteristic 'shoulder' that was reversibly reduced by the local application of 0.2mM Cd. Application of Cd to nearby non-CA neurons was not observed to shorten AP duration (see figure).

After blockade of APs with TTX, voltage-clamp recordings revealed the existence of three different outward currents in both types of neurons. A transient outward current was activated by steps from -60mV to potentials more positive than -40mV, inactivated at depolarized potentials and deinactivated by hyperpolarization. This current was sensitive to 4-aminopyridine. Two sustained outward currents were observed, one senstive to Cd or Co, the other seen in the absence of internal Ca accumulation and sensitive to TEA. Both CA and non-CA neurons responded to GABA with an increase in conductance and membrane current that reversed in polarity near -65mV.

The above results indicate that CA neurons cultured from the embryonic rat mesencephalon and identified by fluorescence microscopy are electrically and chemically excitable. Further, it appears that some CA neurons can be distinguished electrically on the basis of a broad AP that is reduced in duration by Cd.



N.L.Silva is supported by the National Research Council.

322.3 **LIVE ADRENAL CHROMAFFIN CELLS ACCUMULATE THE FLUORESCENT SEROTONIN ANALOGUE 5,7-DIHYDROXYTRYPTAMINE.** R.A. Cruciani*, A.P. Mariani*, U. di Porzio, N.L. Silva and J.L Barker.(SPON: G.D.LANGE). Lab. of Neurophysiol. NINCDS., NIH, Bethesda, MD 20892

It has been shown both in vivo and in vitro that serotonin (5-HT) and 5-hydroxytryptophan (precursor), are substrates of an uptake mechanism in adrenal medullary chromaffin graules of different species. However it is still unclear whether the chromaffin cells themselves synthesize 5-HT. The results of Brownfield et al. support an internal regulating mechanism of 5-HT content, possibly by local synthesis. However Verhofstad and Jonson reported that an inhibitor of tryptophan hydroxylase had no effect on adrenal 5-HT content which suggests that the 5-HT present in chromaffin cells originates externally . Despite this controversy it has been shown in immunohistochemical studies that 5-HT and epinephrine co-exist in chromaffin cells. This methogology requires fixation of the tissue and experiments on living cells have not been performed. 5,7-dihydroxytryptamine (5,7-DHT) is a cytotoxic 5-HT analogue that is accumulated by serotoninergic neurons and fluoresces when excited by UV light allowing the visualization of live indolamine-accumulating neurons as shown in the retina by Vaney. In the present report we show that 5,7-DHT is accumulated by living chromaffin cells both in suspension and in cell culture. Chromaffin cells isolated from the adrenal medulla of 10 day and 6 week old rats were incubated for 1 hr with 2.5 and 25 µM 5,7-DHT and examined in a fluorescence microscope. When excited at 365 nm, the indolamine-accumulating chromaffin cells displayed a well defined "blue" fluorescence. The stained cells were photographed and marked and the cultures fixed in paraformaldehyde. Immunohistochemistry with antisera directed against tyrosine hydroxilase (TH) the rate limiting enzyme for catecholamine synthesis and phenylethanolamine N-methyltransferase (PNMT), demonstrated that all of the cells identified by 5,7-DHT fluoresence contain TH or PNMT. According to Brownfield et al., 75% of the adrenal medullary cells are 5-HT-containing using an antibody to 5-HT and immunohistochemistry. In living cells we have demonstrated that virtually all the medullary TH or PNMT immunoreactive cells can accumulate 5,7-DHT as well. Whether this finding implies that all the chromaffin cells contain 5-HT requires further investigation. Data on the specificity of the uptake mechanism will be also presented.

322.4 MOLECULAR MECHANISMS FOR THE LONG-TERM REGULATION OF TYROSINE HYDROXYLASE (TH) IN CULTURES OF BOVINE ADRENOMEDULLARY CHROMAFFIN (BAM) CELLS.

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The long-term increase in adrenomedullary TH activity by activation of nicotinic receptors and by agents that elevate cAMP is well established. We have recently shown that catecholamine (CA) depletion increases TH activity by a non-transynaptic mechanism (Wilson S.P. et al. Neuroscience 6:71-79, 1981). To further study the molecular mechanism of long-term TH regulation, BAM cells were exposed to a defined medium containing 2 mM Ca^{++} for 3 days with forskolin (FSK, 50 uM), tetrabenazine (TBZ, 100 uM) or to acetylcholine (ACH, 50 uM) with 10 uM physostigmine. All the treatments increased TH activity (radiometric assay), TH protein (quantitative immunoblot) and TH mRNA (blot hybridization), but TH homospecific activity (HSA) was markedly lower after ACH treatment (Table). The low HSA observed after ACH may result from antibody recognition of inactive newly synthesized enzyme or increased rates of inactivation of TH after ACH. Thus, multiple transcriptional and posttranslational mechanisms appear to participate in the long-term regulation of TH activity.

TREATMENT	TH ACTIVITY	TH PROTEIN	HSA	THmRNA
Control	100 ± 4.8%	100 ± 8.3%	1.00	100%
FSK	224 ± 18.6%	287 ± 59.4%	0.78	259 ± 49%
ACH	128 ± 5.7%	285 ± 28.0%	0.45	156 ± 8%
TBZ	194 ± 15.2%	268 ± 27.6%	0.72	148 ± 4%

322.5 THE REGULATION OF TYROSINE HYDROXYLASE IN PC-18 CELLS: A SUBCLONAL CELL LINE DERIVED FROM RAT PHEOCHROMOCYTOMA PC-12 CELLS. H. Houchi, J.M. Mässerano, J.F. Bowyer, A.W. Tank, E. Dreyer, N. Weiner. University of Colorado Health Science Center, Department of Pharmacology, 4200 E. Ninth Ave, Denver CO 80262

Tyrosine hydroxylase can be activated in situ in rat pheochromocytoma PC-12 cells by cyclic AMP-dependent and calcium-dependent mechanisms. In the present report we have evaluated the regulation of tyrosine hydroxylase by cyclic AMP-dependent and calcium-dependent mechanisms in a new pheochromocytoma PC-18 cell line. The PC-18 cells were isolated by Dr. A.W. Tank from rat pheochromocytoma PC-12 cells. Dr. Tank and co-workers have reported that an induction of tyrosine hydoxylase is produced by either cyclic AMP or glucocorticoid treatments (Mol. Pharmacol. 30: 486-496, and 497-503, 1986).

PC-18 cells were grown in RPMI 1640 media containing 10% heat inactivated horse serum and 5% fetal bovine serum. These cells have a dividing time of approximately 24 hours. Treatment of the PC-18 cells for 15 minutes with either 2 mM dibutyryl cyclic AMP or with 1 µM forskolin, an activator of adenylate cyclase, produced a 90% increase in tyrosine hydroxylase activity. In contrast, treatment of the PC-18 cells with 56 mM potassium, 1 µM of the calcium ionophore, A23187, or with 2 µM of the activator of protein kinase C, phorbol myristate acetate (PMA), had no effect on tyrosine hydroxylase activity. $^{45}Ca^{++}$ uptake studies were performed in an attempt to evaluate whether calcium uptake was deficient in the PC-18 cells which might account of the inability of high potassium, A23187 and PMA to activate tyrosine hydroxylase. $^{45}Ca^{++}$ uptake into the PC-18 cells was increased 290% by A23187 and 110% by PMA. However, there was no change in $^{45}Ca^{++}$ uptake in the presence of 56 mM potassium, dibutyryl cyclic AMP or forskolin. These data suggest that tyrosine hydroxylase in PC-18 cells is regulated by only a cyclic AMP-dependent mechanism. The calcium-dependent mechanisms, calcium-calmodulin-dependent or calcium-phospholipid-dependent protein kinases, may either be deficient in these cells or if present, may not phosphorylate tyrosine hydroxylase. In an attempt to evaluate the phosphorylation component, tyrosine hydroxylase was partially purified from PC-18 cells and incubated in vitro with ATP-^{32}P in the presence of calmodulin-dependent protein kinase purified from rat brain and with cyclic AMP-dependent protein kinase purified from bovine brain. Both calmodulin-dependent protein kinase (stoichiometry = 0.30-0.60 mole ^{32}P/mole TH) and cyclic AMP-dependent protein kinase (stoichiometry = 0.50 -0.80 mole ^{32}P/mole TH) phosphorylated tyrosine hydroxylase purified from these cells. The incorporation of phosphate into PC-18 tyrosine hydroxylase in the presence of these kinases was similar to that which occurs with tyrosine hydroxylase obtained from the PC-12 cells. Additional studies are in progress in PC-18 cells to determine the in situ phosphorylation of tyrosine hydroxylase and the levels of the calcium-dependent and cyclic AMP-dependent protein kinases. Supported by USPHS grants NS07927 and NS09199.

322.6 REGULATION OF TYROSINE HYDROXYLASE ACTIVITY IN RAT PC12 CELLS BY NEUROPEPTIDES OF THE SECRETIN FAMILY. R. Roskoski Jr. Dept. of Biochemistry and Molecular Biology, LSU Medical Center, New Orleans, LA. 70119.

Tyrosine hydroxylase, the rate limiting enzyme in catecholamine biosynthesis, is subject to regulation by the cyclic AMP as well as the calcium and cyclic GMP second messenger systems. Treatment of rat PC12 cells with neuropeptides including secretin and vasoactive intestinal peptide (VIP) resulted in an increase in tyrosine hydroxylase activity measured in vitro. For example, activity increased from 242 ± 18 to 691 ± 41 pmol/min/mg protein after treatment with 10 µM secretin (pH 7.2; 0.125 mM 6-methyltetrahydropterine). Porcine histidine isoleucine peptide (PHI) and glucagon, also members of the secretin family, increased tyrosine hydroxylase activity 30-40%. Neuropeptide Y and substance P, which are not members of the secretin family, failed to alter tyrosine hydroxylase activity even at concentrations of peptides up to 50 µM. The efficacy of secretin and VIP was identical in terms of the degree of stimulation of tyrosine hydroxylase activity at 10 µM concentrations. Secretin, however, was more potent. Concentrations as low as 1 nM secretin stimulated tyrosine hydroxylase activity whereas 1 µM VIP was required to produce a significant increase in tyrosine hydroxylase activity. Both secretin and VIP increased the level of cyclic AMP in the PC12 cells. Both agents, moreover, increased the activity of cyclic AMP-dependent protein kinase as measured in vitro. PHI and glucagon also stimulated cyclic AMP-dependent protein kinase activity but to a lesser extent.

Secretin (5-27), the carboxyl terminal portion of secretin, attenuated the response of tyrosine hydroxylase activation to secretin, but not to VIP or PHI. Secretin (5-27) alone had no effect on either tyrosine hydroxylase or cyclic AMP-dependent protein kinase activity. These experiments suggest that PC12 cells contain a secretin preferring receptor (inhibited by secretin (5-27)) and a VIP-preferring receptor (not inhibited by secretin (5-27)). Secretin and VIP also increased the rate of catecholamine biosynthesis in intact PC12 cells. This work was supported by Grant NS-15994 from the U.S. Public Health Service.

322.7 MULTIPLE STATES OF PHOSPHORYLATION OF TYROSINE HYDROXYLASE IN CULTURED DOPAMINE NEURONS. G. Kapatos Laboratory of Neurochemistry, Center for Cell Biology, Sinai Research Institute, Detroit, MI, 48235, U.S.A.

The 59 KDa subunit of tyrosine hydroxylase (TH) contains 3 phosphorylation sites, two of which are differentially recognized by cAMP-dependent, calcium-calmodulin-dependent, or calcium-phospholipid dependent protein kinases (PKs). The amino acid sequence of the third site is atypical and thus involves an unidentified PK. When examined by 2D-gel electrophoresis and Western blotting with a monoclonal antibody, TH within cultured dopamine-containing neurons derived from the embryonic mouse mesencephalon exists as four isoelectric species exhibiting pI's of 6.5, 6.3, 6.23, and 6.17. In a typical culture these forms represent 28, 38, 28, and 6%, respectively, of the total TH detected. Incubation of cultures for 1 hour in the presence of 200 uCi/ml of 32P-orthophosphate, and subsequent immunoprecipitation and analysis as described above demonstrated an absence of change in the relative abundance of the four species and incorporation of isotope into only the 6.3 and 6.23 forms. These data indicate that under basal conditions: 1) 72% of the TH subunits contain one or more moles of phosphate; 2) phosphate is absent from the most basic species of TH (pI 6.5) and; 3) the most acidic form (pI 6.17) is fully phosphorylated and does not turnover rapidly. We propose that the pI forms of 6.3, 6.23, and 6.17 contain 1, 2, and 3 moles of phosphate, respectively, and that the endogenous PKs mentioned above (including the uncharacterized PK) are operative in these dopamine neurons. In order to investigate this possibility cultures were depolarized with 4-amino-pyridine (4AP) or treated so as to mimic the natural stimulation of the known PKs; with a cAMP analogue, a calcium ionophore (A23187), or an active phorbol ester (PMA). As a result of these treatments the following isoelectric species, expressed as a percentage of total TH, were obtained: 4AP: 8, 24, 44, 24%; 8Br-cAMP: 9, 32, 45, 15%; A23187: 7, 23, 47, 23%; PMA: 10, 30, 45, 15%. All treatments thus resulted in a 3-fold decline in the pI 6.5 form, and a 1.6-fold increase in the pI 6.23 form. In contrast to depolarization or A23187 treatment, incubation with 8Br-cAMP or PMA produced an insignificant change in the pI 6.3 form and only a 2-fold rather than 4-fold increase in the amount of the pI 6.17 species. From these data we conclude that: 1) newly phosphorylated TH is primarily recruited from the unphosphorylated pool of pI 6.5 species; 2) activation of cAMP or calcium-phospholipid-dependent PKs are not as effective as stimuli for phosphorylation of TH as are depolarization and elevated calcium; and 3) under these conditions no more than 25% of the TH pool can make the transition to the fully phosphorylated state.

322.8 INCREASED TRANSCRIPTION OF THE GENE FOR TYROSINE HYDROXYLASE INDUCED BY CYCLIC AMP IN A PHEOCHROMOCYTOMA CELL LINE. L.H.Fossom*, N. Weiner and A.W. Tank. Dept. Pharmacology, Univ. Colorado Health Sciences Center, Denver, CO 80262 and Dept. Pharmacology, Univ.of Rochester Medical Center, Rochester, NY 14642.

Tyrosine hydroxylase (TH) has been shown to be regulated in vivo by stress or catecholamine depleting/blocking drugs. During prolonged stress or reserpine treatment, a slowly developing and long-lasting increase in the enzyme levels of TH is produced. The mechanism responsible for this induction of TH is not completely understood, but recent studies have shown that an increase in TH-RNA precedes the increase in TH protein, suggesting that regulation may be at the level of transcription of the TH gene. Although the intracellular messengers that mediate the stress-related in vivo induction of TH and TH-RNA have not been clearly established, two candidates, cAMP and glucocorticoid, have been extensively investigated. We are using rat pheochromocytoma PC-18 cells (a subclonal cell line derived from PC-12 cells), to study induction of TH enzyme both by cAMP and glucocorticoid. We here report initial results on the time course of the cAMP-mediated induction of TH-RNA levels and the cAMP-mediated effects on rate of transcription of the TH gene.

TH-RNA levels in whole cells or nuclei were quantified by dot-blot and Northern blot analysis, as previously reported (Tank et al, Mol. Pharmacol. 30,497,1986), using as probe the 32P-labelled Pst 1-Kpn 1 restriction fragment of the recombinant plasmid pTH.4, which contains only sequences complementary to TH-RNA (Lewis et al, J.Biol.Chem. 258,14632,1983). Rate of transcription of the TH gene was determined by nuclear transcription run-off assays, followed by hybridization of 32P-RNA run-offs to filters bound with pBR322 plasmid with or without the insert containing sequences complementary to TH-RNA. Treatment of PC18 cells with 1 mM 8-bromocAMP for up to 1 hr failed to alter TH-RNA levels, but by 2 hr of treatment levels were increased approximately 3-fold and remained elevated for at least 12 hr. When the transcription rate for the TH gene was determined in nuclei isolated from cells treated for 1.5-2 hr with or without 1 mM 8-bromocAMP, a 3-fold increase in transcription rate was found with cAMP treatment compared with controls (62+5.5 ppm for controls, 170+17 ppm for treated cells, means+SEM, n=5). Nuclei isolated from a cell line which does not express TH enzyme (GH3 cells, an established rat pituitary cell line) showed no measurable transcription of the TH gene. These results suggest that most if not all of the cAMP-mediated increase in TH-RNA in PC18 cells is due to increased transcription of the TH gene. Supported by USPHS grants NS19749, NS24106, NS09199 and NS07927.

322.9 AN ULTRASTRUCTURAL STUDY OF DOPAMINERGIC TERMINALS AND TARGET CELLS IN REAGGREGATE CELL CULTURE. L. Won, A. Heller, P. C. Hoffmann, B. H. Wainer, S. Price* and P. Greengard[1]. The University of Chicago and [1]The Rockefeller University.

We have previously demonstrated at the light microscopic level that dopaminergic axonal varicosities are associated with clusters of dopaminoceptive target cells (DARPP-32-immunoreactive) in three-dimensional reaggregate cultures composed of mesencephalic and striatal cells (Won et al., Soc. Neurosci. Abstr. 12: 1004, 1986). In the present study, we identified dopaminoceptive cells at the ultrastructural level to determine whether they were synaptically contacted by associated dopaminergic (DA) terminals.

Dissociated fetal murine mesencephalic cells, including DA neurons, were coaggregated with dissociated fetal striatal cells in rotatory culture for 20 days before harvesting for immunocytochemistry. A polyclonal rabbit antibody against tyrosine hydroxylase (TH) was employed to identify dopaminergic axon terminals. A mouse monoclonal antibody was used against the phosphoprotein, DARPP-32, which is present in dopaminoceptive striatal cells expressing D-1 type DA receptors (Ouimet et al., J. Neurosci. 4: 111-124, 1984). Both neural antigens were localized in the same tissue section using a two-color sequential immunoperoxidase staining procedure. TH-immunoreactivity was visualized first with diaminobenzidine (DAB), and then DARPP-32-immunoreactivity was visualized with benzidine dihydrochloride (BDHC). The DAB reaction product (TH), which was brown and diffuse, was easily distinguishable from the BDHC reaction product (DARPP-32), which was blue and granular under the light microscope. At the electron microscopic level these two chromogens remained distinct; the DAB reaction product yielded a diffuse, electron dense cytoplasmic staining whereas the BDHC reaction product consisted of large, electron dense crystalline deposits.

Electron microscopic examination of reaggregate sections was focused on areas which contained patches of TH-positive terminals and DARPP-32-labeled cells and processes, as localized at the light microscopic level. The BDHC crystalline reaction product labeled the nucleus and cytoplasm of cells immunoreactive for DARPP-32. These cells were round or oval in shape, medium-size (9-15 um in diameter) and contained a lightly stained nucleus, which occupied most of the cell. Few cytoplasmic organelles were observed. DARPP-32-immunoreactive cells could be divided into 2 types: 1) neurons containing a smooth, non-indented round or oval nucleus and 2) neurons containing a shallow-indented nucleus. Examination of 35 DARPP-32-labeled neurons revealed that 28 of these cells had a non-indented nucleus. Four DARPP-32-labeled perikarya with non-indented nuclei were contacted by TH-positive boutons. In addition, both types of DARPP-32-immunoreactive cells (those with a non-indented or indented nucleus) received synapses from unlabeled boutons upon their perikarya. Symmetrical synapses were observed between TH-positive boutons (0.5-0.7 um in diameter) and DARPP-32-immunoreactive dendrites. Unlabeled terminals also made symmetrical synapses with DARPP-32-labeled dendrites and perikarya . TH-positive boutons were also observed synaptically contacting an unlabeled, medium size neuron and unlabeled dendrites located within the neuropil.

Based on the presence of TH-immunoreactive boutons contacting the dendrites and perikarya of DARPP-32-labeled cells, we conclude that the association of TH- and DARPP-32-immunoreactivity in reaggregates at the light microscopic level results in identifiable synaptic contacts between dopamine axon terminals and their striatal dopaminergic target cells at the electron microscopic level.
(Supported by MH-28942 and L. W. was supported by GM-07151)

322.10 VOLTAGE-CLAMP ANALYSIS OF EMBRYONIC MESENCEPHALIC NEURONS IN CULTURE. L.A. Chiodo and G. Kapatos, Labs. Neurophysiology and Neurochemistry, Center for Cell Biology, Sinai Research Institute, Detroit,MI 48235.

We have previously demonstrated that DA-containing neurons in mouse mesencephalic cultures derived from embryonic day 13 are flat, multipolar cells characterized by diameters which range between 17-33 um and presence of 3-5 large (approx. 5 um in diameter) proximal dendrites. These morphological characteristics are virtually identical to these observed in vivo for mesencephalic DA neurons. We have recently begun to examine the individual ionic currents present in these morphologically identified neurons using discontinuous single-electrode voltage clamp methods. All cells were studied at a temperature of 20-22^{o}C (with 3M K acetate filled electrodes 20-50 megohm) and, although the ionic composition of the media was varied depending on the requirements of individual experiment, osmolarity was maintained between 320-330 mOsmoles (pH 7.2-7.4), contained 10mM glucose, and was buffered with 5mM HEPES.

To date, we have observed the following currents in these neurons: (1) a very large, transient inward current which is activated by depolarizing voltage steps from the resting potential (-55 to -65 mV) to a level of approx. -42 mV, is dependent on external Na ions, and is blocked by TTX (10 uM) I_{Na}; (2) a slower, smaller inward current which is activated by depolarizations to approx. -45 mV from a conditioning hyperpolarization to a potential of -80 mV, reaches a peak activation at 20-50 mV is inactivated with further depolarizations (50-75% inactivation by 75 mV), and is Ca-dependent (blocked by replacing Ca with Co)-- I_{Ca}; (3) a transient outward current which is activated by depolarizations to approx. -65 mV after a conditioning hyperpolarization to -80 mV, and blocked by 4-AP (1-5 mM) I_{A}; (4) a delayed outward current which is activated on depolarization from rest, 4-AP-resistant. At least one component of this last current is blocked by TEA (5-25 mM) and thus appears to be a K-current (I_{K}). Further analysis of these and other currents are being conducted. (Supportd in part by MH-41557).

322.11 EFFECTS OF CHRONIC ANTIDEPRESSANT TREATMENT ON VOLTAGE DEPENDENT CALCIUM CHANNELS IN CULTURED BOVINE ADRENAL MEDULLARY CELLS. M. Arita*, A. Wada*, Y. Uezono* and F. Izumi. Department of Pharmacology, University of Occupational and Environmental Health, School of Medicine. Iseigaoka 1-1, Yahatanishiku, Kitakyushu 807 Japan.

Prolonged tratment with antidepressants has been shown to modulate the neuronal function and change their therapeutic efficacy. We reported previously that short term treatment of cultured adrenal medullary cells with antidepressants inhibited the influx of Na via voltage dependent Na channels and nicotinic receptor associated ionic channels (Soc. Neurosci, abstr., 275-9, 1986), but not the influx of Ca via voltage dependent Ca channels. In cultured adrenal medullary cells, high concentration of potassium (high K) directly activates the voltage dependent Ca channels without alteration of Na permeability (Neurosci., 15:283-297, 1985). In this study, we examined the change of Ca influx through voltage dependent Ca channels by long term exposure with antidepressants.

(1) Imipramine, imipramine-n-oxide, mianserin and mianserin-n-oxide (1 μM - 100 μM) did not inhibit high K-induced Ca influx when these agents were not pretreated. (2) High K-induced influx of Ca was reduced to 52 % and 34 % of control value by 4 and 6 days exposure to imipramine (100 μM) and remained at this level up to 11 days. (3) Imipramine-n-oxide and mianserin also reduced high K-induced Ca influx after 4 days exposure. However, mianserin-n-oxide which lacks antidepressant effect did not inhibit high K-induced Ca influx.

These observations suggest that long term exposure to antidepressants may affect the voltage dependent Ca channels and reduce the Ca influx in cultured bovine adrenal medullary cells.

SEROTONIN RECEPTORS: RADIOLIGAND BINDING STUDIES

323.1 [^{125}I]DOI: A NEW RADIOLABEL FOR 5-HT2 SEROTONIN SITES. R.A.Glennon,*1 M.R.Seggel,*1 W.H.Soine,*1 K.H.Davis,*2 R.A.Lyon,*2 & M.Titeler.*2 (SPON: L. Harris) (1) Dept of Medicinal Chemistry, Sch of Pharmacy, MCV/VCU, Richmond, VA 23298, and (2) Dept of Pharmacol, Albany Med College, Albany, NY.

Serotonin-2 (i.e. 5-HT2) binding sites are commonly labeled with tritiated ketanserin (KET), a 5-HT antagonist. More recently, we have introduced tritiated DOB, i.e. 1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane, as an agonist ligand for 5-HT2 sites. Tritiated DOB appears to label the agonist high-affinity state of 5-HT2 sites (5-HT2H sites) and as such, antagonists possess similar affinity (Ki) regardless of which radioligand is used to label the sites. Agonists, however, display a ten to 100-fold greater affinity when DOB is used as the radioligand. Because of the low specific activity of tritiated DOB (16 Ci/mmol), we synthesized 125-I DOI - the iodo analog of DOB. The radioiodinated agent was prepared to a specific activity of 1625 Ci/mmol (no carrier added) and a radiochemical purity of 98%. Binding assays were conducted with rat (Zivik-Miller Sprague-Dawley) parieto-frontal cortex homogenates using standard techniques. Binding was saturable and of high affinity; Bmax = 4.5 pmol/g wet wt and Kd = 2.2 nM.

Competition studies (triplicate competition curves) were conducted with several serotonergic agents. All competing agents reduced 125-I DOI-binding to the same extent and produced Hill coefficients of 0.75 to 0.9. Ki values were correlated with those obtained using tritiated DOB as the radioligand.

	Ki (nM)		
Ligand:	[^{3}H]KET	[^{3}H]DOB	[^{125}I]DOI
Serotonin	600	6	10.6
R(-)DOB	25	0.4	1.9
(±)DOI	20	0.7	2.8
Spiperone	0.5	1.8	1.2
Ketanserin	1.2	1.3	2.2
Cinanserin	4.5	3.8	6.3

The results suggest that 125-I DOI labels a subset of 5-HT2 sites in a saturable, displaceable, and selective manner and that the signal is stable and reliable. This radioiodinated ligand should prove useful in subsequent serotonin binding studies. (Supported by DA-01642)

323.2 DIFFERENTIAL DEVELOPMENT OF 5-HT$_1$ AND 5-HT$_2$ RECEPTORS IN FRONTAL CORTEX. Dana Dempsey* and Lynda Uphouse.(SPON. John Hines) Department of Biology, Texas Woman's University, Denton, Texas, 76204.

In addition to their role as neurotransmitters, biogenic amines have often been postulated as chemical messengers involved in the maturation and development of neural tissue . A neurohumoral role for serotonin (5-HT) has been especially emphasized . In support of this concept are reports that serotonin levels and the concentration of tryptophan hydroxylase, the rate-limiting enzyme for synthesis of serotonin, appear relatively early in cerebral ontogenesis. Most studies of the development of serotonin receptors have utilized ^{3}H-serotonin as the ligand for measuring receptor density. Serotonin receptors have been divided into the two major groups of 5-HT$_1$ and 5-HT$_2$ receptors, of which only the first has a high affinity for ^{3}H-serotonin. Consequently most previous studies have restricted their examination to the 5-HT$_1$ receptor population. Although functional roles for the receptors are only beginning to be understood, considerable evidence suggests that some of the 5-HT$_1$ receptor population may exist on the presynaptic membrane, while 5-HT$_2$ receptors may mediate many of the postsynaptic pharmacological actions of serotonin agonists. The development of the 5-HT$_2$ receptor has not been systematically pursued so it remains possible that the capability for serotonin neurotransmission matures earlier than previous examinations of receptor development would suggest. The following study has examined this possibility.

5-HT$_1$ receptors were examined by the binding of ^{3}H-5-HT in the presence of unlabelled 5-HT as competitor. 5-HT$_2$ receptors were examined by the binding of ^{3}H-ketanserin with unlabelled mianserin as competitor. Frontal cortices from male rats, aged 5 to 30 days postnatal were used for preparation of membranes for receptor binding. Binding was performed with various concentrations of the tritiated ligands and the data were evaluated by Scatchard analyses. In agreement with previous reports, there was little ^{3}H-5-HT binding until 15 to 20 days of age when the density of binding sites rapidly approached adult levels. In contrast, ^{3}H-ketanserin binding was readily detectable as early as 10 days of age and was clearly present by 5 days of age. Binding levels then gradually increased to reach adult values by 30 days of age. Although additional studies will be required to substantiate these findings, the present data suggest that the 5-HT$_1$ and 5-HT$_2$ receptors mature at different rates in the developing frontal cortex.

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323.3 GUANINE NUCLEOTIDE REGULATION OF THE BOVINE HIPPOCAMPUS $5HT_{1A}$ RECEPTOR. T. B. Kline* and R. Iyengar* (Spon. S. Beck). Department of Pharmacology, Mount Sinai School of Medicine of the City Univeristy of New York, NY 10029

The mammalian hippocampus contains serotonin receptors predominantly of the $5HT_{1A}$ type. Acting via GTP binding proteins (G-protein) the $5HT_{1A}$ receptor is thought to stimulate the opening of the hyperpolarizing K^+ channel and inhibit adenylyl cyclase. The effects of serotonin on the K^+-channel and adenylyl cyclase is attenuated by prior treatment of the system with pertussis toxin. We have used the agonist [^{3}H] 8-hydroxy-2 (di-n-propylamino) tetralin ([^{3}H]DPAT) to study guanine nucleotide regulation of agonist binding to the $5HT_{1A}$ receptor. In the absence of guanine nucleotides [^{3}H]DPAT bound to the receptor with high affinity (K_D=1.7nM). Co-addition of 100uM GMP-P(NH)P resulted in a decrease in affinity (K_D=7nM), without significant decrease in the total number of receptor sites. Competition curves utilizing fixed amounts of [^{3}H]DPAT and varying concentrations of unlabeled DPAT indicate that in the absence of guanine nucleotides there is a single form of high affinity receptors. Upon addition of saturating concentrations of guanine nucleotides there are both high and low affinity forms of the receptor. Formation of the high affinity state of the receptor is not dependent upon added divalent cations (Mg^{2+} or Mn^{2+}). The kact for GTP, guanyl-5'-yl-imidodiphosphate and guanosin-5'-O(2-thiodiphosphate) were 0.19, 0.78 and 0.06 uM respectively.

From these data we conclude that $5HT_{1A}$ receptors directly interact with G-protein(s) that are pertussis toxin substrates, and that high affinity agonist binding arises as a consequence of this interaction. Further, in the presence of saturating concentrations of guanine nucleotides there does not appear to be a complete dissociation of receptor-G protein interactions, as evidenced by high affinity DPAT binding. This is in contrast to other systems such as receptors interacting with G_S (thereby stimulating adenylyl cyclase) in which it is observed that guanine nucleotides completely dissociate G_S from the receptor, thus resulting in exclusively low affinity agonist binding (supported by NIDA grant DA-01875 and NIH grants DK-38761 and CA 44998).

323.4 EVIDENCE FOR DISTINCT SUBTYPES OF SEROTONIN RECEPTORS IN MAMMALIAN RETINA. B. Dvorkin*, X.-C. Qiu*, S.G. Horowitz* and M.H. Makman* (SPON: E.B. Gardner). Departments of Biochemistry and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461

Considerable evidence exists for the presence of pharmacologically distinct serotonin (5HT) receptor subtypes in the CNS. While bovine retina has been shown to contain high affinity ^{3}H-5HT binding sites, these sites were not characterized as to subtype. 5HT and also melatonin have been shown to stimulate adenylate cyclase (AC) activity of rabbit retina. We report here evidence for the presence of $5HT_{1A}$ and $5HT_{1D}$ (Heuring and Peroutka, J. Neurosci., 7:894, 1987) receptor binding sites in mammalian retina. Also we report that 5HT exerts dual effects on retinal AC: an inhibition of transmitter- or forskolin-stimulated activity that is mediated by $5HT_{1A}$ receptors and a stimulation of basal activity that appears to involve another receptor subtype.

Bovine retinal membranes were found to contain high affinity (K_D, 2.7 nM) and saturable (B_{max}, 26 fmoles/mg protein) binding sites for ^{3}H-8-hydroxy-N,N-dipropyl-2-amino tetralin (DPAT), a 5HT receptor agonist with high selectivity for the $5HT_{1A}$ receptor subtype. These receptor sites had high affinity for 5HT itself but not for melatonin, dopamine or norepinephrine. Also they exhibited high affinity for p-aminophenyethyl-m-trifluromethylphenyl piperazine (PAPP), a potent $5HT_{1A}$ agonist, and 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane (WB-4101), a potent $5HT_{1A}$ antagonist. ^{3}H-DPAT binding sites were also present in rat retina (K_D 1.6nM; B_{max}, 43 fmoles/mg protein). With ^{3}H-5HT itself as ligand, and with use of various selective drugs, bovine retina was found to contain in addition to $5HT_{1A}$ sites, sites that corresponded most closely to $5HT_{1D}$ sites, but no detectable $5HT_{1B}$ or $5HT_{1C}$ sites. We have previously shown that retina contains essentially no $5HT_2$ receptor sites labeled by spiroperidol.

Both rat and rabbit retinal membranes were found to contain AC activity that in the presence of forskolin, a potent activator of AC, was inhibited by 5HT, the selective $5HT_{1A}$ agonists DPAT and PAPP, but not by melatonin. This inhibition could be completely prevented by the antagonist WB-4101. Basal AC was stimulated by 5HT under the same conditions of assay. However, this stimulation involved a receptor as yet not identified, but clearly distinct from the $5HT_{1A}$ receptor based on relative agonist potencies. In addition to inhibition of forskolin stimulated AC, $5HT_{1A}$ agonists were also capable of inhibiting both dopamine- and vasoactive intestinal peptide-stimulated AC activities. These last mentioned findings may provide some indication as to the location and function of $5HT_{1A}$ receptors in retina. Also the retina provides a useful system for elucidating the receptor subtypes mediating stimulation and inhibition of AC by 5HT.

323.5 PARTIAL PURIFICATION OF 5-HYDROXYTRYPTAMINE 1a (5-HT1a) RECEPTOR PROTEIN BY PAPP AFFINITY COLUMN AND ^{3}H-p-azido-PAPP. W. Yang* and J.C. Shih (Spon: Arthur Yuweiler) Division of Biological Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90033

We have previously shown that the 5-hydroxytryptamine-1a (5-HT1a) receptor protein was solubilized from bovine hippocampus with 0.3% digitonin and 0.1% Nonident-p40. ^{3}H-5-HT binds to a saturable population of sites in the solubilized fraction with the same high affinity (2-5 nM) as that of the crude membrane. Furthermore, binding of ^{3}H-5-HT to the solubilized receptor is displaceable by a large series of serotonergic agents and the potency of this series corresponds well with the affinity of these agents for the 5-HT1a receptor sites in the crude membranes.

In this report, data was presented to show the partial purification of the 5-HT1a receptor was achieved by using PAPP-affinity column, and ^{3}H-p-azido-PAPP. PAPP has been shown to be a selective ligand for the 5-HT1a receptor (Asarch K, Ransom R, Shih JC, Life Sci 36, 1265-1273 (1985) and ^{3}H-p-azido-PAPP is a selective photoaffinity labeling probe for the 5-HT1a receptor (Shih JC, Asarch K, Ransom R, Psychopharm Bull 22, 818-824, 1986). In these experiments, the solubilized 5-HT1a receptor was effectively bound to the PAPP-affinity column. The bound receptor was eluted by 1 μM ^{3}H-p-azido-PAPP. The eluate was irradiated with a Hanovia 450-W medium-pressure mercury lamp; after the radioligand concentration was reduced to 10 nM using a centricon 30. The photolabeled 5-HT1a receptor protein was then separated by SDS-PAGE. The molecular weight of the labeled protein was 55 KD and 38 KD. When the solubilized fraction was incubated with PAPP affinity gel in the presence of excess 5-HT or PAPP the ^{3}H-p-azido-PAPP elution and labeling of these two polypeptides was significantly decreased. This result suggests that ^{3}H-p-azido-PAPP labeled polypeptides may be related to the 5-HT1a receptor. This notion was supported by the fact that ^{3}H-p-azido-PAPP also photolabeled a 55KD polypeptide in the crude membranes from bovine hippocampus or bovine cortex (Ransom RW, Asarch KB, and Shih, JC J. Neurochem 47:1066-1072, 1986). The nature of the 38KD polypeptide will be discussed. (Supported by NIMH Grant No. 37020 and MH 39085)

Abbreviation used:
PAPP: 1-(2-4-aminophenyl)ethyl)-4-(3-trifluoromethylphenyl) piperazine
p-azido-PAPP: 1-(2-4-azidophenyl)ethyl)-4-(3-trifluoromethylphenyl) piperazine

323.6 ^{3}H-SPIROXATRINE: A NOVEL HIGH AFFINITY RADIOLIGAND FOR BRAIN 5-HT_{1A} SEROTONIN RECEPTORS. M. Titeler and K.H. DAVIS*, Dept. Pharmacol. Toxicol., Albany Medical College, Albany, New York 12208

The 5-HT_{1A} serotonin receptor was originally detected with ^{3}H-5-HT (1). The first specific 5-HT_{1A} radioligand was the 5-HT_{1A} agonist ^{3}H-8-OH-DPAT (2). Recently it was reported that spiroxatrine is a potent, competitive 5-HT_{1A} receptor antagonist in an isolated organ preparation (3). In order to develop the first antagonist radioligand for the 5-HT_{1A} receptor, spiroxatrine was radiolabelled by New England Nuclear. We conducted in vitro radioligand binding studies with ^{3}H-spiroxatrine and ^{3}H-8-OH-DPAT in order to determine if ^{3}H-spiroxatrine would radiolabel the 5-HT_{1A} receptor and also display binding properties generally associated with antagonist radioligands. Table one lists the K_I values of five drugs in competing for ^{3}H-spiroxatrine in rat cortical homogenates.

Drug	KI (nM)
5-HT	3.4
8-OH-DPAT	1.0
Ipsapirone	3.9
(+/-)DOB	7200
Methysergide	8.1
Mesulergine	190

The high affinity of the agonists 5-HT, 8-OH-DPAT and ipsapirone indicate that ^{3}H-spiroxatrine is probably labelling the 5-HT_{1A} receptor. However, it has been noted that antagonist radiolabelled receptors generally display far lower affinities for agonists than agonist radiolabelled receptors (4). Therefore the high affinities displayed by 5-HT, 8-OH-DPAT, and ipsapirone for ^{3}H-spiroxatrine labelled 5-HT_{1A} receptors are somewhat anomalous and may indicate that ^{3}H-spiroxatrine is an agonist radioligand. We will report on further studies examining the in vitro regulation of ^{3}H-spiroxatrine and ^{3}H-8-OH-DPAT labelled 5-HT_{1A} receptors with a view to determing if ^{3}H-spiroxatrine is indeed an antagonist 5-HT_{1A} radioligand.

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323.7 LSD AND PHENYLISOPROPYLAMINE HALLUCINOGEN INTERACTIONS WITH MULTIPLE RADIOLABELLED BRAIN SEROTONIN RECEPTORS. R.A. Lyon*, M. Titeler, and R.A. Glennon*. (SPON: J. Popp). Dept. Pharmacol. Toxicol., Albany Medical College, Albany, New York 12208. Dept Med. Chem., Virginia Commonwealth Univ., Richmond, Va. 23298

We recently reported that the potencies of a large series of phenylisopropylamine hallucinogens and LSD strongly correlate with their affinities for the ^{3}H-ketanserin labelled brain 5-HT_2 receptor (1). This observation was consistent with our working hypothesis that brain 5-HT_2 receptor stimulation underlies the psychoactive properties of the hallucinogenic drugs-of-abuse. In order to further investigate the interactions of hallucinogens with brain 5-HT_2 receptors we recently reported the development of ^{3}H-DOB, an hallucinogenic 5-HT_2 radioligand (2). In the present study we investigated the potencies of phenylisopropylamine hallucinogens and LSD in competing for ^{3}H-DOB labelled 5-HT_2 receptors as well as for radiolabelled 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} receptors (Table 1).

	K_i (nM)			
Drug	5-HT_2	5-HT_{1A}	5-HT_{1B}	5-HT_{1C}
d-LSD	0.54	0.43	6.6	3.8
(-)DOB	0.39	2332	683	47
(-)DOM	1.80	4004	1840	94
2,5-DMA	268	1131	8435	1217
(-)MDA	198	4167	>10000	2079

LSD displays high affinity for the four 5-HT receptor sub-types assayed (Table 1). However the phenylisopropylamine hallucinogens generally display 10-100 fold higher affinities for the ^{3}H-DOB labelled 5-HT_2 receptors than for the other 5-HT receptor sub-types, implicating the 5-HT_2 receptor as a key site-of-action of the phenylisopropylamine hallucinogens. We are currently investigating the potencies of eleven other hallucinogens for radiolabelled 5-HT_2, 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} receptors to determine if one or more of these serotonin receptor sub-types may contribute to the psychoactive properties of these drugs.

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323.8 CHARACTERIZATION OF SEROTONIN-1B BINDING SITES IN RAT CENTRAL NERVOUS SYSTEM TISSUES USING ^{125}I-IODOCYANOPINDOLOL. P.J. MONROE and D.L. NELSON. Dept. Pharmacology and Toxicology, Univ. of Arizona College of Pharmacy, Tucson, AZ 85721.

It has recently been reported that the radioligand ^{125}I-iodocyanopindolol (ICYP) labels serotonin$_{1B}$ (5HT_{1B}) sites in several mammalian CNS tissues (Eur. J. Pharmacol. 118 (1985), 13-23). The experiments in the current study were undertaken to further examine the characteristics of ICYP binding sites in three rat CNS regions: spinal cord (sc), cortex (ctx), and corpus striatum (cs). Preliminary experiments were performed based on the method described in the above reference. Later experiments were designed to verify that no changes in binding characteristics resulted from altering the assay protocol to that used in ^{3}H-5HT binding assays. Once it had been determined that no differences were observed, all subsequent assays were performed according to standard ^{3}H-5HT binding assay protocol.

Saturation studies using ICYP in a concentration range of 25 pM to 2.5 nM revealed that the ligand was binding to a single population of sites in all three tissues examined. Affinity constant (K_d) values were found to not vary within the three tissues (ANOVA, $p>0.05$), and were calculated to be 0.53, 0.39, and 0.57 nM, respectively, for the sites labeled in the sc, ctx, and cs. The relative proportion of ICYP binding site densities within these tissues agreed with what has previously been reported for 5HT_{1B} sites (sc = ctx < cs). Furthermore, the ability of 5HT to compete for sites labeled by ICYP in the striatum (apparent K_i = 36 nM) is consistent with that seen for striatal 5HT_{1B} sites (by definition, those sites labeled by ^{3}H-5HT in the presence of 100 nM 8-OH-PAT; apparent K_i = 8.2 nM), and also agrees with values which have been previously reported.

The relative affinities of the ICYP binding sites for 8-OH-PAT, quipazine, phenylpiperazine (PP), and TFMPP are indicated by the K_i values shown below:

DRUG	SPINAL CORD	CORTEX	STRIATUM
8-OH-PAT	14.5 uM	15.0 uM	-------
QUIPAZINE	0.44 uM	0.40 uM	0.34 uM
PP	0.55 uM	0.67 uM	0.45 uM
TFMPP	0.04 uM	0.02 uM	0.03 uM

These results are in agreement with the premise that ICYP labels 5HT_{1B} sites in these tissues. However, it should be noted that in the spinal cord, all three piperazine compounds were able to reduce ICYP binding to a greater extent than 10 uM 5HT. Thus in this tissue, ICYP is capable of labeling sites in addition to those which have been designated 5HT_{1B}. Care must therefore be taken when analyzing data from experiments in which this ligand is used to label 5HT binding sites in certain tissues.

Supported by NIH grants NS16605 and NS01009.

323.9 APPARENT ABSENCE OF 5-HT_{1B} BINDING SITES IN HUMAN BRAIN. MEMBRANE BINDING AND AUTORADIOGRAPHIC STUDIES ON CORTICAL BIOPSIES AND POST-MORTEM TISSUES. J. Martial*, C. DeMontigny, A. Olivier*, S. Lal* and R. Quirion (Spon: B. Collier). Douglas Hospital Research Centre and Dept. of Psychiatry, McGill Univ., 6875 Blvd LaSalle, Verdun, Québec H4H 1R3.

At least three distinct sub-types of serotonin (5-HT)$_1$ receptors have been characterized using binding assays namely 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} types. The 5-HT_{1B} sub-type is of special interest since it displays nanomolar affinity for 5-HT itself and various well known drugs "classically" classified as beta-blockers such as propranolol and pindolol. Moreover, there is evidence that the 5-HT_{1B} receptor sub-type could act as an autoreceptor on 5-HT neurons. However, there is little evidence for the existence of this 5-HT receptor type in human brain tissues. Thus far, the presence of 5-HT_{1B} receptors have been convincingly demonstrated only in mouse and rat brain. It is possible that the apparent lack of 5-HT_{1B} sites in human brains could be related to the use of post-mortem tissues. Thus, we have studied the possible existence of 5-HT_{1B} receptor binding sites in humans in both fresh cortical biopsies and post-mortem tissues using membrane binding assays and receptor autoradiography. Fresh cortical (temporal, frontal, parietal) biopsied tissues were obtained from patients undergoing surgery for the removal of epileptogenic foci. Only apparently normal (outside foci) tissues were used without delay for binding assays. [^{3}H]5-HT binding to putative 5-HT_{1B} sites were investigated as follows: 200 µl of membrane preparations (3.5-4.0 mg protein/ml) were incubated for 60 min at 25°C in 170 mM Tris.HCl buffer, pH 7.4 containing 4 mM $CaCl_2$, 0.01% ascorbic acid, 10 µM pargyline, 100 nM 8-OHDPAT to block binding to 5-HT_{1A} sites and various concentrations of [^{3}H]5-HT (20.4 Ci/mmol, NEN, Boston) ranging between 0.25 - 10 nM. Specific binding was defined as the difference in ligand bound in absence and presence of 1.0 µM 5-HT. In parallel, autoradiographic studies were also performed using 20 µM sections obtained from fresh cortical biopsies as well as post-mortem tissues (post-mortem delays up to 24 hours). Sections were incubated with 2.0 nM [^{3}H]5-HT as described above or for 120 min with 50 pM [^{125}I](-) cyanopindolol (CYP; 2200 Ci/mmol) in 170 mM Tris.HCl buffer, pH 7.4 at 25°C in presence of 150 mM NaCl and 10 µM (-) isoproterenol to block binding to beta adrenergic receptors. In all preparations studied, only very low specific binding of either [^{3}H]5-HT and [^{125}I]CYP was observed suggesting the apparent absence of 5-HT_{1B} receptor binding sites in human brain tissues. Moreover, the presumed lack of 5-HT_{1B} receptors in human brain does not appear to be related to the use of post-mortem tissues.

323.10 5-HT_{1b} RECEPTORS DO OCCUR IN HUMAN BRAIN: AUTORADIOGRAPHIC AND HOMOGENATE BINDING STUDIES. A. Biegon, and P.M. Whitaker-Azmitia. Weizmann Institute of Science, Rehovot, Israel and Dept. of Psychiatry, SUNY, Stony Brook, NY 11794.

Previous studies, using autoradiographic techniques have failed to detect 5-HT_{1b} receptors in human brain (Hoyer et al., Brain Research, 376:85, 1986). However, there is clinical evidence for their existence (Mueller et al., J. Clin. Endocrin. Metabol. 61:1179, 1985; Heninger and Charney, Soc. for Neurosci., Abst. 11:1256, 1985). We, therefore, decided to re-examine the question, using the direct 5-HT_{1b} label ^{125}I-iodocyanopindolol (^{125}I-CYP) and expanding the study to include brain regions not used by the earlier researchers.

Brains were obtained at autopsy and included three subjects (aged 31, 44 and 66) with no known psychiatric or neurological illnesses. Autoradiographic studies, were performed on 40 micron sections. Sites were labelled directly with ^{125}I-CYP (150 pM) in the presence of 100 µmolar norepinephrine or with ^{3}H-5-HT (2 nM) displaced by 200 nM spiperone. Homogenate binding studies were used to determine pharmacological profiles of the two radioligands in four brain regions: substantia nigra, globus pallidus, cortex and hippocampus. 5-HT displaceable ^{125}I-CYP binding to globus pallidus, in the presence of excess norepinephrine, is saturable with a linear Scatchard plot, a K_D of 98 pM and a B_{max} of 14 fm/mg protein.

Our results show the presence of 5-HT_{1b} sites in human globus pallidus and substantia nigra. This is indicated by the autoradiographic studies which show selective labelling by ^{125}I-CYP which is displaceable by TFMPP (a selective 5-HT_{1b} ligand) but not 8-OH-PAT (a selective $_{1a}$ ligand) and the binding of ^{3}H-5-HT which is not displaceable by spiperone. In homogenate binding studies, the selective $_{1a}$ agonists 8-OH-PAT, LY-165,163 and spiperone have IC_{50} values from 1 to 10 µmolar while TFMPP has a value of less than 100 nM.

This receptor distribution is qualitatively similar to that previously reported in rat.

323.11 DETECTION AND CHARACTERIZATION OF THE PUTATIVE 5-HT1D SEROTONIN RECEPTOR IN RAT, PORCINE, AND BOVINE BRAIN. K.H. DAVIS* AND M. TITELER. (SPON: D. Poulos). Dept. Pharmacol. Toxicol., Albany Medical College, Albany, New York 12208

^{3}H-5-HT (serotonin) binding studies in mammalian brain have revealed the existince of three distinct receptors, 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} (1). In order to study 5-HT_{1C} receptors in our laboratory we co-incubated 1 uM pindolol, a non-specific blocker of both 5-HT_{1A} and 5-HT_{1B} receptors, with ^{3}H-5-HT. Rather than detecting only 5-HT_{1C} receptors, binding studies revealed evidence for an additional high affinity ^{3}H-5-HT binding site. In order to selectively examine this site we have developed an assay involving 1 uM pindolol, 100 nM mesulergine, and 2 nM ^{3}H-5-HT. Table 1 lists the K_I values for six drugs in competing for the site labelled under these conditions in rat cortex, porcine striatum, and bovine cortex.

	K_I (nM)		
Drug	rat cortex	porcine striatum	bovine cortex
serotonin	2.0	1.7	2.5
8-OH-DPAT	1300	390	300
d-LSD	15	2.6	1.6
TFMPP	330	320	280
methysergide	100	7.4	16
(+/-)DOB	3400	1200	1300

These data indicate that the site is present in at least three species and is present in at least two major brain regions. The pharmacological characteristics reported herein are similar to those reported by Heuring and Peroutka (2), who studied and named a putative "5-HT_{1D}" receptor in bovine caudate. We will report on further pharmacological characterization of the site, on in vitro regulation, and on the development of potent and specific drugs for this putative receptor.

1. Lyon, R.A., Davis, K.H., Glennon, R.A., and Titeler, M., Biochem. Pharmacol., in press (1987)
2. Heuring, R.E., Peroutka, S.J., J. Neuroscience, vol. 7, 894-903 (1987)

323.12 PERIPHERAL NEURAL SEROTONIN RECEPTORS (5-HT_{1P}): LABELING WITH ^{3}H-5-HYDROXYINDALPINE. T. A. Branchek, G. M. Mawe, and M.D. Gershon. Department of Anatomy & Cell Biology, Columbia University, P & S, New York, NY.10032.

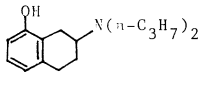
Peripheral neural 5-HT receptors, initially identified in the enteric nervous system (ENS), were first called "M" receptors (Gaddum and Picarelli, 1957). Since these receptors are different from both the 5-HT_1 and 5-HT_2 classes of receptor it has been suggested that they be re-named "5-HT_3" receptors (Bradley et al., 1986). Recently, however, it has been shown that, as in the CNS, there is more than a single type of enteric neural receptor for 5-HT (Mawe et al., 1986). One of these, called 5-HT_{1P}, has a high affinity for ^{3}H-5-HT, initiates a long lasting depolarization of enteric neurons associated with increased input resistance, and is the physiological receptor through which enteric serotoninergic neurons mediate slow epsp's. The other receptor, called 5-HT_{2P}, is not labeled by ^{3}H-5-HT, initiates a brief depolarization with decreased input resistance, but a physiological action of 5-HT mediated by these receptors has not yet been identified. Hydroxylated indalpines (Pharmuka) have been found to be agonists, and dipeptides of 5-hydroxytryptophan (5-HTP-DP) to be antagonists, at 5-HT_{1P} receptors, while 2-methyl-5-HT and ICS 205-930 (Sandoz) have been shown to be, respectively, an agonist and an antagonist at 5-HT_{2P} receptors. We have now examined the possibility that hydroxylated indalpines can be used as probes with which to label 5-HT_{1P} receptors. The action of 5-hydroxyindalpine (5-OHIP) on enteric neurons was determined electrophysiologically and compared with that of 5-HT; the binding of ^{3}H-5-OHIP to isolated enteric membranes was studied by rapid filtration and to frozen sections of tissue by radioautography. ^{3}H-5-OHIP binding was compared with that of ^{3}H-5-HT. 5-OHIP, like 5-HT, induced a triphasic response in most enteric neurons; an intial short-lived depolarization, during which input resistance fell, was followed by recovery, and then a long-lasting depolarization, during which the input resistance increased. 5-OHIP bound saturably, reversibly, and with high affinity to enteric membranes (K_D = 7.6 ± 0.7 nM; B_{max} = 76 ± 14 fmol/mg pr). Binding of ^{3}H-5-OHIP was not inhibited by agents that bind to 5-HT_1 (8-OH-DPAT, D-LSD), 5-HT_2 (ketanserin, spiroperidol, D-LSD, methysergide), or 5-HT_{2P} (ICS 205-930, MDL 72222 [Merrell Dow]) receptors, but was displaced by substances, such as hydroxylated indoles and 5-HTP-DP, which antagonize the binding of ^{3}H-5-HT to enteric membranes or tissue sections. K_i's were: 6-OHIP = 4.4 nM; 5-OHIP = 7.6 nM; 5-HT = 44 nM; 5-HTP-DP = 44 nM; 2-methyl-5-HT > 100 nM. Radioautographs demonstrated enteric ^{3}H-5-OHIP binding sites in the lamina propria of the intestinal mucosa and in the submucosal and myenteric plexuses. Extraenteric sites of ^{3}H-5-OHIP binding were also found in skin, heart, and brain. In pharmacology and in anatomical location peripheral ^{3}H-5-OHIP binding sites were similar to those of ^{3}H-5-HT; therefore, 5-OHIP mimics the effects of 5-HT on 5-HT_{1P} receptors and labels them. On the other hand, 5-OHIP, also has additional effects. Binding of ^{3}H-5-OHIP in the CNS was antagonized by D-LSD and ICS 205-930, as well as by 5-HTP-DP. Moreover, 5-OHIP (and ICS 205-930) inhibited the specific neuronal uptake of ^{3}H-5-HT; however, antagonists of this uptake did not block peripheral ^{3}H-5-OHIP binding. It is concluded that 5-OHIP is a relatively specific agonist at 5-HT_{1P} receptors and that ^{3}H-5-OHIP can be used as a probe for these receptors. Supported by NIH (NS12969, NS22637), Council for Tobacco Res. and the PMAF.

323.13 NEW DERIVATIVES OF 8-HYDROXY-2-(DI-N-PROPYLAMINO)-TETRALIN AND THEIR INTERACTION WITH CENTRAL SEROTONIN RECEPTORS: L. Klaesson[a]*, B. Backlund-Höök[b]*, U. Hacksell[b]* and N.E. Andén[c]* (SPON: J. Engel). [a]Dept.of Med. Pharmacol., [b]Dept. of Org.Pharm. Chem., Biomedical Center, University of Uppsala, [c]Dept. of Pharmacol., Karolinska Institute, Stockholm, Sweden.

8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) is a potent and centrally acting serotonin (5-hydroxytryptamine, 5-HT) receptor agonist with pronounced selectivity for the 5-HT_{1A}-site. It has, however, very low stereoselectivity, the (2R)-enantiomer is only twice as potent as its (2S) antipode.[1)]

We have previously synthesized and tested the C3-methylated derivatives of 8-OH-DPAT. They showed, in contrast to 8-OH-DPAT, clear stereoselectivity, with one stereoisomer acting as an agonist on the 5-HT receptor and the other beeing inactive as a 5-HT receptor agonist. The active isomers appear to be selective for the 5-HT_{1A}-site, but were less potent than 8-OH-DPAT.[2)]

In order to further characterize the structural requirements for the 5-HT receptor activation, a new series of 8-OH-DPAT derivatives has been synthesized and the pharmacological effects have been examined by means of biochemical tests and behavioural studies. The derivatives will be discussed with respect to their potency, specificity and stereoselectivity in the interaction with central 5-HT receptors.



8-OH-DPAT

1) Arvidsson LE et al, J. Med. Chem. 24, 921, 1981. Hjorth S et al, J. Neural Transm. 55, 169, 1982.
2) Arvidsson LE et al, J. Med. Chem. 27, 45, 1984. Klaesson L et al, Soc. Neurosci. Abstr. 12:140, 1986.

324.1 DIFFERENTIAL UP- AND DOWN-REGULATION OF TYPE I AND TYPE II ADRENOCORTICOSTEROID RECEPTORS. W.G. Luttge and M.E. Rupp*, Dept. of Neuroscience, Univ. of Florida Coll. of Medicine, Gainesville, Florida 32610.

Previous studies have reported that adrenalectomy and/or treatment with exogenous glucocorticoids can lead to an up- or down-regulation, respectively, of cytosolic Type II adrenocorticosteroid receptors in brain and other tissues. [Type II receptors have a very high affinity for synthetic fluorinated glucocorticoids such as dexamethasone (DEX), a somewhat lower affinity for naturally occuring glucocorticoids such as corticosterone (CORT) and a very low affinity for naturally occuring mineralocorticoids such as aldosterone (ALDO).] In other recent studies it has been claimed that the comparatively low concentration of Type I receptors in brain results in their near total occupancy in adrenally-intact animals, whereas the comparatively high concentration of Type II receptors remain only partially saturated under these same conditions. [Type I receptors have a very high affinity for ALDO and CORT and a very low affinty for DEX.] As a result of this differential occupancy and affinity it has been claimed that, unlike Type II receptors, Type I receptors do not undergo an up- and down-regulation similar to that seen for Type II receptors. The results from our recent studies refute this claim.

In the first study adult female CD-1 mice were adrenalectomized and ovariectomized (Adrex-Ovx) and 0, 1, 3, 6, 12, 24, 48, 96, 192 or 384 h later the concentration of specific Type I and Type II receptors in whole brain, kidney and liver cytosol determined by a 24 h incubation at 0°C with [^{3}H]ALDO (+ RU 26988 to prevent binding to Type II receptors) or [^{3}H]DEX, respectively. Non-specific binding was determined by adding [^{1}H]steroids. Type I receptor binding in brain was found to undergo dramatic up-regulation, with levels 12-14 times those in intact animals by 4-8 days post-Adrex-Ovx. By 16 days, however, Type I specific binding capacity returned to intact levels. Similar, but less dramatic fluctuations were seen in kidney and liver whereas much smaller fluctuations were seen for Type II receptors in all 3 tissues. In a follow-up study with Scatchard analyses we observed a similar transient up-regulation in maximal binding for Type I, and to a lesser extent Type II binding in all 3 tissues. As expected, the apparent binding affinity for both receptors increased after removal of competing endogenous steroids. In the last study we found that exogenous CORT prevented the Adrex-Ovx-induced up-regulation of Type I and Type II receptors in all 3 tissues. Exogenous ALDO was also able to fully prevent Type I receptor up-regulation, however, it was much less effective in preventing Type II receptor up-regulation. Therfore, both Type I and Type II receptors appear to be subject to up- and down-regulation under the influence of both endogenous and exogenous adrenocorticosteroids. (Supported by NS 24404.)

324.2 DIFFERENTIAL INACTIVATION OF TYPE II RECEPTORS FOR ADRENOCORTICOSTEROID HORMONES IN BRAIN: RECOVERY OF INTACT TYPE I RECEPTORS AND DISCOVERY OF A NEW CLASS OF TYPE II RECEPTORS. S.M. Emadian and W.G. Luttge. Dept. of Neuroscience and Center for Neurobiological Sciences, Univ. of Florida Coll. of Medicine, Gainesville, FL 32610.

Recently we reported that whereas Type II receptors for adrenocorticosteroids in brain cytosol preparations required a sulfhydryl reducing reagent for optimal ligand binding, Type I receptors manifested maximal binding capacity in the absence of reducing reagents (Emadian et al., J. Steroid Biochem. 24:953-961, 1986). In this same study we found that removal of endogenous reducing reagents from unlabeled cytosol (prepared in the absence of a reducing reagent) by dextran-coated charcoal (DCC) further reduced the ligand binding capacity of Type II, but not Type I, receptors. As an extention of other observations reported previously from this laboratory (Densmore et al., Life Sci. 35: 2237-2246, 1984), in the experiments outlined below we found that unoccupied Type II, but not Type I, receptors lose their ligand binding capacity in the presence of 300 mM KCl, especially when the unoccupied receptors "aged" at 0°C. In the present study, we sought to use these differences in Type I and Type II receptors to differentially inactivate the latter, in order to facilitate the purfication and further characterization of Type I receptors in future studies.

Brain cytosol prepared in 20 mM HEPES (pH 7.6) was isolated from ovariectomized-adrenalectomized CD-1 mice and subjected to various potentially inactivating treatments prior to assessment of Type I and Type II receptor specific binding capacity by incubation for 24 h at 0°C with [^{3}H]aldosterone (ALDO) (plus [^{1}H]RU 26988 to prevent ALDO binding to Type II receptors) or [^{3}H]dexamethasone (DEX), respectively. Our attempts to selectively inactivate unoccupied Type II receptors revealed that 10% of the DEX binding capacity remained even after extensive and repeated pretreatment with DCC. As expected this treatment had no effect on Type I receptor binding capacity. Further analysis revealed that the DCC-resistant DEX binders do not require endogenous or exogenous sulfhydryl reducing agents to ratain full ligand binding capacity, do not lose their ligand binding capacity when aged at 0°C and remain intact when exposed to buffers with high ionic strength. Noting that these treatments inactivate Type II receptors, but have no effect on Type I receptors, we propose that the DEX binding capacity remaining after treating crude brain cytosol with DCC represents a new class of receptors whose physiological and physicochemical properties remain to be characterized.

Supported by N.I.N.C.D.S. Grant NS 24404

324.3 POTENTIATION OF PHORBOL ESTER-INDUCED UPREGULATION OF ANGIOTENSIN II RECEPTORS IN NEURONAL CULTURES BY A CALCIUM IONOPHORE. C. Sumners, S.M. Rueth*, F.T. Crews and M.K. Raizada*. Depts. of Physiology and of Pharmacology and Experimental Therapeutics, University of Florida, Gainesville, FL 32610.

In previous studies we have shown that phorbol esters, which are agonists of protein kinase C, upregulate angiotensin II (Ang II) receptors in neuronal cultures prepared from the hypothalamus and brainstem of one-day-old rats. The present study was designed to examine the role of Ca^{2+} in phorbol ester-stimulated increases in Ang II receptors, because the activation of C-kinase requires Ca^{2+}. We have studied the effects of a Ca^{2+} ionophore (A23187) on Ang II receptors, alone or in combination with phorbol esters. Treatment of neuronal cultures with the phorbol ester TPA (12-0-tetradecanoylphorbol-13-acetate) at 0.8 μM for 1 hr resulted in significant increases (80-100%) in Ang II binding. This effect was significantly enhanced by co-treatment with the Ca^{2+} ionophore A23187 (0.19-19 μM). A maximum enhancement of 80-90% above TPA stimulation was obtained with 1.9 μM A23187, but higher doses of the ionophore were less effective. Treatment of neuronal cultures with A23187 alone gave the opposite effect, ie 1.9 - 19 μM A23187 caused significant decreases in Ang II binding with a maximum effect at 19 μM of 40-50%. These doses of A23187 had no toxic effects on the cells, as evidenced by Trypan Blue exclusion. Saturation and Scatchard analyses were performed to determine the effects of TPA and A23187 on Ang II receptors, and results are summarized as follows.

Treatment	Kd (nM)	Bmax(fmol/mg protein)
Control	0.82 ± 0.05 (6)	113 ± 17.0 (6)
TPA (0.8 μM, 1hr)	1.31 ± 0.06 (6)*	244 ± 31.4 (6)*
TPA+A23187(0.8μM/1.9μM)	1.54 ± 0.08 (3)*	432 ± 51.2 (3)*
A23187 (1.9 μM, 1hr)	0.85 ± 0.14 (3)	70.4 ± 9.3 (3)*

Data are means ± SEM. Numbers in parentheses = # of experiments.
(*) significantly different from control values.

It is concluded that the TPA induced upregulation of Ang II receptors is greatly enhanced in the presence of A23187 (ie. increased intracellular Ca^{2+}). Conversely, without TPA the ionophore downregulates Ang II receptors. Although the precise mechanisms involved are not established, these results suggest two possibilities of Ang II receptor regulation by Ca^{2+}; (i) via C-kinase and (ii) via influx of Ca^{2+} into the cell.

Supported by NIH grants NS-19441 and HL-33610.

324.4 HETEROGENEOUS DISTRIBUTION AND UPREGULATION OF MU, DELTA AND KAPPA OPIOID RECEPTORS IN THE AMYGDALA. C.M. Paden, S. Krall* and W.C. Lynch. Depts. of Biology and Psychology, Montana State Univ., Bozeman, MT 59717.

We have undertaken a quantitative autoradiographic study of upregulation of mu, delta and kappa receptors throughout the rat brain in order to provide a data base for investigations of the functional importance of receptor plasticity in specific opioid systems. In this initial report the analysis of four nuclei of the amygdala is presented. While upregulation of mu receptors has been observed in the amygdala (Tempel, A., et al., PNAS, 81:3893, 1984), this phenomenon has not been analyzed within individual nuclei, nor has upregulation of delta or kappa receptors been investigated in this structure.

Adult male Holtzman rats were implanted sc with Alzet osmotic minipumps containing saline (SAL; n=3) or 150 mg/ml naloxone HCl (NAL; n=3), yielding a nominal dosage of 155 µg/kg per hour. Pumps were removed after 14 days, and animals were sacrificed by decapitation 24 hours later. Freeze-dried 30 micron horizontal cryostat sections of whole brain were prepared as described by Herkenham and Pert (J. Neurosci., 2:1129, 1982). Following preincubation in 0.17 M tris containing 120 mM NaCl and 50 µM GTP, sections were incubated for 30 minutes at 25° C in either 1.5 nM ^{3}H-dihydromorphine (mu receptors), 3 nM ^{3}H-DADLE plus 5 nM morphine (delta receptors), or 3.8 nM ^{3}H-bremazocine plus 30 nM morphine and 100 nM DADLE (kappa receptors). Non-saturable binding was estimated by inclusion of 1 µM levallorphan in the incubation. Following a 45 day exposure on LKB Ultrofilm, quantitative autoradiography was performed using a digital image analysis system. Specific binding was estimated by comparison to ^{3}H-Microscale standards after subtraction of background gray values.

A significantly different basal distribution of each receptor subtype was observed, with mu binding greatest in the lateral nucleus (La), delta greatest in the basolateral (Bl), and kappa greatest in the medial (Me). Levels of all three receptors were very low in the central nucleus (Ce). Chronic NAL treatment resulted in statistically significant increases in mu receptors in the Me, Bl and La, while increases in delta and kappa receptors were significant only in the Bl. These results indicate that all three receptor subtypes are subject to upregulation following chronic antagonist treatment, but it is clear that the degree of upregulation varies across different brain regions in a manner which is not predictable solely on the basis of basal receptor distributions.

Supported by MONTS-NSF project ISP-801149 and a grant from the Proctor and Gamble Corp. to WCL, and by a Grant-In-Aid from the Montana Heart Association to CMP.

324.5 INHIBITION OF MEZEREIN- BUT NOT AGONIST-INDUCED LOSS OF VIP RECEPTOR/ADENYLATE CYCLASE ACTIVITY BY AN INHIBITOR OF PROTEIN KINASE C. J. T. Turner, D. W. Bollinger*, and M. L. Toews*, Department of Pharmacology, University of Missouri, Columbia, MO 65212.

Previous studies from our laboratory have indicated both a loss of cell surface receptors for vasoactive intestinal peptide (VIP) and a desensitization of VIP-stimulated adenylate cyclase (AC) activity in HT29 human colonic adenocarcinoma cells following preincubation with either VIP receptor (VIP-R) agonists or activators of protein kinase C (PKC), including phorbol esters and mezerein (MEZ). Differences in the changes induced by PKC activators as compared with VIP-R agonists prompted us to investigate whether inhibition of PKC produced similar effects on agonist- and PKC activator-induced loss of VIP receptors and AC activity.

HT29 cells were treated with the VIP-R agonist peptide histidine isoleucineamide (PHI) or MEZ with or without pretreatment with the PKC inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7). Changes in the VIP-R/AC system were assessed by three methods: ^{125}I-VIP binding to intact HT29 cells on ice; intact cell cyclic AMP production using the [^{3}H]adenine preloading method; and cell lysate AC assays.

These results were obtained in ^{125}I-VIP binding assays:

Treatment	Specific binding, % of control
None	100
0.1 μM PHI	37 ± 1
300 μM H-7	96 ± 4
PHI + H-7	36 ± 7
1 μM MEZ	62 ± 5
MEZ + H-7	91 ± 10

Similar results were obtained in AC assays. The finding that H-7 reverses MEZ- but not PHI-induced changes in VIP-R/AC activity indicates that the mechanisms through which VIP-R agonists and PKC activators act are different and suggests that PKC is not involved in agonist-mediated regulation of the system. Supported by NIH grant DE05339.

324.6 DOWN REGULATION OF PROTEIN KINASE C IN AtT-20 CELLS: ITS EFFECT ON THE NEUROPEPTIDE SECRETION AND BIOSYNTHESIS. Sheela Vyas*, John Bishop* and Jitendra Patel* (SPON: J. Garcia-Arraras). NIMH, NIH, Bethesda, Maryland 20892, USA

AtT-20 cells were treated with 100 nM phorbol 12 myristate 13 acetate (TPA) for 24-48 hours. It was found that whereas the phospholipid, Ca^{2+} dependent activity of protein kinase C (PKC) virtually disappears in these cells (98% decrease), that of cAMP and Ca^{2+}/Calmodulin dependent kinases remain unchanged. Treatment of cells with TPA at different time intervals showed that the onset of disappearance of PKC activity occurs after 1-2 hours, negligible activity is seen after 8 hours. When the cells were incubated with varying concentrations of TPA (1-100 nM) for 24 hours, higher doses than 10 nM cause a drastic reduction in PKC. In order to see whether the TPA treatment also has an effect on the PKC substrate levels, the phosphorylation of endogeneous substrates was carried out using exogeneous PKC. In TPA treated cells, a time dependent decrease in phosphorylation of proteins with M_r=35 and 50 K_D was seen.

In cells preincubated with TPA for 24 hours, the cAMP-mediated secretion of immunoreactive β-endorphin in response to forskolin or CRF remained unimpaired. However, in these cells, TPA stimulated secretion of β-endorphin was abolished. A homologous desensitization of the secretory response has been previously observed by Zatz and Reisine (P.N.A.S.: 82:1286-1290, 1985). In the absence of TPA preincubation, POMC mRNA levels were increased in response to forskolin, TPA and CRF. After treatment of cells with TPA, the responses to forskolin and CRF remained elevated whilst the TPA induced increase in POMC mRNA levels was abolished. The above finding suggests that multiple regulatory mechanisms are involved in the regulation of POMC mRNA levels and β-endorphin secretion.

324.7 DYNAMIC OF HIGH-AFFINITY VASOACTIVE INTESTINAL PEPTIDE (VIP) BINDING SITES IN SUBCLONES OF A HUMAN NEUROBLASTOMA (SK-N-SH) EXPRESSING VIP: POTENTIAL AUTOCRINE ACTION OF VIP. J.M. Muller*, L. Eiden*, C.-M. Hsu* and J. Waschek* (SPON: P.A. Anderson). Lab. of Cell Biology, National Institute of Mental Health, Md 20892.

Three cell lines (SH-EP, SY5Y and SH-IN) corresponding to subclones isolated from a human neuroblastoma (SK-N-SH) (R.A. Ross, B.A. Spengler, and J.N., Biedler, J. National Cancer Institute, 71:4, 741-747, 1983) appear to be a promising model to study the divergence of transformed neuronal cells originating from the same tissue. For example, SY5Y and SH-IN which contain neuronal markers, possess measurable levels of mRNA encoding VIP and synthesize this peptide. On the other hand, the SH-EP cell line, which appears epithelial in morphology, contains no detectable VIP mRNA (cf. Waschek et al., this volume).

The ability of these different cell lines to specifically bind [3-iodotyrosyl-^{125}I] vasoactive intestinal peptide was investigated. Competitive inhibition of ^{125}I VIP binding on these different cells by unlabeled peptide was analyzed by the mathematical transformation of Scatchard. The results indicate that the three cell lines possess high affinity receptors for VIP. However, SH-EP possess numerous high affinity VIP receptors (7000 sites/cell; Kd = 0.5 nM), while SH-IN and SY5Y have a 7 fold lower binding capacity for radioiodinated VIP with a similar Kd. In addition, in SH-EP cells, VIP induces a rapid and pronounced down regulation of VIP receptors (up to 70% loss of binding capacity compared to control); this process is time and dose dependent (t1/2 = 8 min. in response to 10^{-7} M VIP) and is reversible upon removal of VIP. Dilution of SY5Y and SH-IN in culture medium appears to cause an increase in VIP binding capacity which can be seen within 1 hour. Moreover, the culture medium of SY5Y and SH-IN cells significantly inhibits binding of ^{125}I-VIP on different target cells (HT 29, SY-EP) possessing a high binding capacity for the peptide.

These data are consistent with the possibility that VIP or a related peptide and/or fragments of this peptide secreted by SY5Y and SH-IN cells into their culture medium could act as autocrine factors causing self regulation of the membrane VIP binding capacity of these cells. The differences observed in VIP production and membrane binding capacity among the three cell lines suggest that this autoregulation could be involved in the divergence of these three types of transformed neuronal cells originating from a common precursor. Work is now in progress to check if agents like dibutyryl-cAMP or forskolin which induce changes in the morphology of the SH-EP (epithelial like) cells can also affect their binding capacity for VIP and induce the production of neuron specific markers.

324.8 VASOPRESSIN (AVP) RECEPTOR REGULATION FOLLOWING LESION OF AVP INNERVATION OF VENTRAL SEPTAL AREA OF THE RAT BRAIN. P.Poulin, T. Malkinson, W.L. Veale and Q.J. Pittman. Neuroscience Research Group. University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Recently, immunocytochemical studies have detected AVP cell bodies within the bed nucleus of the stria terminalis (BST) that project to the lateral septal area and the nucleus of the diagonal band and its immediate surroundings, including the ventral septal area (VSA). AVP microinjected into the VSA is known to cause motor disturbances via an interaction with specific receptors. Long term castration (3 months) has been shown to provide a selective "lesion" of AVP fibers projecting to the VSA and lateral septum (LS). Receptors are known to be in a dynamic state of turnover and typically cells respond to a decrease in neurotransmitter levels by increasing their receptor number and/or altering their affinity. We have, therefore, tested the possibility that AVP receptors in the VSA of castrated male rats may be "supersensitive" to the AVP-induced motor disturbances and that changes would occur in AVP receptor numbers or properties. In a behavioral study, when castrated or control animals were challenged with AVP, given into the VSA, no difference in response (motor disturbances) to either a first injection of AVP, or of the characteristic augmented response seen after a second injection was seen. In binding studies, done on synaptic plasma membrane fractions, Scatchard analysis revealed no significant differences in Kd or Bmax in VSA membranes from castrated or control animals (Kd 2.78 nM vs 2.27 nM and Bmax 30 fmol/mg protein vs 28 fmol/mg protein respectively), in LS membranes (Kd 1.7 nM vs 1.3 nM and Bmax 47 fmol/mg protein vs 40 fmol/mg protein), or in hippocampal membranes (Kd 1.3 nM vs 1.3 nM and Bmax 25 fmol/mg protein vs 28 fmol/mg protein). Thus reduction in AVP content of VSA and LS has not altered AVP action in mediating motor disturbances, nor has it caused a change in AVP receptor properties or numbers. Supported by MRC. P.P. is an AHFMR student.

324.9 EFFECT OF SYSTEMICALLY ADMINISTERED CAERULEIN ON RECEPTOR-ADENYLATE CYCLASE COUPLING IN THE RAT BRAIN. T. Saito, Y. Hatta*, F. Tsuchiya*, H. Ikeda* and N. Takahata*. Dept.of Neuropsychiatry, Sapporo Med. Col., S.1, W.16, Chuou-ku, Sapporo 060, Japan.

Caerulein has various central activities similar to those of cholecystokinin octapeptide (CCK-8). Many reports have stated that CCK seems to have a role as a neuromodulator and much interest has been focused on the interaction between CCK and dopamine (DA). Although recent studies have indicated that systemically administered caerulein altered beta-adrenergic receptor function, caerulein's effect on receptor-effector coupling is still unclear. In the current study we examined caerulein's effects on receptor-adenylate cyclase (AC) coupling in the cortex and striatum of the rat brain. Male Wister rats (240-280 g) were used. In the acute caerulein treatment, animals were injected intraperitoneally (i.p.) with caerulein (100 ug/kg) or saline and sacrificed by decapitation at 1 hour after injection. In the chronic treatment, rats were injected with caerulein (200 ug/kg/day, i.p.) or saline twice a day, for 5 days and sacrificed by decapitation at 1 hour after the last injection. After acute injection with caerulein, the concentration of magnesium required for the half-maximal activation of AC (EC_{50}) was decreased slightly from 3.8 mM to 3.1 mM in the cortical membrane. EC_{50} for magnesium in the cortical membrane was shifted significantly from 3.9 mM to 2.8 mM after chronic caerulein treatment. The ^{125}I-pindolol binding to the beta-receptor in the cortical membrane was not altered after acute or chronic caerulein treatment. However, chronic treatment with caerulein resulted in an increase in the IC_{50} for isoproterenol in displacing ^{125}I-pindolol binding to the rat cortical membrane. The EC_{50} for isoproterenol activation of AC in the cortical membrane was also increased after chronic caerulein treatment.

Our data indicate that caerulein treatment in vivo may alter the N-protein function in the rat cerebral cortex. Effect of caerulein on the striatal receptor-AC coupling are currently being investigated.

324.10 CHRONIC MORPHINE TREATMENT INCREASES ADENYLATE CYCLASE ACTIVITY AND PERTUSSIS TOXIN-STIMULATED ADP-RIBOSYLATION OF G-PROTEINS IN A REGION-SPECIFIC MANNER IN RAT BRAIN. J.J. Erdos*, R.S.Duman, J.F.Tallman, and E.J. Nestler, Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508

The molecular changes that occur in neural cells following chronic opioid treatment have not been extensively investigated on a regional basis. Studies on cultured neuroblastoma x glioma cells suggest that the adenylate cyclase complex plays a role in the development of tolerance, dependence, and withdrawal. Few data, however, have been obtained in intact brain on the effects of chronic morphine treatment on adenylate cyclase activity or on the cellular components comprising the adenylate cyclase complex. We have systematically investigated on a regional basis the effects of chronic morphine treatment on adenylate cyclase activity and on levels of pertussis toxin-catalyzed ADP-ribosylation of Gi and Go.

Male Sprague-Dawley rats (150 g) were implanted subcutaneously with one 75 mg morphine pellet daily for 5 days. On day 6 the rats were sacrificed and brain regions isolated by punches taken from coronal cross sections. Punches were sonicated and a particulate fraction obtained by centrifugation for assay of adenylate cyclase activity and of ADP-ribosylation of G proteins.

Chronic morphine treatment was found to enhance basal, as well as GTP- and forskolin-stimulated, adenylate cyclase activity by approximately 30% in the locus coeruleus (LC), but to have no effect on adenylate cyclase activity in the neostriatum (NS) and dorsal raphe (DR), and variable effects in the frontal cortex (FC). In a similar manner, chronic morphine treatment was found to increase the level of pertussis toxin-catalyzed ADP-ribosylation of G-proteins by approximately 25% in the LC, but to have no effect in the NS, and variable effects in the FC. By one dimensional SDS polyacrylamide gel electrophoresis, it was not possible to determine whether the observed increase in the LC occurred in Gi or Go.

These results along with others reported in this volume (Nestler et al., Duman et. al.) indicate that the adenylate cyclase complex and cyclic AMP system can be characterized in discrete brain nuclei, and that regional differences exist in their regulation. Following chronic morphine treatment, there appears in the LC to be a specific increase in adenylate cyclase activity, the level of Gi/Go, and cyclic AMP-dependent protein kinase activity. These increases may contribute to an understanding of the molecular basis of known electrophysiological and behavioral phenomena of opioid tolerance, dependence, and withdrawal.

324.11 CHRONIC MORPHINE TREATMENT INCREASES CYCLIC AMP-DEPENDENT PROTEIN KINASE ACTIVITY IN THE RAT LOCUS COERULEUS. E.J. Nestler and J.F. Tallman. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

The biochemical mechanisms underlying the chronic effects of opiates remain unknown. Studies on cultured neuroblastoma x glioma cells have implicated the enzyme adenylate cyclase in the development of tolerance, dependence, and withdrawal, but it has been difficult to extend these findings to neurons, and steps in the cyclic AMP system other than adenylate cyclase have not been investigated as possible sites of action of opiates. We chose to study the chronic effects of opiates on the cyclic AMP system in the rat locus coeruleus (LC). The LC contains cell bodies of virtually one type of neuron, which develops tolerance, dependence, and withdrawal upon chronic opiate treatment, as shown by electrophysiological studies.

Male Sprague-Dawley rats (150 g) were implanted subcutaneously with one morphine pellet (75 mg) daily for 5 d and were used on day 6. Control rats underwent identical surgery, but were not implanted with pellets. Brain regions were obtained from isolated brains either by gross dissection or by making punches from coronal cross-sections of pons or midbrain. The brain regions were sonicated and particulate and soluble fractions prepared by ultracentrifugation. Cyclic AMP-dependent protein kinase activity was determined in both fractions by use of histone phosphorylation assays.

Chronic morphine treatment was found to increase cyclic AMP-dependent protein kinase activity in both the particulate and soluble fractions of the LC. Study of the time-course by which morphine regulates this protein kinase showed that chronic treatment is required for this effect: no increase in cyclic AMP-dependent protein kinase activity was observed in response to 1 d of treatment, regardless of whether kinase activity was assayed on day 2 or day 6. This increase in protein kinase activity appeared to be specific to the LC. Chronic morphine treatment did not alter cyclic AMP-dependent protein kinase activity in fractions of the frontal cortex, neostriatum, or dorsal raphe. Protein kinase activity was also unaltered in fractions of pons cross-sections from which LC punches were obtained and of punches taken just medial or lateral to the LC.

We have found in related studies (Erdos et al., this volume) that, concomitant with increased AMP-dependent protein kinase activity, chronic morphine treatment increases the level of adenylate cyclase activity and of certain G-proteins, effects also observed specifically in the LC. In addition, we have found (unpublished data) that chronic morphine treatment alters the phosphorylation of a number of individual phosphoprotein substrates for cyclic AMP-dependent protein kinase in the LC. These various effects may represent feedback mechanisms by which LC neurons adapt to chronic opiate exposure and may be part of the molecular basis of tolerance, dependence, and withdrawal.

325.1 EVALUATION OF TOXIN F AS A PROBE FOR NICOTINIC RECEPTORS IN RAT BRAIN. D.W. Schulz and R.E. Zigmond. Department of Pharmacology, Harvard Medical School, Boston, MA 02115.

Toxin F is present along with α-Bungarotoxin (BGT) in the venom of Bungarus multicinctus. Unlike BGT, toxin F (< 100 nM) has been shown to block nicotinic transmission in chick ciliary ganglia and cultured rat sympathetic neurons. In these tissues, toxin F appears to bind to two sites, only one of which is recognized by BGT. Thus it has been suggested that it is the toxin F-selective site that mediates nicotinic responses to acetylcholine.

We have attempted to determine if toxin F is an appropriate compound for studying nicotinic receptors in rat brain. Our studies have focused primarily on the corpus striatum, due to 1) the high concentrations of acetylcholine and choline acetyltransferase in this region, 2) reports of relatively high densities of [^{3}H]-nicotinic agonist binding sites, and 3) evidence that nicotinic receptors that modulate dopamine release are present on the terminals of nigrostriatal neurons.

[^{125}I]-Toxin F bound to striatal membranes with a K_d of 2 nM, a B_{max} of 20 fmol/mg protein, and a Hill coefficient of 0.9. Of 6 brain regions assayed, binding was highest in thalamus, hippocampus, striatum, and hypothalamus, moderate in cortex, and much lower in cerebellum. However, among the first four of these areas, there was considerable variation in the ratio of [^{125}I]-toxin F:[^{125}I]-BGT binding. As is the case with sympathetic neurons, 1 μM BGT competed for only a portion of specific striatal [^{125}I]-toxin F sites (27 + 3%), while unlabeled toxin F completely inhibited [^{125}I]-BGT binding. Kinetic studies revealed that specific binding did not reach equilibrium until after 2 hr at 37° C, and the K_{off} was 0.25/hr. In competition experiments, a variety of nicotinic agonists and antagonists competed for some toxin F-selective sites at appropriate concentrations, but failed to consistently inhibit more than 50% of specific binding. Furthermore, [^{3}H]-nicotine binding in striatum was inhibited only 15% by 1 μM toxin F.

When superfused striatal slices were exposed to the nicotinic agonist DMPP (10μM) for 1 min, a > 100% increase in efflux of endogenous dopamine was observed over the next 3 minutes. This response was reversed over 60% by the nicotinic antagonist mecamylamine (100 μM). However, Toxin F at concentrations up to 1 μM failed to inhibit this augmentation of dopamine release.

In summary, toxin F displays some of the characteristics expected of a nicotinic ligand. However, in view of the lack of convincing pharmacological evidence from binding and release studies that toxin F interacts with striatal nicotinic sites, caution should be exercised when considering this compound as a probe for central nicotinic receptors. Future experiments will utilize other brain regions and other functional assays to address this question. Supported by NS12651 and MH00162.

325.2 **ELECTRON MICROSCOPIC EVALUATION OF INTERNALIZATION OF (125I) ALPHA-BUNGAROTOXIN BINDING INTO RAT HYPOTHALAMIC NEURONS.** **M. M. Miller and E. Antecka.* Departments of Obstetrics and Gynecology and Experimental Medicine and The Center for the Study of Reproduction, McGill University, Montreal, Canada, H3A 1A1.**

The time course of internalization of labeled alpha-bungarotoxin (α-BTX) binding sites was examined by electron microscopic autoradiography in the suprachiasmatic nucleus (SCN) of the adult female rat. (125I) α-BTX was infused into the third ventricle and 4, 8, and 12 hours later animals were perfused percardially with aldehyde fixatives. The dorsal-lateral aspect of the SCN was processed for autoradiography. The ultrastructural distribution of silver grains was studied both by line source and 50% probability circle analysis. The distribution of grains evaluated by 50% probability circle analysis was compared with the distribution of randomly generated hypothetical grains. In electron microscopic autoradiographs, silver grains overlaid either single structures (exclusive grains) or multiple neuronal and/or glial profiles (shared grains). The distribution of real grains was significantly different from that of hypothetical grains at all time points. Non-specific binding was also assessed at 4, 8, and 12 time points in rats infused with (125I) α-BTX in combination with radioinert α-BTX; labeling in these controls was not above background at any time point. Quantification of silver grain localization by 50% probability circle analyses indicated that membrane bound sources were mainly associated with axo-dendritic appositions, regardless of the length of time the tissue was exposed to radioligand. At 4, 8, and 12 hours, the compartment containing synaptic terminals was the most enriched when comparing real to hypothetical grains. Probability circle analysis of exclusive grains indicated that by 8 hours after intracerebroventricular infusion of specifically labeled α-BTX, binding sites were most likely to be within neurons and dendrites. Radiolabel was associated with vesicular structures within the cytoplasm, but not with lysosomal structures. The present study demonstrated that the majority of α-BTX binding sites remain membrane bound with respect to time and may be associated with synaptic transmission. A significant proportion of the silver grains are internalized into soma and dendrites of SCN neurons. Supported by NIH RO1 HD - 19431 and Fonds du Recherche en Santé du Québec.

325.3 TOPOGRAPHIC, NONCOLLATERALIZED BASAL FOREBRAIN PROJECTIONS TO ANTERIOR CINGULATE CORTEX, HIPPOCAMPUS, AND AMYGDALA IN RHESUS MONKEY. V.E. Koliatsos†, L.J. Martin*†, L.C. Walker†, R.T. Richardson§, M.R. DeLong§ and D.L. Price†. †Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; §Neurophysiology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Although neocortical projections of the magnocellular basal forebrain complex (MBFC) appear to be topographic and not widely collateralized, the possibility of single neurons innervating multiple nonneocortical targets has not been studied in primates. The present study investigates the differential organization of MBFC neurons projecting to anterior cingulate cortex (ACC) and hippocampus (HC), and to amygdala (AM) and HC. All of the above targets receive abundant cholinergic innervation, and each pair of targets receives projections from overlapping sectors of MBFC. However, ACC and HC are interconnected to a minor degree, whereas AM and HC are extensively interconnected. The retrograde tracer Fast Blue was used for HC injections, and Diamidino Yellow was used for injections of AM and ACC. Neurons projecting to ACC were present in rostral septum, vertical limb of diagonal band of Broca (dbB), and dorsal anteromedial nucleus basalis of Meynert (nbM). Cells projecting to AM were widely distributed in anterolateral nbM, intermediate nbM, ventral posterior nbM, and, to a lesser degree, in medial septal nucleus, vertical limb of dbB, and anteromedial nbM. Neurons projecting to HC were situated in almost all sectors of MBFC, clustering most densely in medial septum/dbB/anteromedial nbM and, to a lesser degree, in posterior nbM. In the vertical limb of dbB, neurons projecting to HC mingled with neurons projecting to ACC, which tended to be more laterally and dorsally located, and cells projecting to AM, which were more medially and ventrally located. In anteromedial nbM, neurons projecting to HC were ventral to cingulopetal cells; in anterolateral nbM, they were randomly mixed with amygdalopetal neurons; in posterior nbM, they mingled with amygdalopetal cells but tended to be located more dorsally and laterally. Cells projecting to both ACC and HC were found very rarely in areas of maximum overlap of cingulopetal and amygdalopetal neurons; cells projecting to both AM and HC were approximately 2% of the total number of amygdalopetal and hippocampopetal neurons. These findings indicate that: nonneocortical projections of MBFC neurons are topographically organized; and the degree of interconnectivity and/or functional relatedness of terminal fields does not seem to be related to the degree of collateralization of MBFC axons. Moreover, although separate targets may receive afferents from widely overlapping MBFC regions, the vast majority of these afferents does not originate in the same neurons.

325.4 **THE CHOLINERGIC TELENCEPHALIC SYSTEM OF THE NEW WORLD CEBUS MONKEY: ANALYSIS OF THE PROJECTION TO AMYGDALA.** J.H.Kordower, R.T.Bartus[1], F.F. Marciano*, and D.M. Gash. Dept. Neurobiology and Anatomy, University of Rochester Sch. Med. Rochester N.Y. 14642 and [1] Medical Research Division, Lederle Laboratories, Pearl River N.Y. 10065 USA.

The telecephalic cholinergic system has received considerable attention in recent years due to its putative role in normal cognition and dementing disorders. While cortical projection systems from the basal forebrain have received detailed scrutiny, there have been few and conflicting reports concerning the cells of origin of the cholinergic projection to the amygdala. This study describes the telencephalic cholinergic system of the Cebus monkey and analyzes the pathway and cells of origin for this cholinergic limbic input.

Nine Cebus apella monkeys were anesthetized and perfused with 0.9% saline followed by a 4% paraformaldehyde/0.1% glutaraldehyde fixation. Four animals received an intra-amygdaloid injection of horseradish peroxidase conjugated to wheat germ agglutinin (2.5-5%; 1 ul volume) 48-72 h prior to sacrifice. Sections were processed for choline acetyltransferase (ChAT) immunoreactivity (antibody AB8/dilution 1:20), acetylcholinesterase (ACHE), and Nissl substance. To assess the cells of origin for the projection to amygdala, other sections were processed via cobalt stablized tetramethylbenzidine (TMB) histochemistry either alone, or in combination with ChAT or ACHE staining.

Magnocellular ChAT-immunoreactive neurons were observed in the CH2-CH4 regions of the basal forebrain. These cells were up to 50 μm in diameter, were rounded in shape and often contained an eccentric nucleus. These cells had multipolar processes emanating from their perikarya. ChAT-immunoreactive cells in the CH1 region were oval in shape with their long axis oriented in the vertical direction. Apical and basal processes were observed emanating from these cells. Smaller cells were observed in the striatum and an occasional cell observed in the bed nucleus of the stria terminalis. Cholinergic somata were never observed in the cortex or hippocampus. Many processes from cells in the CH3-4 region were observed to project in apposition to proximal blood vessels.

Double labeled cholinergic cells projecting to amygdala (TMB+/ChAT+ or TMB+/ACHE+) were restricted to the sublenticular portions of the basal forebrain. Most of these cells were found in the anterolateral portion of the CH4 region. Fewer were found in the anteromedial portion of the CH4 and in the CH3 region. No double labeled cells were found in the CH1-2 complex and virtually no double labeled cells were found in the medullary laminae of the globus pallidus. A few double labeled cells were located just caudal to the amygdaloid complex and thus project rostrally to their terminal region.

These data will be discussed in terms of the possible role this cholinergic pathway might play in terms of both experimental studies of cognition and in neurodegenerative diseases in which cognitive dysfunction is a central feature.

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325.5 CHOLINE ACETYLTRANSFERASE CONTAINING NEURONS OF RAT AND MONKEY BRAIN. H. Tago*, P.L. McGeer, L.B. Hersh, and G. Bruce* (SPON: P.C.K. Leung). Kinsmen Lab. of Neurol. Res., Dept. of Psychiatry, Univ. B.C., Vancouver, B.C. and Dept. Biochem., Univ. of Texas Health Sci Centre, Dallas, Texas.

We have used a highly sensitive polyclonal anti-human placental choline acetyltransferase (ChAT) rabbit serum, combined with a nickel ammonium sulfate second antibody intensification method, to identify ChAT-containing neurons in rat and monkey brain. The method permitted the identification of a number of new neuronal groups, as well as the confirmation of several other neuronal groups that had formerly been regarded as uncertain. Intense fiber staining permitted the direct tracing of some cholinergic tracts.

The most prominent new ChAT neuronal system was located in the hypothalamus. Small neurons in the infundibular (arcuate) nucleus had fibers extending towards the infundibulum. Other ChAT-positive hypothalamic cells were scattered loosely in the surrounding matrix. A medially distributed group was close to the third ventricle running in a rostro-caudal fashion and a lateral one was distributed principally in the region of the medial forebrain bundle, the lateral dorsal aspect of which contained mainly cholinergic fibers. The identification of these cells helps to account for previous biochemical and pharmacological studies which have strongly indicated the presence of intrinsic cholinergic neurons in the hypothalamus.

Other new neuronal groups include strongly positive cells in the parabigeminal nucleus and a few positive cells in the central gray and near the roof of the aqueduct, and in the cerebellum.

The method permitted confirmation of an extensive system of bipolar neurons in the cerebral cortex of the rat, but not the monkey. A few cholinergic neurons were seen in the amygdala and hippocampus. A strongly positive group was located in the medial habenula. Other, previously reported brainstem groups, were all positive (Kimura et al. in Handbook of Chemical Neuroanatomy, Vol. 3, pp. 51-65, Eds. A. Bjorklund and T. Hokfelt, 1984).

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325.6 DISTRIBUTION OF CHOLINERGIC STRUCTURES IN THE CD-1 MOUSE BRAIN DEMONSTRATED BY IMMUNOCYTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE (ChAT). M.G. Cunningham* and E.J. Mufson (SPON: J. Rogers). Harvard Medical School, Boston, MA 02115 and Institute for Biogerontology Research, Sun City, AZ 85351.

A monoclonal antibody (AB8) (Levey et al., J. Neurosci., 3:1-9, 1983) against ChAT was used to localize central cholinergic structures in the CD-1 mouse brain.

Cholinergic perikarya were observed within five general nuclear groups: 1) striatum, including caudate, putamen, nucleus accumbens, and olfactory tubercle; 2) basal forebrain including the medial septal region (Ch1), vertical limb nucleus of the diagonal band (Ch2), horizontal limb nucleus of the diagonal band (Ch3), and substantia innominata-nucleus basalis complex (Ch4); 3) pontomesencephalic nuclei including the pedunculopontine nucleus (Ch5), lateral dorsal tegmental nucleus (Ch6), and parabigeminal nucleus (Ch8); 4) epithalamus including the medial habenular nucleus (Ch7); and 5) cranial nerve nuclei. Cholinergic cell bodies were also found in the cerebral cortex, hippocampus, nucleus tractus solitarius and medullary reticular formation.

Fiber staining was seen within several axonal tracts including the corpus callosum, cingulum bundle, anterior commissure, fornix, fasciculus retroflexus and portions of the cranial nerves. Fine varicose fibers were found within cortex, striatum, amygdala, hippocampus, thalamus and superior colliculus. ChAT immunoreactive fibers were observed swirling around the densely stained interpeduncular nucleus. Intense ChAT staining was also seen in the lateral amygdala and in the parabigeminal nucleus.

The present study indicates that the distribution and characteristics of cholinergic structures in the CD-1 mouse are similar to those previously reported in the rat and monkey. These factors, combined with our finding of age-related shrinkage of cholinergic basal forebrain neurons in the CD-1 mouse (Mesulam, Mufson, and Rogers, Ann. Neurol., 1987), make this animal a potential model for the investigation of age-associated changes of central cholinergic systems.

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325.7 ASCENDING BRAINSTEM PROJECTIONS TO FOREBRAIN CHOLINERGIC NEURONS. L. Zaborszky, W.E. Cullinan, and L. Heimer. Department of Otolaryngology, University of Virginia School of Medicine, Charlottesville, VA 22908.

We have reported previously that following large meso-diencephalic knife cuts, degenerating terminals are found on cholinergic dendrites in the forebrain (Zaborszky et al., Soc. Neurosci. Abst. 12:571, 1986). Furthermore, the specific removal of monoaminergic afferents (6-hydroxydopamine and 5,7 -dihydroxytryptamine) resulted in decreased choline acetyltransferase (ChAT) activity in the forebrain and areas containing the cholinergic projection neurons (Zaborszky and Luine, J. Cell Biochem., Suppl. 11D:187, 1987). In the present study we used the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) in order to determine the areas of origin of brainstem afferents to the cholinergic neurons.

PHA-L was iontophoretically injected in adult rats in the following brainstem areas: ventral tegmental area (A-10 cell group), various portions of the substantia nigra (A-9 cell group), cell groups in the vicinity of the superior cerebellar peduncle (pedunculopontine tegmental nucleus, A-7 cell group, Kolliker-Fuse nucleus, medial and lateral parabrachial nuclei), A-5 cell group, the locus coeruleus (A-6 cell group), subcoeruleus nucleus, Barrington's nucleus, rostroventrolateral reticular nucleus (C-1 cell group), parvocellular reticular nucleus, and the rostral portion of the A-1 cell group. Following 10-day survival periods, brains were fixed via transcardial perfusion, sectioned at 40 um in the coronal plane, and processed for PHA-L immunoreactivity following the conventional avidin-biotin peroxidase (ABC) protocol (Hsu et al., 1981). Another series was processed for the simultaneous visualization of PHA-L labeled fibers and terminals and ChAT containing cell bodies using several different double-labelling techniques. These included the double fluorescence method (FITC/RITC), and the ABC technique utilizing the same (DAB-intensified/DAB; Gorcs et al., 1986) or different chromagens (DAB/BDHC; Levey et al., 1986). Following all successful PHA-L injections, terminal and 'en passant' varicosities surrounding cholinergic cell bodies and dendrites were found in varying amounts in the lateral part of the nucleus of the vertical limb of the diagonal band (DB), the dorsal and medial portions of the horizontal limb of the DB, substantia inominata, ventral pallidum, globus pallidus, and lateral hypothalamus. Since in many cases the PHA-L deposit labeled heterogenous neuronal populations, studies are in progress to determine the neurotransmitter content of these fibers.

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325.8 CHOLINERGIC PEDUNCULOPONTINE TEGMENTAL N. PROJECTIONS TO THE FOREBRAIN. A. E. Hallanger and B. H. Wainer. Com. on Neurobiology, The University of Chicago, Chicago, Il 60637.

We have previously demonstrated that the pedunculopontine tegmental nucleus (PPT) in the midbrain provides cholinergic afferents to all thalamic nuclei, including the principal relay nuclei. The laterodorsal tegmental nucleus (LDT) is a major second source of cholinergic afferents to some thalamic nuclei, including the laterodorsal, anteroventral, and mediodorsal nuclei. We have now investigated projections from the PPT and surrounding mesopontine tegmental neurons to other subcortical regions. The anterogradely transported lectin phaseolus vulgaris leukoagglutinin (PHA-L; 2.5%) was iontophoretically injected into the PPT in 10 rats. The tracer was immunohistochemically visualized, with diaminobenzidine as the chromogen, and tissue sections were counterstained with cresyl violet. Anterogradely labelled fibers and varicosities were observed in the thalamic nuclei, confirming the findings of our previous retrograde studies. In addition, PHA-L-labelled fibers and varicosities suggestive of terminal fields were observed in the tuberal and posterior lateral hypothalamic regions, the anterior hypothalamic area, the ventral pallidum in the region of the nucleus baslis of Meynert, the dorsal and lateral septal regions, and in the central and medial nuclei of the amygdala.

To determine whether the cholinergic neurons of the PPT project to these subcortical structures in which axons anterogradely labelled from the region of the PPT are found, the retrograde tracer WGA-HRP was injected into the hypothalamus (n=8), septum (n=7), ventral pallidum (n=5), and amygdala (n=4). Tissue sections were processed for both the retrograde tracer (using TMB) and for ChAT immunohistochemistry (using DAB). Single- (retrograde only) and double- (retrograde + ChAT) labelled neurons in the mesopontine tegmentum were counted in a one of four series of 50 micron sections from these animals. Numerous ChAT-immunoreactive neurons in the PPT and LDT were retrogradely labelled following placement of WGA-HRP into the tuberal and posterior lateral hypothalamus and the anterior hypothalamic area.These retrogradely labelled cholinergic neurons comprised an average of 10% of the total number of retrogradely labelled neurons in the mesopontine tegmentum. Noncholinergic neurons of the central tegmental field, retrorubral field, and cuneiform nucleus were also retrogradely labelled. Following placement of WGA-HRP into dorsal and lateral septal regions, the vast majority of retrogradely labelled neurons were cholinergic neurons of the PPT and LDT, with few noncholinergic retrogradely labelled neurons in the adjacent tegmentum. In contrast, few cholinergic neurons were retrogradely labelled following placement of tracer into the nucleus basalis of Meynert or into the central, medial, and basolateral nuclei of the amygdala. Noncholinergic neurons of the central tegmental field rostral to the PPT and of retrorubral field adjacent to the PPT were retrogradely labelled in these cases. In summary, these anterograde and retrograde studies demonstrate that the cholinergic PPT provides a substantial proportion of mesopontine tegmental afferents to the hypothalamus and lateral septum, while its projections to the nucleus basalis and the amygdala are minimal. Supported by PHS NS 17661 and HD-04583 (B.H.W.) and PHS 5T32 GM-07281 (A.E.H.).

325.9 Changes in the Density of Cortical Cholinergic Innervation in Human Aging and Alzheimer's Disease. Changiz Geula and Marsel Mesulam, Harvard University and Beth Israel Hospital, Boston, MA.

Several lines of evidence indicate that an AChE-rich staining pattern provides a reliable marker for the cholinergic axons of the cerebral cortex. We used a photomontage-based intersect analysis to measure the density of acetylcholinesterase (AChE)-rich fibers in the cerebral cortex (entorhinal area, cingulate gyrus, inferotemporal area TE) of four control subjects (aged 22, 43, 68 and 91 years) with no history of dementia and four confirmed cases of Alzheimer's disease (AD) (aged 78, 88, 88 and 86 years). The AChE-rich fibers were visualized using a modified form of the histological method described by Tago et al. (J. Histochem Cytochem 34:1431, 1986).

In the control subjects, all cortical areas contained a dense net of AChE-rich axons displaying numerous synaptic swellings. Each cortical area had an individual pattern of lamination and density. In keeping with our prior observations in the monkey brain, (Ann. Neurol 19:144, 1986) the entorhinal and cingulate regions contained a denser net of cholinergic axons than inferotemporal visual association cortex. In order to assess the effects of aging and AD, we analyzed "best-stained" areas within comparable sectors of the three architectonic regions under investigation. Our results therefore emphasize changes in the "peak density" of cortical cholinergic innervation in these regions.

Aging was associated with a relatively modest decrease in the density of AChE-rich fibers. With the possible exception of entorhinal cortex, no consistent loss of density could be discerned from 22 to 43 and 68 years of age. When the 91 year old subject was compared to the 22 year old, the reduction in fiber density was in the order of 17-25% (25% in entorhinal cortex, 23% in area TE and 17% in cingulate cortex). The age-dependent reduction in fiber density appeared more pronounced in the deep cortical layers as compared with the superficial layers. In the brains of AD patients, AChE fiber density was markedly and consistently reduced even when compared to the 91 year old control subject. In all AD cases, area TE contained practically no AChE-positive fibers whereas in the cingulate cortex, varying densities of these fibers were detectable in two of the cases (60% and 20% reduction when compared to the 91 year old brain). The entorhinal cortex in AD showed a near-total loss of AChE-rich axons but this was not as complete as in TE. Our results provide the morphological basis for prior neurochemical reports showing a greater loss of cholinergic markers in the temporal lobe than elsewhere in the senile form of AD (Rossor et al. Brain 105:313, 1982). While based on a relatively small sample, these observations also suggest that the age-related alterations of cortical cholinergic innervation are quite modest, becoming established only in the late senium.

Supported by the McKnight Foundation, Javits Neuroscience Investigator Award (NS20285) and an Alzheimer's Disease Research Center Grant (AGO5134).

325.10 TIME OF ORIGIN OF CHOLINERGIC NEURONS IN THE RAT BASAL FOREBRAIN. K. Semba and H.C. Fibiger. Division of Neurological Sciences, Department of Psychiatry, University of British Columbia, Vancouver, B.C. V6T 2A1 Canada.

The basal forebrain contains a major group of cholinergic neurons which is distributed from the medial septum rostrally to the region of the ventromedial globus pallidus caudally. These cholinergic neurons project to the cerebral cortex, hippocampus, amygdala, and olfactory bulb. In the present study, the development of the basal forebrain cholinergic neurons was investigated using tritiated thymidine autoradiography combined with choline acetyltransferase (ChAT) immunohistochemistry. Tritiated thymidine was injected into timed pregnant rats (conception designated day 0), and the brains of their progeny as young adults were processed for immunohistochemistry followed by autoradiography. The combined techniques permit the determination of the time of final mitotic division of cholinergic neurons.

The first ChAT-immunoreactive neurons to become post-mitotic were located in the caudal globus pallidus and the substantia innominata. In these regions, the peak of final mitotic division of ChAT-immunoreactive neurons occurred on embryomic (E) day 12. In the rostral globus pallidus, the ventral pallidum, and the magnocellular preoptic area, the peak of cholinergic neurogenesis occurred slightly later, on E13. In all the above regions, most ChAT-immunoreactive neurons had left the mitotic cycle by E15. In the horizontal and vertical limbs of the diagonal band, most ChAT-positive neurons became post-mitotic on E13-15. In the medial septum, the peak of final mitotic activity occurred on E15, although some ChAT-positive neurons underwent final mitosis earlier. In all basal forebrain regions, cholinergic neurogenesis was complete by E17.

These results indicate that there is a caudal to rostral gradient in the neurogenesis of cholinergic neurons in the basal forebrain, with peak final mitosis occurring in the caudal cells approximately 3 days earlier than in the rostrally located neurons. It has previously been shown that (1) the cholinergic projection to the cerebral cortex arises in caudal parts of the basal forebrain, whereas that to the hippocampus originates from rostral basal forebrain neurons, and (2) the neurogenesis in the cortex largely preceeds that in the hippocampus. Together, these findings point to a correlation between the gradient of neurogenesis of the cholinergic basal forebrain neurons, and that of their target neurons.

Supported by the Medical Research Council.

325.11 LOCALIZATION OF mRNA ENCODING THE ENZYME ACETYLCHOLINESTERASE IN THE TORPEDO ELECTROMOTOR SYSTEM BY IN SITU HYBRIDIZATION. M.W. Newton, D. Healy, M. Schumacher* and P. Taylor*. Depts. of Pharmacology, Univ. of Calif., San Diego, CA 92093 and Mt. Sinai Medical School, New York, NY 10029.

The electric organ of Torpedo californica is an abundant source of the asymmetric and globular forms of acetylcholinesterase (AChE). AChE is synthesized in the electrocytes of the electric organ and in the cholinergic neurons of the electromotor nucleus of the brain, from which it is transported along the axons to the electric organ. In situ hybridization is a technique which can potentially localize the cellular sites of biosynthesis of the molecular forms of AChE and which may be useful for studying the regulation of AChE expression. We have used in situ hybridization to analyze the distribution of mRNA coding for AChE at the cellular level in the Torpedo electromotor system. The ^{35}S-labeled RNA probes were prepared by transcription of the AChE cDNA recently isolated in our laboratory with SP6 and T7 RNA polymerases. The presence of large molecular weight RNA species coding for AChE has previously been demonstrated by Northern blot analysis of these tissues.

Frozen tissue sections were cut from fixed or fresh frozen electric organ and electromotor nucleus, fixed onto slides and hybridized with antisense (experimental) or sense (control) strands of the labeled RNA probe encoding AChE. After washes and RNAase treatment, the sections were analyzed by autoradiography.

Autoradiograms of transverse sections through the electric lobe of the brain after hybridization with antisense ^{35}S-RNA revealed dense, punctate labeling in the electromotor nucleus. This pattern was not seen in adjacent sections hybridized with the sense strand. High resolution emulsion autoradiography indicated that the dense punctate labeling was due to the labeling of the large electromotor neurons, whereas the surrounding glial cells were labeled at only low density. Hybridization of the electric organ sections with the antisense ^{35}S-RNA probe resulted in a diffuse pattern of labeling, which was more dense than that observed in sections hybridized with the sense probe. High resolution emulsion autoradiography indicated that the antisense RNA label was frequently associated with the nucleus and perinuclear regions in the electrocytes.

These results indicate that mRNA coding for AChE may be localized at the cellular level by in situ hybridization. Because the catalytic subunits of asymmetric and globular AChE forms may differ in primary structure near the carboxy terminus, specific probes may be developed which can localize expression of the different enzyme forms at the cellular level by in situ hybridization. Supported by USPHS fellowship NS 07622 to MN and by GM-8360.

325.12 LANDMARKS IN NEUROCHEMISTRY I. HENSING AND THUDICHUM. L.H. Marshall and H.W. Magoun, Brain Research Institute, University of California, Los Angeles, CA 90024.

The earliest systematic analysis of mammalian brain chemistry was carried out by Johann Becker Hensing (1683-1745), a Hessian scholar, physician, and chemist. He looked for, and found, phosphorous in beef brain and published his findings in 1719. His detailed study establishes Hensing as the first modern neurochemist,* but his contributions were largely forgotten.

A century-and-a-half later, another Hessian, Johann Ludwig Wilhelm Thudichum (1829-1901), wrote and published the next important study in brain chemistry and also acknowledged his fellow-countryman's work. After receiving his M.D. from the University of Giessen as had Hensing, Thudichum emigrated to England. In A Treatise on the Chemical Constitution of the Brain (1884), he identified the colloidal nature of the brain "bioplasm" and related it to the phosphatides which he knew occurred in plant as well as animal bioplasm. His findings were assailed and discounted until they were published 17 years later in Germany. Many of the substances he isolated from brain tissue--lecithins, cephalins, and myelins--he identified, classified, and named, and he prepared pure samples that exist today. Thudichum is credited with prophesizing psychochemistry by suggesting that many forms of insanity are due to effects upon the brain-substance of "poisons fermented within the body". Unfortunately, in his treatise he did not discuss any functional aspects of the compounds he discovered.

*Tower, D.B. Hensing, 1719. New York: Raven Press, 1983

325.13 LANDMARKS IN NEUROCHEMISTRY II. NEUROTRANSMISSION TO 1940. H.W. Magoun and L.H. Marshall. Brain Research Institute, University of California, Los Angeles, CA 90024.

In the 19th century the roots of functional neurochemistry were gathering evidence not from the central nervous system, but from the periphery with studies of the nerve-muscle preparation. In 1877 Eduard du Bois-Reymond stated that contractile tissue is stimulated at its boundary by "a thin layer of ammonia, lactic acid, or some other powerful stimulating substance. Or the phenomenon must be electrical". This chemical concept stemmed from Fabbrioni's explanation of excitation from contact of dissimilar metals (1792). In 1894 F.S. Locke, in Germany, recognized that calcium ions were necessary to effect neuromuscular transmission. By the early years of the 20th century, his compatriot, Ernest Overton, not only confirmed Locke but showed that sodium ions were required for nerve excitability and suggested a sodium-potassium exchange was involved in conductance. William Howell in the United States and Thomas R. Elliott and John N. Langley in Great Britain published observations that strengthened the idea of the chemical nature of nerve action. Otto Loewi's dual frog heart preparation and its refinement by Kahn were the means of proving that point. Langley in 1905 proposed his receptor theory for a chemical synapse, postulating a "receptive substance" to receive the stimulus from the nerve and pass it on to the postsynaptic cell.

The identification of adrenalin and noradrenalin, involving both British and Swedish investigators, established the concept of adrenergic neurotransmission by the early 1930s. In that same decade David Nachmansohn proposed that acetylcholine, in conjunction with certain enzymes, control bioelectric signals. By the next decade some of the biophysicists and neurochemists had identified themselves as molecular neurobiologists by their concentration on events associated with neural activity.

ACETYLCHOLINE: METABOLISM II

326.1 CHOLINE PROTECTS AGAINST STIMULATION-INDUCED DECREASE IN BRAIN MEMBRANE PHOSPHATIDES. I.H. Ulus, R.J. Wurtman, C. Mauron* and J.K. Blusztajn. Department of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, USA

Rat brain slices continue to make and release large amounts of acetylcholine (Ach) without exhibiting reductions in their contents of free choline or Ach, even when superfused with a choline free medium. This study examines the possibility that the choline in membrane phospholipids is the source of the choline used for Ach synthesis under these conditions.

Male Sprague-Dawley rats, 200-250 g., were decapitated, their striata rapidly dissected, and 300 μm slices prepared using a McIlwain tissue chopper. The slices were superfused (0.6 ml/min. 37°C) with a physiological medium containing (mM): NaCl, 120; KCl, 3.5;$CaCl_2$,1.3;$MgSO_4$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; glucose 10 and eserine salicylate, 0.02 (a cholinesterase inhibitor). The medium was continuously bubbled with 95% O_2 and 5% CO_2 (pH = 7.4). After 20 min equilibration, samples of slices were removed for the determination of "initial" tissue contents of phospholipid phosphate. The remaining slices were superfused at rest for 20 min. then electrically stimulated (1 ms pulses: 15 Hz, 30 V amplitude) for 20 min. and then maintained at rest for another 20 min. This stimulation-rest procedure was repeated 8 times. At the end of the 2nd, 4th, 6th and 8th stimulation-rest period, samples of slices were removed for determination of phospholipid. Effluents were collected during every period for determination of Ach.

Total tissue phospholipids were found to have declined from 0.746± 0.014 (μg P/μg DNA: initial) to 0.666 ± 0.040; 0.621 ± 0.02b; 0.576 ± 0.025; and 0.569 ± 0.028 after the 2nd, 4th, 6th and 8th stimulation periods, respectively. Phospholipid levels were inversely related (r = -0.98) to the number of the stimulation period. Addition of choline to the medium (10, 20 or 40 μM) markedly enhanced Ach release, both basal and during stimulation; it completely protected the slices from the phospholipid depletion observed in its absence. Stimulation in the choline-free medium caused proportionate decreases in tissue phosphatidylcholine (PC), phosphatidylethanolamine and phosphatidylserine, so that their ratio to total phosphatides did not decline. Addition of choline to the superfusion medium, while blocking the reduction in phosphatide content, also failed to affect this ratio.

These data support the hypothesis that the phospholipids in brain membranes serve as a reservoir of choline to be used for Ach synthesis. They also indicate that when the supply of free choline is inadequate to sustain Ach synthesis, the cell obtains more choline not by selectively utilizing PC, but by diminishing the total amount of membrane.

326.2 N-METHYLCARBAMYL CHOLINE, A NOVEL NICOTINIC AGONIST, ENHANCES ACETYLCHOLINE RELEASE FROM RAT BRAIN SLICES. D. M. Araujo, P. A. Lapchak, B. Collier and R. Quirion. Douglas Hospital Res. Ctr. and Depts. of Psychiatry and Pharmacology, McGill University, Montreal, Canada H4H 1R3.

[^{3}H]N-methylcarbamyl choline (MCC) binds specifically, saturably, and with high affinity to nicotinic sites in rat cerebral cortex (Abood and Grassi, Biochem. Pharmacol. 35:4199-4202, 1986; Boksa and Quirion, in press). The receptor binding characteristics of [^{3}H]MCC to rat brain are similar to those of [^{3}H]nicotine. The present study attempted to clarify the possible function of the [^{3}H]MCC/nicotinic sites in rat brain. [^{3}H]MCC binding to membranes from rat frontal cortex and hippocampus was characterized as follows: membranes were prepared by homogenizing brain tissue in a Brinkmann Polytron in buffer containing (mM) Tris.HCl 50, NaCl 120, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, pH 7.4, after which homogenate was centrifuged (49000g/10min/3x). The final membrane pellet was resuspended in fresh buffer and incubated with various concentrations of [^{3}H] MCC (60min, at 4°C), with non-specific binding defined in the presence of 10μM nicotine. The results show that [^{3}H]MCC binds to both frontal cortex (B_{max}=52.4fmol/mg protein, k_d=14.9nM) and hippocampus (B_{max}=21.7fmol/mg protein, k_d=14.0nM) with high affinity and to an apparently single class of sites. The effect of unlabeled MCC on acetylcholine (ACh) release from slices of rat frontal cortex and hippocampus was then tested. Slices were prepared using a McIlwain tissue chopper and pre-incubated in Krebs medium (pH 7.4) containing eserine (30μM) and choline (10μM) for 60min (37°C). Medium was then replaced with fresh buffer and tissue was reincubated for 10min. This incubation was repeated either with or without (controls) various concentrations of MCC. The drug enhanced spontaneous ACh release; this effect was concentration-dependent, with the maximal increase (164 and 74%) evident at concentrations of 1 and 10μM, for hippocampus and frontal cortex, respectively. Similarly, nicotine (1μM) also augmented ACh release from rat brain slices (by 78% in hippocampal slices), although it was not as potent as MCC. The increase in spontaneous ACh release measured in the presence of MCC would appear to be mediated by a nicotinic receptor since it was abolished by the nicotinic antagonists, d-tubocurarine (1μM) and dihydro-β-erythroidine (1μM), but not by the muscarinic antagonist, atropine (1μM). In contrast to its effect on spontaneous ACh release, MCC did not alter evoked ACh release induced by high K^+ (50mM) medium. In summary, the present results are compatible with the idea that presynaptic nicotinic receptors may be involved in the regulation of ACh release in rat brain, at least in the frontal cortex and in the hippocampus. (Supported by MRC, Canda and FRSQ and FCAR, Quebec)

326.3 EFFECTS OF CHOLINERGIC AGENTS ON RAT BRAIN ACETYLCHOLINE METABOLISM: EVIDENCE FOR REGIONAL DIFFERENCES IN METABOLIC REGULATION. A. Enz* (SPON: P.H. Kelly), Preclinical Research, Sandoz Ltd., CH-4002 Basle/Switzerland.

Interest in the metabolism of acetylcholine (ACh) in the brain and the factors that control or modulate it was greatly stimulated by the discovery that an impairment of cholinergic function may play a crucial role in Alzheimer's disease. Muscarinic agonists like oxotremorine, aceclidine and RS86, a spirosuccinimide, increase ACh levels in striatum, cortex and hippocampus of the rat brain in a dose dependent manner, but not in the pons/medulla region. Consistent with these findings, no alteration in ACh turnover is observed after pulse injection of deuterated choline. AChesterase inhibitors (AChE-I) such as tacrine, physostigmine and the miotine derivative RA7 reduce ACh turnover and enhance ACh levels in rat striatum, cortex and hippocampus, but again these drugs exert no influence on pons/medulla. However, the drugs examined stimulate metabolism of dopamine in the striatum, of noradrenaline in the cortex and of serotonin in hippocampus and cortex, whereas no change in noradrenaline metabolism is observed in the pons/medulla region.

The results demonstrate regional differences in the regulation of ACh metabolism. In the forebrain, ACh levels and turnover rates were affected by muscarinic agonists and endogenous ACh, whereas in the pons/medulla these parameters were remarkably constant, suggesting region-specific mechanisms regulating ACh turnover.

326.4 THE RELEASE OF ENDOGENOUS ACETYLCHOLINE FROM RAT TISSUE SLICES PREPARED FROM THE NUCLEUS BASALIS. R.H. Metcalf* and R.J. Boegman (SPON: C. Romero-Sierra). Department of Pharmacology and Toxicology, Queen's University, Kingston, Canada.

Physiological and neuroanatomical studies indicate that there is a cholinergic input to the nucleus basalis (NB) of the rat. It has been proposed that this input arises from the magnocellular cholinergic neurons intrinsic to this region (Kristt et al., Brain Res. 337: 19-39, 1985; Lamour et al., Brain Res. 362: 122-131, 1986). The objective of our study was to measure the release of endogenous ACh from tissue slices of the NB following depolarization with 35 mM K^+ or exposure to 3,4-DAP, a compound known to enhance the release of neurotransmitter from spontaneously active neurons (Thesleff, Neurosci. 5: 1413-1419, 1980). In our study, three strategies were employed to determine the location of the NB. Firstly, choline acetyltransferase (ChAT) activity was determined according to the method of Fonnum (1951). Secondly, quinolinic acid (QUIN), an excitotoxin that produces a 50-60% reduction in cortical ChAT, was stereotaxically injected into the right NB. ChAT activity was measured in both ipsilateral and contralateral regions seven days later. Thirdly, immunohistochemical staining for ChAT-positive neurons was assessed. Having established our dissection, endogenous ACh release from the slices was determined by gas chromatography - mass spectrophotometry (GC-MS). Values were expressed as the mean ± SEM of 6-8 animals unless otherwise stated.

ChAT activity in the NB was 101.84 ± 2.95 nmoles ACh formed/mg protein/hr in the right and 95.37 ± 4.35 nmoles/mg protein/hr in the left. There was no significant difference between the hemispheres. Unilateral injection of 1 µl, 120 mM QUIN into the right NB produced a 66.36% ± 2.82% reduction in ChAT activity at the injection site which was significantly different from control ($p \leq 0.05$). ChAT immunohistochemistry demonstrated the presence of large, sparsely distributed neurons in the tissue slices.

Depolarization (35 mM K^+) of the tissue slices produced a significant increase in the release of ACh. ACh release in the right NB was 1.669 ± 0.088 nmoles/g wet wt/min and in the left was 1.796 ± 0.165 nmoles/g wet wt/min. These values were significantly different from spontaneous release, which varied between 0.168 and 0.225 nmoles/g wet wt/min, but not from each other. The K^+-induced release of ACh was totally dependent on the presence of Ca^{2+} (1.3 mM added to the incubation buffer). 1 mM 3,4-DAP caused the release of 0.448 ± 0.034 nmoles/g wet wt/min ACh (n=4), which was well above spontaneous release. However, it was below that seen in the presence of 35 mM K^+. Our data indicate that ACh can be released from the large cholinergic neurons in the NB by a calcium-dependent process.

Supported by the Medical Research Council of Canada.

326.5 ACTIVATION OF PRESYNAPTIC OPIATE RECEPTORS DECREASES THE RELEASE OF ENDOGENOUS ACETYLCHOLINE FROM RAT AND GUINEA-PIG CORTICAL SLICES. P.A. Lapchak, D.M. Araujo and B. Collier. Dept. Pharm. and Ther., McGill University, Montreal, Quebec, Canada, H3G 1Y6.

There is considerable evidence in the literature suggesting that opiates may have a role in the modulation of neurotransmitter release in the rat brain. Receptor autoradiography has shown that mu, delta and kappa opiate receptors are located in the rat and guinea-pig cortex. However, little direct evidence for a functional link between these opiate receptors and cholinergic function is available. The present study attempted to identify the opiate receptor subtype(s) involved in the regulation of acetylcholine (ACh) release from the cortex of the rat and guinea-pig.

In rat cortical slices, k specific agonists U-50,488H and dynorphin A(1-13) (10 µM) decreased the K^+ evoked release of ACh in the frontal (23.0 ± 5.4% and 24.2 ± 5.9%), parietal (37.5 ± 4.5% and 23.3 ± 4.9%) and occipital cortex (25.2 ± 8.7% and 31.9 ± 4.2%). The µ selective agonists, DAGO and FK-33824 (10 µM), also decreased the release of ACh from the parietal cortex (29.1 ± 4.5% and 29.7 ± 7.3%), but did not have any effect on the evoked release of ACh from either the frontal or occipital cortex. The δ selective agonists DADLE and DSLET (10 µM)were ineffective in altering ACh release from all cortical areas, suggesting that δ opiate receptors appear not to be involved in the regulation of cortical ACh release.

In the guinea-pig, agonists (1 µM) of each of the three opiate receptor subtypes depressed evoked ACh release from the parietal cortex. This decrease was 27% for k (U-50,488H), 22% for δ (DSLET and DADLE) and 24% for µ (FK-33824). In the occipital cortex, k (U-50,488H) and δ (DSLET) agonists (1 µM) inhibited evoked ACh release by 35% and 32%, respectively; µ receptor ligands were ineffective. In the frontal cortex, both k and δ opiate receptor agonists depressed evoked release. However, this effect was much less (16-22%) than in the occipital or parietal cortex, and was apparent only at higher concentrations (10 µM) of these drugs. The opiate agonists were without effect on the basal release of ACh from either rat or guinea-pig cortical slices. The effects of the opiate agonists were reversed by incubation in the presence of 10uM naloxone.

The possible mechanism(s) whereby the opiate receptor agonists tested may alter ACh release is not clear but may be that Ca^{+2} is involved in this effect. This is supported by results which show that none of the opiates which clearly altered evoked release had any effect on basal (non-calcium dependent) ACh release. In conclusion, these results suggest that presynaptic opiate receptors are involved in the regulation of endogenous ACh release from a population of cholinergic neurons in the cortex, but this effect appears to be species dependent. (Supported by MRC, FCAR, Canada).

326.6 A TRANSMEMBRANE SIGNAL IN THE ACETYLCHOLINE STORAGE SYSTEM OF SYNAPTIC VESICLES. K.Nornberg and S.M. Parsons.Department of Chemistry,University of California,Santa Barbara,CA 93106.

The acetylcholine(ACh) storage system of Torpedo electric organ synaptic vesicles is composed of an ATPase,an ACh transporter and a receptor for the inhibitory compound 2-(4-phenylpiperidyl)cyclohexanol (vesamicol).Highly purified synaptic vesicles submitted to hyposmotic shock reseal in isosmotic buffer during20 min incubation at 23°C as measured by [^{14}C]ribitol retention.Some properties of the vesamicol receptor in lysed and resealed synaptic vesicles were studied.Vesicle ghosts exhibit an increase of [^{3}H]vesamicol binding up to 170% compared to that of intact vesicles.[^{3}H]Vesamicol bound to lysed vesicles was displaced in a dose-dependent manner by a nonpermeable analog of vesamicol with potency similar to that in the intact vesicles.Thus, the vesamicol receptor faces the cytoplasmic surface in both intact and lysed vesicles,and some factor on the inside of the intact vesicles controls the vesamicol receptor conformation on the outside.In intact synaptic vesicles ACh added exogenously does not compete with vesamicol binding.In contrast, [^{3}H]vesamicol binding to vesicles ghosts was inhibited by ACh with IC_{50} 5-50 mM,depending on the vesicles preparation.The transport of^{50}[^{14}C]ACh by vesicles ghosts was studied to determine whether the exogenous ACh was acting on the outside of the membrane to displace bound vesamicol.Cholinergic vesicle ghosts rapidly accumulated [^{14}C]ACh in the same amounts under active or passive transport conditions,and the inside concentration of [^{14}C]ACh was the same as that in the incubation buffer.Thus,the question of the sidedness for the ACh effect on vesamicol binding to vesicles ghosts remains to be answered.It also was found that the inhibitory effect of ACh on [^{3}H]vesamicol binding was much less potent (by at least 100 fold) at low vesicles concentration,suggesting that an unknown dissociable factor mediates the ACh effect on vesamicol binding.Taken together, these results suggest that an internal factor,possibly ACh,sends a regulated signal to the vesamicol receptor which reports about the internal status of the vesicles.

326.7 INHIBITION OF HIGH AFFINITY CHOLINE TRANSPORT WITH A-4 ATTENUATES THE EFFECT OF ETHYLCHOLINE MUSTARD AZIRIDINIUM (AF64A) ON BOTH CHOLINERGIC AND SEROTONERGIC PARAMETERS. P.E. Potter*, C.E. Tedford*, G.H. Kindel* and I. Hanin (SPON. A. Karczmar). Department of Pharmacology and Experimental Therapeutics, Loyola University Stritch School of Medicine, 2160 S. First Ave., Maywood, IL 60153, U.S.A.

Intraventricular (i.c.v.) administration of AF64A in rats leads to a marked and longlasting reduction in indices of hippocampal cholinergic function, such as acetylcholine (ACh) levels and release, high affinity choline transport (HAChT), and the activities of choline acetyltransferase and acetylcholinesterase[1,2]. We have also found decreases in 5-hydroxytryptamine (5-HT) levels after i.c.v. injection of 2 nmoles of AF64A into each lateral ventricle[1,3]. However, in contrast to the changes in cholinergic parameters, this reduction was transient, with a maximal effect at 4 days after treatment, followed by eventual recovery to control levels. This suggests that, rather than being due to a direct toxic action of AF64A on serotonergic neurons, the changes might result from increased 5-HT release as a consequence of removal of inhibitory cholinergic input. If so, decreasing the effect of AF64A on cholinergic neurons by blocking the HAChT site should also prevent the decrease in 5-HT, whereas if the changes were due to direct toxicity of AF64A, this treatment should not protect serotonergic neurons.

In the present study, A-4, a specific inhibitor of HAChT[4], was given i.p. (20 mg/kg) one hour before bilateral i.c.v. administration of 2 nmoles AF64A. The rats were killed 4 days after treatment by head focussed microwave irradiation and hippocampal content of ACh, choline, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were determined by HPLC with electrochemical detection. AF64A treatment reduced ACh content from 198.5 ± 11.7 to 112.3 ± 21.1 pmoles/mg protein ($p < 0.01$) and decreased 5-HT by from 2.96 ± 0.18 to 2.19 ± 0.24 pmoles/mg tissue ($p < 0.05$). The content of 5-HIAA was not changed at this time. Pretreatment with A-4 markedly attenuated the effect on ACh and completely prevented the decrease in 5-HT. These results strongly support the hypothesis that the changes observed in serotonergic parameters after AF64A treatment are related to the loss of cholinergic input to these neurons.

1. Potter et al., Neurochem. Int. 8, 199-206, 1986.
2. Leventer et al., Neuropharmacol., 26, 361-365, 1987.
3. Hörtnagl et al., Neuroscience, in press.
4. Tedford et al., J. Pharm. Exp. Ther. 240, 476-485, 1987.
Supported by NIMH Grant # MH 42572.

326.8 LACK OF GABAERGIC MODULATION OF ACETYLCHOLINE TURNOVER IN THE RAT THALAMUS. C. Cosi* and P.L. Wood (SPON: E. Snowhill). Neuroscience Res. Dept., Pharmaceuticals Div., CIBA - GEIGY Corp., Summit, N.J. 07901 U.S.A.

Electrophysiological, radioenzymatic and immunohistochemical studies (see Fibiger, Brain Res. Rev. 4:327, 1982) describe the existence of cholinergic projections from the midbrain and pons to the thalamus. However, the modulation of this projection by other neurotransmitter systems has not been defined. A GABAergic innervation of thalamic nuclei has been described (Fagg and Foster, Neuroscience 9:701, 1983). Since a GABAergic modulation of other central cholinergic systems, including the septohippocampal and substantia innominata-cortical pathways, is known (Wood, Can. J. Physiol. Pharmacol. 64: 325, 1986), the present study investigated the effects of GABAergic drugs on acetylcholine (ACH) turnover in the thalamus.

ACH turnover was measured in the thalamus, frontal cortex and parietal cortex after the intraperitoneal injection of THIP, a GABAergic agonist, and the benzodiazepine receptor agonist, diazepam. Animals were infused intravenously with deuterated phosphorylcholine for 9 min, 21 min after the administration of drug. The percentage of deuterium incorporated into regional brain choline and acetylcholine, was monitored by gas chromatography-mass spectrometry in positive chemical ionization mode (Wood and Peloquin, Neuropharmacology 21:349. 1981).

THIP (20 mg/kg) reduced ACh turnover in the frontal cortex and in the parietal cortex to 53 % and 48 % of control, respectively ($p < 0.05$) while not affecting ACh turnover in the thalamus of the same animals. Diazepam (5 mg/kg) decreased ACh turnover in the frontal cortex and in the parietal cortex to 65 % and 48 % of control, respectively ($p < 0.05$), but did not affect ACh turnover rate in the thalamus in the same animals.

This study confirms the previous finding of a GABAergic inhibition of the cortical cholinergic system (Wood, 1986) and indicates that the thalamic cholinergic projection does not appear to be modulated by GABAergic inputs.

326.9 EVIDENCE FOR CARRIER-MEDIATED SPONTANEOUS RELEASE OF ACETYLCHOLINE FROM RAT HIPPOCAMPAL TISSUE. Michael T. Ivy* and Paul T. Carroll. Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Spontaneous release of acetylcholine (ACh) from brain tissue differs from evoked release in that it appears to occur from the nerve terminal cytosol rather than from vesicles; also it is not calcium (Ca^{++})-dependent (Carroll, P.T. and Aspry, J.M., Neurosci., 6: 2555, 1981; Carroll, P.T., Neurochem. Res., 8: 1271, 1983). Recent evidence suggests that the spontaneous release of ACh from brain tissue may be carrier-mediated; 2-(4-phenylpiperidino) cyclohexanol (AH 5183), a drug which blocks vesicular ACh transport, reduces the spontaneous release of ACh from both mouse forebrain and rat striatum (Carroll, P.T., Brain Res., 358: 200, 1985; Ricny, J. and Collier, B., J. Neurochem., 47: 1627, 1986) at low concentrations. In the present study, the inhibitory effects of the L- and D-stereoisomers of AH 5183 on the spontaneous ACh release from rat brain hippocampal tissue were compared. The temperature and energy-dependence of spontaneous ACh release were also studied. The results indicated that both of the stereoisomers inhibited spontaneous ACh release in a concentration dependent fashion to produce parallel responses. The L-stereoisomer of AH 5183 had a half-maximal inhibitory concentration (IC_{50}) of 263 nM, whereas the D-isomer had an IC_{50} of 25.2 µM. The L-isomer of AH 5183 reduced the spontaneous release of ACh in both the presence and absence of extracellular Ca^{++}. Spontaneous ACh release was also temperature-dependent; a Q_{10} of approximately 3.2 was obtained. It did not, however, appear to be energy-dependent; 2, 4-dinitrophenol did not significantly reduce it. These results suggest that the spontaneous release of ACh from rat hippocampal tissue is carrier-mediated (supported in part by a Tarbox postdoctoral Fellowship to MTI and NINCDS 212189-03 to PTC).

326.10 IS THE VERATRIDINE INDUCED RELEASE OF ACh FROM RAT HIPPOCAMPAL TISSUE LINKED WITH AN ACTIVATION OF CHOLINE-O-ACETYLTRANSFERASE ACTIVITY? Paul T. Carroll and Michael T. Ivy* (SPON: J.B. Lombardini), Texas Tech University Health Sciences Center, Lubbock, Texas 79430.

It is well established that K^+ and veratridine depolarization of rat striatal tissue in vitro augment the calcium dependent activity of a water soluble fraction of tyrosine hydroxylase (El Mestikawy et al, Nature, 302: 830, 1983; El Mestikawy et al, J. Neurochem, 45: 173, 1985; Knorr et al, Biochem. Pharmacol. 35: 1929, 1986). To ascertain whether depolarization of rat hippocampal tissue might similarly activate choline-O-acetyltransferase (E.C. 2.3.1.6; ChAT) activity, the effect of varying concentrations of veratridine on both ACh release and on the activity of various ChAT fractions, isolated from rat hippocampal tissue according to the procedure of Benishin, C.G. and Carroll, P.T., J. Neurochem. 41: 1030, 1983, was determined. The results indicated that veratridine treatment stimulated the activity of water and detergent soluble ChAT fractions over the same concentration range as it stimulated ACh release. Conversely, it did not augment or reduce the activity of the major ChAT fraction, the pH 7.4 salt soluble fraction, at any of those concentrations which stimulated ACh release. Those treatments which decreased the amount of ACh released in response to veratridine depolarization such as tetrodotoxin, Ca^{2+} omission, and the ACh transport blocking drug 2-(4-phenylpiperidino) cyclohexanol (AH 5183) - 75 nM, also decreased the veratridine induced activation of the detergent soluble ChAT fraction. In contrast, L-AH 5183 (75 nM) did not block the veratridine induced activation of the water soluble ChAT fraction. When hippocampal minces were loaded with [^{14}C]choline and then depolarized with veratridine in the presence of hemicholinium, veratridine augmented the synthesis and release of [^{14}C]ACh. Both of these increases were abolished by L-AH 5183 (75 nM). When subcellular fractions containing occluded ACh were prepared by sucrose density gradient centrifugation, ChAT, but not lactate dehydrogenase (EC 1.1.1.27; LDH), could be solubilized from organelles in the 0.4 M sucrose fraction upon addition of detergents. Veratridine depolarization of hippocampal minces doubled the activity of the detergent soluble ChAT fraction in the 0.4 M sucrose layer and this increase was blocked by L-AH 5183 (75 nM). These results suggest that the evoked release of ACh from rat hippocampal nerve terminals may be linked with an activation of a detergent soluble ChAT fraction associated with synaptic vesicles (Supported in part by NINCDS 1 RO1 NS2-1289-03, 2 RO1 NS2-1289-04).

326.11 REVERSAL BY ANESTHETICS OF CARDIORESPIRATORY EFFECTS OF ACETYLCHOLINE ACCUMULATION. A.S. Foutz*, M.P. Morin-Surun*, J. Champagnat* and M. Denavit-Saubié. Laboratoire de Physiologie Nerveuse, C.N.R.S. 91190 Gif-sur-Yvette, France.

We have shown that the proportion of medullary respiratory neurons inhibited by iontophoretic application of ACh was much higher in pentobarbital anesthetized cats than in decerebrate cats (Morin-Surun et al., Naunyn Schmiedeb. Arch. Pharmacol. 1984, 325 : 205). We have now extended these results to the functional level by using different types of preparations : (1) Local intracerebroventricular administration of the anticholinesterase paraoxon (3 mg) in the 4th ventricle of chronic cats stimulated respiration (frequency and minute volume) in the conscious state but depressed respiration in the same animals anesthetized with pentobarbital. Decerebrated cats after recovery from halothane anesthesia responded to paraoxon by an increased ventilation. (2) In cats pretreated with methylatropine (0,2 mg/kg), paralyzed and artificially ventilated, paraoxon (3 mg/kg i.v.) induced an immediate and long lasting arrest of phrenic nerve respiratory discharges during anesthesia with pentobarbital, alpha-chloralose or halothane but did not suppress central respiratory activity in cats decerebrated or ventilated with nitrous oxide/oxygen. (3) Blood pressure was increased by paraoxon (i.v.) in cats which were either awake (chronic), decerebrated or ventilated with nitrous oxide/oxygen, but it was decreased in all anesthetized animals. It is concluded that anesthetics may reverse the action of ACh at both cellular and functional levels and thus may considerably alter the normal functioning of cardiorespiratory cholinergic mechanisms.

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326.12 MEMBRANE PEROXIDATION, ALUMINUM AND CHOLINERGIC FUNCTION. J. Judkins*, M.K. Raizada, and E.M. Meyer. Depts. of Pharmacology and Physiology, Univ. of Florida School of Medicine, Gainesville, FL.

Aging reduces the calcium-dependent release of brain acetylcholine (ACh) apparently by reducing the potency of calcium ions for triggering release. Since membrane lipid peroxides may accumulate with age, we studied the effects of artificially induced lipid peroxidation on a variety of cholinergic processes. Adding ferrous (0-200 uM) and ascorbate (0-10 uM) ions together (inducing the Fenton reaction) in creasing concentrations to cerebral cortical synaptosomes induced a biphasic degree of lipid peroxidation as measured by malonaldehyde production. A similar biphasic effect was observed on ACh release. Low levels of peroxidation induced a reduction in calcium-dependent release, while higher levels increased both ACh and choline efflux via a non-physiological process. Higher levels of peroxidation were also associated with a reduction in high affinity choline uptake and ACh synthesis, without affecting low affinity uptake.

Since the permeability to cations such as choline seemed to be increased with membrane peroxidation, we attempted to increase the accumulation of aluminum ions by primary rat brain neuronal cultures with the Fenton reaction. Combined, the Fenton reaction plus aluminum (at pH 5.5) blocked ACh synthesis after an overnight incubation. Individually, each treatment reduced synthesis by about 20%. A surprising result in this preliminary study was that protein levels increased by 140% after the overnight treatment compared to controls, suggesting a large increase in protein synthesis induced by the combined treatment. Together, these results suggest that impairment of membrane function with the Fenton reaction may be a model for studying age-related changes in transmitter release as well as aluminum-sensitivity.

326.13 EFFECTS OF HALOTHANE ON CENTRAL CHOLINERGIC SYNAPSES. C.W. Bazil and K.P. Minneman*, Department of Pharmacology, Emory Univ. School of Medicine, Atlanta, GA 30322

We reproduced anesthetic concentrations of halothane in rat brain tissue in vitro and examined aspects of cholinergic functioning. Halothane was introduced into Krebs Ringer bicarbonate buffer in 95% O_2/5% CO_2, and an equilibrating atmosphere maintained throughout. We found previously (Bazil et al, J. Neurochem. in press) that equilibration with approximately 1.0% halothane containing buffer in vitro results in brain tissue concentrations similar to those associated with complete anesthesia in vivo.

Previous reports showed that high concentrations of halothane lock muscarinic receptors into a high affinity state for agonist binding (Aronstam et al, Biochem. Pharm., 35:667, 1986). We prepared membranes from rat midbrain, and found no effect on the displacement of ^{3}H-N-methylscopolamine by carbachol (K_i or Hill coefficient) or on the shift in displacement curves induced by Gpp(NH)p in the presence of 1.25% halothane. Carbachol-induced increases in inositol phosphate accumulation were examined in slices of rat cerebral cortex. There was no difference in maximal stimulation in the presence of 1.25% halothane. These results suggest that anesthetic concentrations of halothane do not alter muscarinic receptor binding affinity or coupling to inositol phospholipid metabolism.

High concentrations of halothane have also been shown to depress ^{3}H-acetylcholine release from synaptosomes (Johnson and Hartzell, J. Neurochem. 44:1838, 1985). Basal and potassium-evoked release were examined using superfused slices of rat cerebral cortex preloaded with ^{3}H-choline. The slices were perfused with Krebs-Ringer bicarbonate buffer for 1 hr, and the perfusate discarded. The perfusate was then collected in 4 minute fractions. Control pulses of high K^+ buffer (52 mM) for 4 min were given to all slices. Various concentrations of halothane were then introduced into the perfusing buffer of some chambers and further pulses of high K^+ buffer given to all chambers. Depolarization-evoked release of acetylcholine was markedly reduced by equilibration with as little as 0.75% halothane, which gives tissue concentrations comparable to in vivo concentrations in anesthetized animals.

These results suggest that clinically relevant concentrations of volatile anesthetics do not indiscriminantly affect membrane-associated proteins such as receptors and transducer proteins, but may be more specific in their actions. However, acetylcholine release is reduced substantially at concentrations of halothane found in the anesthetized brain. The relevance of this phenomenon to the production of anesthesia remains to be determined.

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327.1 ^{3}H-CHOLINE MUSTARD AZIRIDINIUM ION UPTAKE AND BINDING IN RAT BRAIN SYNAPTOSOMES. R.Jane Rylett and E.H. Colhoun . Departments of Physiology and Pharmacology, Univ. Western Ontario, London, Canada.

Choline used as substrate for the synthesis of acetylcholine (ACh) must be transported into the nerve ending by a specialized transmembrane carrier localized almost exclusively in cholinergic presynaptic terminals. We determined that choline mustard (ChM Az) and ethylcholine mustard aziridinium ion (ECM Az) are irreversible ligands specific for the high-affinity choline carrier in rat brain synaptosomes (Rylett and Colhoun, 1980, 1984) and may be useful tools as inhibitors of choline transport in in vitro studies and as ligands for labelling and isolating the carrier protein (Rylett and Whittaker, 1987). ECM Az has also been used as a potential neurotoxin to produce deficits in central cholinergic neuron function (Hanin and Fisher, 1986). The interaction of ChM Az or ECM Az with the choline carrier in rat brain synaptosomes has not been characterized fully, nor have the mechanism(s) of action of these compounds as putative in vivo cholinotoxins been elucidated.

In the present study, the uptake and binding of ^{3}H-ChM Az was investigated in purified synaptosomes prepared from hippocampus and striatum of rat brain. ^{3}H-ChM Az bound to synaptosomal membranes was separated from that transported into synaptosomes by lysing the nerve endings and collecting the TCA-precipitated membranes onto filters. ^{3}H-ChM Az was transported into the nerve endings by a HC-sensitive mechanism, but at a rate much slower than ^{3}H-choline; uptake of ^{3}H-ChM Az was only 18.4% compared to uptake of ^{3}H-choline after 5 min and fell to only 10.6% of ^{3}H-choline uptake by 30 min. Kinetic analysis of ^{3}H-ChM Az (0.2-8 µM) transport at 5 min (linear uptake) revealed a K_m of 2.14 ± 0.98 µM and V_{max} of 2.54 ± 0.75 pmol/mg/min compared to a K_m of 1.44 ±0.15 µM and V_{max} of 13.0 ± 2.7 pmol/mg/min for ^{3}H-choline. Following 30 min incubation with 0.2 - 1 µM ^{3}H-ChM Az, approximately 50% of the ^{3}H-label associated with the nerve endings was membrane-bound (about half of which was bound specifically demonstrated by HC-3), 30% was taken up by a HC-sensitive carrier and 15-20% was taken into the synaptosomes by some other mechanism; the latter amount could represent accumulation of ^{3}H-labelled breakdown products of the mustard. In summary, ^{3}H-ChM Az could be accumulated into cholinergic nerve endings, but this uptake was only a fraction of ^{3}H-choline transport measured under the same conditions.

Rylett,R.J. and Colhoun,E.H. 1980. J.Neurochem.,34:713-719.
Rylett,R.J. and Colhoun,E:H. 1984. J.Neurochem.,43:787-794.
Rylett,R.J. and Whittaker,V.P. 1987. Int.Soc.Neurochem.,11th meeting
Hanin,I. and Fisher,A. 1986. Ann.Rev.Pharm.Tox.,26:161-181.
(Supported by the Medical Research Council of Canada)

327.2 SELECTIVE REDUCTION IN HIGH AFFINITY CHOLINE UPTAKE AFTER SYSTEMIC ADMINISTRATION OF A-4, A CENTRALLY-ACTING HEMICHOLINIUM-3 ANALOG. C.E. Tedford*, E. Wulfert*, J.G. Cannon*, J.P. Long* and I. Hanin. Dept. of Pharmacology, Loyola Univ., Stritch School of Medicine, Maywood, IL 60153 and UCB s.a. Pharmaceutical Sector, Brussels, Belgium.

A-4, a tertiary amine 4-methyl piperidine analog of hemicholinium-3, has been shown to produce a selective reduction in cholinergic function (Tedford et al., 1986 and 1987). Diminished cholinergic function was indicated by inhibition of neuromuscular transmission, reduction in ACh synthesis in rat striatal slices, and decreased ACh content in various rat brain regions after i.p. administration of A-4. It has been postulated that the effects of A-4 are due to inhibition of high affinity choline uptake (HAChT). In the present study the effect of systemic administration of A-4 (40 mg/kg), on several central cholinergic indices was investigated. These include HAChT, low affinity choline uptake (LAChT), choline acetyltransferase (ChAT) activity, acetylcholinesterase (AChE) activity and QNB binding.

Male Sprague-Dawley rats (200-300 grams) were given 40 mg/kg of A-4 or saline i.p. and killed 60 minutes later by decapitation. Sodium-dependent high affinity and sodium-independent low affinity choline uptake, as well as QNB binding were assessed in synaptosomes prepared from the hippocampus, cerebral cortex and striatum. ChAT and AChE activities were determined in brain homogenates from the hippocampus, cerebral cortex and striatum.

A selective reduction in HAChT was seen 60 minutes after administration of A-4. Hippocampal HAChT was reduced by 45% (from 6.7 ± 0.3 to 3.7 ± 0.3 pmoles/mg protein/8 min). Similar reductions were seen in the striatum and the cortex. Striatal HAChT was reduced by 54% (from 17.6 ± 2.6 to 8.1 ± 0.9 pmoles/mg protein/8 min) and cortical HAChT was reduced by 62% (from 4.0 ± 0.2 to 1.5 ± 0.1 pmoles/mg protein/8 min). A-4 did not affect LAChT in any of the brain regions examined, moreover, no differences were seen in ChAT activity, AChE activity, or total QNB binding in the control versus A-4 treated rats in any of the brain regions examined.

In summary, the results indicate a selective reduction in HAChT after systemic administration of A-4, which is consistent with the decrease in central ACh content seen 60 minutes after i.p. administration of 40 mg/kg of A-4 (Tedford et al., 1987). Therefore, it appears that reduced central cholinergic function can be produced after acute i.p. administration of A-4 and, furthermore, reduction in central cholinergic function is produced by selective impairment in HAChT.

Tedford et al.,1986, Eur. J. Pharmacol. 128, 231-239.
Tedford et al.,1987, J. Pharmacol. Exp. Therap. 240 (2), 476-485.

327.3 DEMONSTRATION OF A REVERSIBLE AND REPEATABLE IN VITRO ACTIVATION OF THE HIGH AFFINITY CHOLINE UPTAKE SYSTEM IN BRAIN. I. Vincent and J.R. Simon. Depts. of Biochemistry and Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46223.

The in vitro approach most frequently used to activate high-affinity choline uptake (HACU) involves a pre-exposure to elevated potassium ion (K^+) concentrations. This pretreatment results in a subsequent increase in HACU when measured under conditions of normal potassium. In previous studies we examined the effects of elevated K^+ on HACU using rat brain hippocampal and striatal synaptosomes, and observed an approximate two-fold increase in HACU in the hippocampus, while the uptake in the striatum exhibited an increase of 20%. It was also observed that these effects were dependent on the concentration of potassium in the preincubation buffer, and that maximal activation in both regions was achieved at the same concentration, i.e., 48 mM K^+. In the present study, the effect of preincubation time on the K^+-induced activation of HACU has been investigated. Our results indicate that the "activation" of HACU appears to be due to a time-dependent decrease in the control uptake, and that the presence of high concentrations of potassium in the preincubation medium prevent such a decrease. This was found to be the case for both hippocampal and striatal preparations. Based on these findings, a new protocol has been adopted, which involves "equilibrating" synaptosomes in normal Krebs buffer for 30 min at 30°C prior to any additional preincubations. Using this approach, we have re-examined the time course for K^+-activation and its dependence on the K^+ concentration using equilibrated preparations obtained from hippocampus and striatum. Maximal activation in both regions was observed following a 10 min exposure and occurred at a concentration of 48 mM K^+. In addition, we have investigated the reversibility and the repeatability of the activation of HACU. To reverse the stimulated uptake, activated synaptosomes were reincubated in normal Krebs for various times, thereby establishing a deactivation time course. These studies indicated that complete reversal of activation in both regions could be achieved by reincubation for 20 min. Thus, one complete cycle of activation and deactivation could be accomplished by a 10 min exposure to elevated K^+ followed by a 20 min reincubation in normal Krebs. When synaptosomal preparations were subjected to 3 such cycles, the activation in HACU was found to be repeatable as well as reproducible in magnitude. The plasticity of HACU demonstrated in the present study, utilizing in vitro depolarization, resembles closely the well documented alterations in uptake that have been observed following various in vivo treatments. Furthermore, the results validate the use of this in vitro paradigm in studying mechanisms involved in the modulation of HACU.

327.4 HIGH AFFINITY CHOLINE UPTAKE IN SYNAPTOSOMAL FRACTIONS FOLLOWING POTASSIUM-DEPOLARIZATION OF HIPPOCAMPAL SLICES. J.R. Simon and S.K. DiMicco,* Depts. of Psychiatry and Biochemistry, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46223.

Alterations in cholinergic neuronal activity in vivo have been shown to result in parallel changes in high affinity choline uptake (HACU) measured in vitro. Since the mechanisms underlying the modulation of the HACU system are not known, several attempts have been made to reproduce the in vivo-induced activation by utilizing in vitro models. Most of these models employ a pre-incubation of synaptosomes under various test conditions followed by uptake measured in normal buffer. In the present study, synaptosomal HACU has been measured following preincubation of hippocampal slices, thereby providing a system in which the time delay from treatment to uptake assay more closely approximates that which exists following in vivo treatments. Initial time course experiments indicated that a significant decrease in synaptosomal HACU occurred over the first 30 min of incubation of the slice. Similar time course experiments conducted in the presence of elevated concentrations of K^+ failed to show such a time-dependent decrease in uptake. Thus, it appears that elevated concentrations of K^+ prevent the decrease in HACU rather than actually stimulating transport. Based on these observations, we have taken the approach of allowing the hippocampal slices to equilibrate for 30 min at 30°C prior to any further perturbations. Using this protocol, HACU was found to be activated by elevated concentrations of K^+ in a time- and concentration-dependent manner. Maximal activation was achieved following a 30 min exposure to 48mM K^+. The activation elicited by 24mM K^+ and 36mM K^+ was not calcium-dependent, whereas that produced by 48mM K^+ and 62mM K^+ was attenuated but not abolished in the absence of external calcium. Similar studies involving the effects of magnesium indicated that the activation produced by all elevated concentrations of K^+ (24mM-62mM) was less in the presence of 12mM magnesium. Nonetheless, the uptake under these conditions still remained significantly activated relative to control. These results suggest that the inhibitions of K^+-stimulated HACU caused by calcium omission and by elevated magnesium are qualitatively different and that these alterations may affect different components of the cholinergic nerve terminal. The results suggest further that the activation in uptake produced by K^+ concentrations above 36mM may involve mechanisms consistent with transmitter release whereas the HACU stimulated by K^+ concentrations below 36mM appear to involve a separate mechanism.

327.5 CHOLINE CSF LEVELS ARE INCREASED IN ALZHEIMER PATIENTS. C. Higgins*, R. Elble and E. Giacobini (SPON: H.B. Clark). Depts. Medicine and Pharmacology, Southern Illinois University School of Medicine, P.O. Box 3926, Springfield, IL 62708 USA

Several authors (see for review Greenwald, B.S. et al., Biol. Psychiatry, 20:367-374, 1985; Sherman, K.S. and Friedman, E., Advances in Behavioral Biology, 30:291-301, 1986) have reported that plasma and red blood cell (RBC) choline levels do not differ between Alzheimer patients and age-matched controls. We measured lumbar cerebrospinal fluid (CSF) choline levels with a radiometric method in 20 controls (mean + SD = 1.91 + 0.60 nmole/ml), 19 mildly demented patients (2.58 + 0.83 nmole/ml) and 24 severely demented patients (2.81 + 1.02 nmole/ml). The CSF choline values of our Alzheimer patients were significantly different from controls ($p < 0.005$). Our patients have undergone serial lumbar punctures over time intervals as long as 30 months, but thus far, we have been able to demonstrate only a statistically insignificant trend for increasing choline values with time. Given the lack of change in plasma and RBC levels, the changes in CSF choline may reflect changes in brain phospholipid metabolism which could relate to the cholinergic deficit of Alzheimer disease. Furthermore, the mechanism of this pathologic rise in CSF choline may have important bearing on the clinical ineffectiveness of choline precursor therapy in Alzheimer disease. Of particular interest is the significant elevation of CSF choline in young adults with Down syndrome (Schapiro, M.B. et al., Neurobiology of Aging, In Press, 1987) and the lack of change in patients with Parkinson disease and Huntington disease (Welch, M.J. et al., J. Neurol. Neurosurg. Psych., 39:367-374, 1976; Aquilonius, S.M. et al., J. Neurol. Neurosurg. Psych., 35:720-725, 1972) and other CNS disorders (Flentge, F. et al., J. Neurol. Neurosurg. Psych., 47:207-209, 1984; Haber, B. and Grossman, R.G., Neurobiology of Cerebrospinal Fluid, 26:345-350, 1983). The rise in CSF choline may, therefore, be a useful marker of the cholinergic deficit in Alzheimer patients. (Supported by NIA grant #AG05416-01A1).

327.6 BRAIN CHOLINERGIC PARAMETERS DIFFERENTIATE INBRED STRAINS OF MICE J.I. Nurnberger, Jr., J.R. Simon, J.N. Hingtgen. Institute of Psychiatric Research and the Departments of Psychiatry and Biochemistry, Indiana University School of Medicine, Indianapolis, IN 46223.

Bailey (1971) introduced a method by which a number of genetically distinct but very similar strains may be studied and compared. His recombinant inbred strains are the result of continued brother-sister matings among the descendants of the F2 generation following a cross between two initial inbred strains. At least seven strains resulting from an initial cross between BALB/cBy and C57BL/6BJ have been described. The continued brother-sister matings result in animals that are essentially homozygous at all loci. The resultant strains may then be backcrossed with one of the parental strains for genetic analyses. Genetic markers with known chromosomal locations have been analyzed in the seven strains, so that each marker may be said to display a "strain-distribution pattern." A pharmacologic response or other biologic characteristic displaying the same "strain distribution pattern" as the marker may be presumed to be linked to that marker.

In the present study, we have investigated the high affinity choline uptake (HACU) and choline acetyltransferase activity (ChAT) in three brain regions of the C57BL/6J and DBA/6J inbred strains of mice. These are ancestor strains for a set of recombinant inbred strains produced by Jackson Laboratories. HACU was determined in crude synaptosomal preparations of the cerebral cortex, striatum and hippocampus using 0.5 uM choline as the substrate. ChAT activity was assayed from the same samples by a radiometric assay employing 0.1 mM acetylcoenzyme A and 10 mM choline. No significant difference between strains were found for either cholinergic parameter in cerebral cortical tissue. On the other hand, HACU was significantly greater in striatal (34%) and hippocampal (17%) preparations from C57BL/6J than from DBA/6J mice. The activity of ChAT was found to be somewhat greater (23%) in striatal synaptosomes obtained from the DBA/6J strain than that found in similar preparations from C57BL/6J mice. No statistically significant difference was found for ChAT activity in hippocampal preparations.

Behavioral studies are in progress to assess possible relationships between neurochemical parameters and ambulatory/rearing responses in an open-field maze. Promising differences will be followed into the recombinant lines.

This work is partially funded by Indiana Department of Mental Health grant #47-869-36.

327.7 ARACHIDONIC ACID INHIBITS CHOLINE UPTAKE AND DEPLETES ACETYLCHOLINE CONTENT IN CEREBRAL CORTICAL SYNAPTOSOMES. P. Boksa, S. Mykita* and B. Collier. Douglas Hosp. Res. Ctr., Depts. Psychiatry and Pharmacology, McGill Univ., Montreal, Canada, H4H 1R3

During cerebral ischemia, hypoxia and some other conditions, there is a rapid liberation of free fatty acids, particularly arachidonic acid, in brain. The cholinergic system is known to be particularly sensitive to impairment by even mild hypoxia. In the present study we have examined the effects of arachidonic acid on choline uptake and acetylcholine (ACh) content and release in rat cerebral cortical synaptosomes. Arachidonic acid (10-150 μM) produced a dose-dependent inhibition of high affinity [^{3}H]choline uptake. Low affinity [^{3}H]choline uptake was inhibited to the same degree. Free fatty acids inhibited high affinity [^{3}H]choline uptake with an order of potency: arachidonic>palmitoleic>oleic>lauric; stearic acid had no effect. Arachidonic acid inhibition of high affinity [^{3}H]choline uptake was reversed by the fatty acid binding protein, bovine serum albumin. To test the possibility that arachidonic acid may have produced a generalized disruption of synaptosomes, the integrity of the synaptosomes was monitored by measurement of a cytosolic marker, choline acetyltransferase. Arachidonic acid had no effect on the recovery of choline acetyltransferase with the synaptosomes. Medium choline was increased by arachidonic acid, however the resulting dilution of radiolabelled choline could not account for the observed inhibition of [^{3}H]choline uptake.

In synaptosomes incubated with [^{3}H]choline, arachidonic acid (150 μM) produced an 87% depletion of [^{3}H]ACh content. This decrease in [^{3}H]ACh content was equal in magnitude to the inhibition of high affinity [^{3}H]choline uptake produced by the same concentration of the fatty acid. A depolarization-induced increase in [^{3}H]ACh content was also completely inhibited by 150 μM arachidonic acid. Arachidonic acid (150 μM) depleted the endogenous ACh content of the synaptosomes by 71%. In the presence of eserine, 150 μM arachidonic acid had no effect on release of endogenous ACh; however depletion of ACh content by arachidonic acid was minimal in the presence of eserine, indicating that eserine may have interfered with the actions of arachidonic acid. The results suggest that arachidonic acid may deplete ACh content by inhibiting high affinity choline uptake and subsequent ACh synthesis. This raises the possibility that arachidonic acid may play a role in the impairment of cholinergic transmission seen in cerebral ischemia. Supported by the Medical Research Council of Canada.

327.8 DIETARY REPLACEMENT OF CHOLINE BY N-AMINODEANOL IN RATS, MEASURED BY HPLC. B. Knusel*, S.D. Lauretz*, R.A. Booth* and D.J. Jenden (SPON: F.J. Ehlert). Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.

N-Amino-N,N-Dimethylaminoethanol (N-Aminodeanol, NADe) is a precursor of a cholinergic false transmitter (Newton & Jenden, TIPS 7:316-320 (1986)). In the present study rats at age 29 days were put on a controlled choline free diet, to which either choline (Ch) or NADe was added at a concentration of 5 gm kg^{-1}. Free and lipid-bound choline and NADe were measured in blood at various times during the first 87 days and Ch, NADe, and their acetate esters were determined in tissues after 120 days of diet. The compounds were separated and quantitated by HPLC. In the blood of controls lipid-bound Ch was constant from days 0 to 87 with a mean of 2.1 mM in plasma and 3.0 mM in cells. Free Ch, in plasma and cells respectively, increased from 20.1 and 25.6 μM at weaning to 38.5 and 42.2 μM on day 10 and then decreased steadily to 22.3 and 19.8 μM on day 87. In NADe-treated animals free and bound Ch decreased to a plateau, which was reached after 10 to 38 days. The final levels in plasma and cells were 300 and 900 μM (bound) and 5 and 4 μM (free). The totals (NADe + Ch) of free and bound compounds in cells and free compounds in plasma followed the pattern of free and bound Ch in controls, being mostly equal to or higher than the respective levels of Ch in controls. The total of bound compounds in plasma after day 10 was only 33% of control. After 120 days the animals were sacrificed. Bound Ch in the NADe group was reduced in cortex, myenteric plexus and liver to 22.5 - 32.4% of control. Free Ch was reduced in liver to 73.3%, in cortex, hippocampus, striatum, heart, diaphragm and myenteric plexus to 17.7 - 30.5% of control. ACh in the same tissues was reduced to 34.9 - 49.0% of control. The total of bound compounds in NADe-treated animals was in all tissues equal, the total of free NADe and Ch was higher and the total of acetyl esters was lower than in controls. As these results show, NADe replaces Ch in all known metabolic pathways, but the degree of replacement, as expressed by the ratio NADe:Ch decreases from the precursor in blood to the transmitter in tissues. Lipid-bound NADe replaces lipid-bound Ch as an element of cell membranes in a 1:1 manner, whereas the kinetics of replacement of the free compounds and of lipid-bound Ch in plasma seem more complex.

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328.1 IN VIVO DOPAMINE RECEPTOR PROFILE OF NEUROLEPTICS

Peter H. Andersen, Department of Pharmacology, NOVO INDUSTRI A/S, Pharmaceuticals R&D, Novo Allé DK-2880 Bagsvaerd, Denmark.

Using binding in vivo to mouse brain tissue of the D2-selective benzamide ^{3}H-Raclopride and the D1-selective benzazepine ^{3}H-SCH 23390, the dopamine receptor profile of a variety of neuroleptics and dopaminergic agonists were investigated. Compounds exhibiting receptor selectivity in vitro retained this selectivity in vivo, thus the butyrophenones (e.g. spiroperidol and haloperidol), benzamides (e.g. sulpiride, clebopride and raclopride), (-)-NPA and LY 171555 were D2-selective whereas the benzazepines (e.g. SCH 23390, SKF 38393 and SKF 75670) were D1-selective.

Most compounds with a dual dopamine D1-D2 receptor profile in vitro retained this profile in vivo i.e. the thioxantenes (e.g. cis-flupentixol), the phenothiazines (e.g. chlorpromazine), the dibenzepines (e.g. clozapine, tilozepine and loxapine) and butaclamol. However, some compounds changed receptor profile in vivo as compared to in vitro. Thus, fluperlapine and fluphenazine, which in vitro were non-selective (D1:D2; 1:3 and 5:1, respectively) and pergolide which in vitro were D2-selective (D1:D2; 50:1) exhibited in vivo D1-selectivity, D2-selectivity and non-selectivity (D1:D2; 1:15, 54:1 and 1.9:1), respectively.

In conclusion, these data indicate that the dopamine D1 receptor is an important target for several different types of neuroleptics, and it is tempting to speculate that blockade of brain dopamine D1 receptors convey the antipsychotic efficacy of some of these compounds.

The generous gift of ^{3}H-Raclopride from Dr. H. Hall, Astra Alab, Sweden, is appreciated.

328.2 IN VIVO LABELING OF BRAIN D-1 AND D-2 DOPAMINE RECEPTORS. C.A. Leslie[1]* and J.P. Bennett, Jr.[1,2,3]. Departments of (1) Behavioral Medicine and Psychiatry, (2) Neurology, and (3) Pharmacology, University of Virginia Medical School, Charlottesville, VA 22908.

Mammalian brain dopamine (DA) receptors appear to exist in two major classes; D-1 DA receptors are positively linked to stimulation of adenylate cyclase activity, and D-2 DA receptors are not linked or negatively linked to adenylate cyclase. Radiolabeled spiperone and spiperone derivatives have been extensively utilized to examine D-2 DA receptors in vitro and in vivo; radiolabeled SCH 23390, a benzazepine neuroleptic, is currently the ligand of choice for examining D-1 DA receptors.

We have characterized in detail the kinetic, saturation, and pharmacological properties of in vivo binding to rat brain of ^{3}H-spiperone and ^{3}H-SCH 23390 and compared these in vitro properties to these occurring in vitro in striatal homogenates.

250-300 gram male rats received tail vein injections of 25-50 µCi ^{3}H-spiperone (s.a. 17-23 Ci/mmol) or 10 µCi ^{3}H-SCH 23390 (s.a. 85-90 Ci/mmol), with or without unlabeled D-1 or D-2 drugs, and were decapitated at varying times thereafter. Brains were removed, chilled on ice, dissected in regions, and homogenized in 50 volumes cold 0.05 M Tris buffer. 0.5 ml alqiuots of homogenate were rapidly vacuum filtered through GFB filters, washed 3X with Tris buffer, and assayed with liquid scintillation spectrometry.

Our data indicate that ^{3}H-SPIP and ^{3}H-SCH bind in vivo to striatal sites having pharmacological characteristics similar to D-2 and D-1 sites, respectively, labeled in vitro. The in vivo kinetic rate constants for association and dissociation for both ligands are reduced compared to in vitro. The estimated binding dissociation constants, measured in saturation experiments, are increased 120-200-fold (i.e., lower affinity) compared to in vitro.

Our data indicate that striatal D-1 and D-2 DA receptors can be labeled in vivo with ^{3}H-SCH and ^{3}H-SPIP, respectively, but that homogenization of tissue induces alterations in the kinetic properties of these ligands.

328.3 **^{3}H-SCH-23390 AND ^{125}I-SCH-23982 LABEL BOTH SEROTONIN AND DOPAMINE RECEPTORS IN THE CHOROID PLEXUS.** K. J. Nicklaus*, P. McGonigle, D. Lau* and P. B. Molinoff. Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104-6084.

^{3}H-SCH-23390 and, more recently, ^{125}I-SCH-23982, have been used to label the D-1 subtype of dopamine receptors. These ligands have been reported to show high selectivity for the D-1 receptor. Quantitative autoradiographic studies with ^{3}H-SCH-23390 have revealed high levels of saturable binding in many brain regions including the caudate-putamen, nucleus accumbens, substantia nigra, and choroid plexus. The binding of ^{3}H-SCH-23390 was further characterized in homogenates of dog choroid plexus. Inhibition of the binding of ^{3}H-SCH-23390 (5 nM) by serotonin resulted in markedly biphasic curves that were best fit by a two-site model. The high-affinity site had a nanomolar affinity for serotonin, consistent with the properties of the 5-HT$_{1c}$ site previously found to be present in high density in choroid plexus. The low-affinity site had a micromolar affinity for serotonin. Scatchard analysis of the binding of ^{3}H-SCH-23390 (0.3-9 nM) resulted in a curvilinear plot when 5 µM (+)-butaclamol was used to define nonspecific binding. Nonlinear regression analysis of untransformed saturation data suggested the existence of two distinct classes of binding sites. The high-affinity site (K_d = 0.5 nM) had the properties expected of the D-1 receptor. The low-affinity site (K_d = 9 nM) was present at an equal or greater density. Quantitative autoradiography of ^{3}H-SCH-23390 and ^{125}I-SCH-23982 binding was also performed. Serial sections of rat brain (20 µ) were labeled with either 1 nM ^{3}H-SCH-23390 or ^{125}I-SCH-23982. Approximately 50% of total binding of either ligand was displaced by 100 nM serotonin in the choroid plexus, while over 95% of total binding was displaced by 1 µM (+)-butaclamol. These findings demonstrate that dog and rat choroid plexus contain both dopamine and serotonin receptors, and both of these classes of receptors are labeled by ^{3}H-SCH-23390 and ^{125}I-SCH-23982. These ligands may label 5-HT$_{1c}$ receptors as well as D-1 receptors in various brain regions. (Supported by USPHS Grant GM 34781 and an NSF predoctoral fellowship to K.J.N.)

328.4 **DOPAMINE D_2 RECEPTORS: EFFECTS OF IONS ON [^{3}H]NEUROLEPTIC BINDING.** K.R. Jarvie, H.B. Niznik and P. Seeman. Dept. Pharmacology, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

Dopamine D_2 receptors are selectively labeled in a sodium-sensitive manner by the benzamide ligand [^{3}H]YM-09151-2. In the presence of 120 mM NaCl, [^{3}H]YM-09151-2 had a dissociation constant of 55 pM and a maximal binding capacity of 34 pmol/g of tissue (canine striatum). In the absence of NaCl, the dissociation constant was 440 pM with no change in the binding capacity. The binding of [^{3}H]YM-09151-2 (52 pM) was increased by 700% with 150 mM Na^+, 440% with 500 mM Li^+, and 290% with 500 mM K^+. The ion concentrations producing half-maximal increases in binding were 4 mM Na^+, 8.5 mM Li^+, and 115 mM K^+. The ionic strength control N-methyl-D-glucamine (NMG) (0.5-500 mM) did not increase [^{3}H]YM-09151-2 binding. [^{3}H]Spiperone binding was much less affected by monovalent cations. Analysis of saturable binding showed that these changes were due to changes in binding affinity, independent of changes in binding capacity.

The sulfhydryl alkylating agent N-ethylmaleimide inactivates [^{3}H]YM-09151-2 binding. The sodium ion at a concentration of 120 mM can protect approximately 40% of the susceptible sites while lithium and potassium ions (120 mM) do not.

The anion exchange blocker 4,4' diisothiocyano-2,2'-stilbene disulfonic acid (DIDS) has been shown to be capable of blocking the effect of Na^+ on the binding of agonists to alpha$_2$-adrenoceptors. Pretreatment with DIDS had no effect on the ability of Na^+ to alter [^{3}H]YM-09151-2 binding (120 mM NaCl: DIDS pretreated, K_D = 69 pM; Control, K_D = 53 pM).

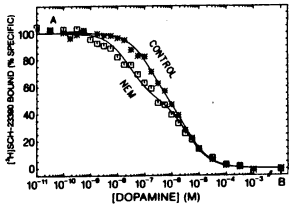
Consistent with their actions on other benzamide ligands, monovalent cations are capable of affecting the interactions of the potent benzamide [^{3}H]YM-09151-2 with the dopamine D_2 receptor. The insensitivity to NMG and extent of stimulation by low millimolar Na^+ concentrations indicate that this effect is not solely due to ion strength.

This work was supported by the Medical Research Council of Canada (MRC) and the Ontario Mental Health Foundation (OMHF). H.B.N. is a recipient of a MRC post-doctoral Fellowship.

328.5 **DOPAMINE D_1 RECEPTORS: EFFECTS OF THIOL MODIFYING REAGENTS**
Natalie H. BZOWEJ*, Hyman B. NIZNIK, Dimitri E. GRIGORIADIS and Philip SEEMAN. Dept. of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

In this study we detail the effects of N-ethylmaleimide (NEM), a sulfhydryl group alkylating agent and of dithiothreitol (DTT), a disulfide bond reducing agent, on [^{3}H]SCHERING 23390 binding to canine striatal D_1 receptors. Pretreatment of striatal membrane preparations with increasing concentrations of NEM (100 nM to 100 mM) and of DTT (100 nM to 100 mM) for 20 min at 37°C reduced [^{3}H]SCH 23390 binding by about 90% with IC_{50}'s of 40 µM and 3 mM, respectively. This apparent reduction in binding was shown to be a result of a decrease in the number of binding sites with only a 3-fold decrease in the affinity of the remaining sites, as determined by [^{3}H]SCH 23390 saturation curves. Pretreatment of membranes with increasing concentrations of various dopaminergic antagonists (100 pM to 10 µM) and agonists (1 pM to 10 µM) prior to the incubation with 200 µM NEM or 10 mM DTT afforded protection against the reduction in binding by these agents.

NEM has been found to mimic the effect of guanine nucleotides on agonist binding of the D_2 dopamine receptor, with a loss of the agonist high-affinity form of the receptor. In marked contrast, the high-affinity form of the D_1 receptor is not abolished by pretreatment with 200 µM NEM. Moreover, competition of dopamine for [^{3}H]SCH 23390 binding resulted in an apparent 5-fold leftward shift in the affinity of dopamine for the high-affinity state (figure below). Pretreatment with 10 mM DTT did not significantly alter either the affinity or the proportion of high- and low-affinity states of the D_1 dopamine receptor as detected by dopamine/[^{3}H]SCH 23390 competition. Furthermore, pretreatment of membranes with either NEM or DTT had no effect on guanine nucleotide regulation of the high-affinity state. Further effects of NEM and DTT on the regulation of the D_1 receptor will be discussed.



This work was supported by The Medical Research Council of Canada (MRC) and the Ontario Mental Health Foundation (OMHF). N.H.B. and D.E.G. are supported by OMHF Student- ships. H.B.N. is a recipient of an MRC post-doctoral Fellowship.

328.6 ALTERATIONS IN MUSCARINIC M-2 AND DOPAMINE D-2 RECEPTORS IN THE RAT BRAIN FOLLOWING INTRASTRIATAL INJECTION OF THE CHOLINOTOXIN, AF64A: AN AUTORADIOGRAPHIC STUDY.
V.L. Dawson, J.K. Wamsley, F.M. Filloux and T.M. Dawson. Dept. Psych., Pharm., Univ. Utah Sch. Med., SLC, UT 84132.

The aziridinium ion of ethylcholine (AF64A) has been shown to produce a selective decrement in Na^+-dependent high affinity choline transport sites (HAChT), choline acetyltransferase activity and acetylcholine levels without affecting other neurotransmitter systems. These results suggest that AF64A is a neurotoxin selective for the cholinergic nerve. Although, nonselective cell destruction can be observed at the site of injection, adjacent to this site, the toxin appears to be selective for cholinergic cells. By means of in vitro quantitative autoradiography, the effect of stereotaxic intrastriatal lesions with AF64A on muscarinic receptors, labeled with [^{3}H]-Quinuclidinyl benzilate (QNB), and [^{3}H]-Pirenzepine (PZ); Na^+-dependent HAChT, labeled with [^{3}H]-Hemicholinium-3 (HC-3); and dopamine D-1 and D-2 receptors, labeled with [^{3}H]-SCH 23390 and [^{3}H]-Sulpiride respectively, has been examined.

Unilateral stereotaxic lesions of rat caudate-putamen (CPu) with AF64A were performed. Control animals were stereotaxically injected with vehicle. Ten micron thick, slide-mounted, serial tissue sections of rat forebrain were incubated in radiolabeled ligand. Dopamine D-1 and D-2 receptors were labeled with 1.0 nM [^{3}H]-SCH 23390 and 20 nM [^{3}H]-Sulpiride respectively, under previously described conditions. Total muscarinic receptors were labeled with [^{3}H]-QNB and M-2 receptors were identified as the residual after displacement with pirenzepine. M-1 receptors were labeled with [^{3}H]-PZ. In order to determine the efficacy of the lesions, Na+-dependent HAChT were labeled with [^{3}H]-HC3. Three areas within the CPu were quantitated, a site near the lesion (zone #1), an area farthest away from the lesion (zone #3) and an intermediate site (zone #2).

The results showed a significant decrease of HAChT of 62% and 20% at zones #1 and #2 respectively, and no decrease of binding sites at zone #3. Total muscarinic receptors and the M-2 subtype were also decreased at zones #1 and #2. The M-1 subtype showed no change. There were no observable changes in the dopamine D-1 receptors, but a significant decrease in D-2 receptors at zone #1 was observed.

These results provide evidence that, under the conditions used, AF64A is a selective cholinergic neurotoxin. Furthermore, a population of D-2 receptors are postsynaptic and a population of M-2 receptors are presynaptic on cholinergic interneurons within the CPu.

328.7 PURIFICATION OF RAT STRIATAL DOPAMINE (DA) RECEPTORS BY FAST PROTEIN LIQUID CHROMATOGRAPHY (FPLC).
J.Y. Lew* and M. Goldstein. (SPON: C. Adler) Department of Psychiatry, Neurochemistry Research Labs, New York University Medical Center, New York, NY 10016.

We have investigated whether FPLC might be a useful procedure for purification of rat striatal DA receptors. The CHAPSO solubilized striatal DA receptors were prelabeled with 2 nM ^{3}H-spiroperidol (^{3}H-Spi) and the protein-bound radioactivity was separated from the unbound by passing through a Sephadex G-25 column. Samples were then applied on a Mono Q anion exchange column. The column was preequilibrated with 0.05 M Tris-HCl buffer, pH 7.0, containing 0.1% digitonin (Buffer A). The proteins were eluted from the Mono Q column with a linear gradient from 0 to 2.0 M NaCl in Buffer A. The ^{3}H-Spi protein-bound radioactivity was associated with two peaks and their DA receptor binding properties are now under investigation.

In another experiment we covalently labeled the striatal membrane-bound DA receptors with the photoaffinity ligand ^{3}H-7-azidofluphenazine (^{3}H-7-AF) (J.Y. Lew, E. Meller and M. Goldstein, Eur. J. Pharmacol. 113:145, 1985), and following solubilization with CHAPSO the labeled proteins were submitted to FPLC. Elution with the linear gradient from 0 to 2 M NaCl in Buffer A showed that the radioactivity is associated with two peaks. The two ^{3}H-7-AF labeled protein peaks separated by FPLC on the Mono Q column are now being further characterized on SDS-gel electrophoresis. Following SDS-gel electrophoresis of the glycoproteins isolated from the rat striatum, ^{3}H-7-AF covalently labels one major peptide (92 KDa) and a minor peptide (72 KDa). The incorporation of radioactivity into the 92 KDa peptide is reduced by approximately 70-80% in presence of 10^{-6}M butaclamol, suggesting that this peptide represents the D-2 DA receptor binding protein.
These studies were supported in part by NINCDS 06801 and NIMH 02717.

328.8 **AFFINITY CHROMATOGRAPHY FOR SELECTIVE PURIFICATION OF D_1 DOPAMINE RECEPTORS: SYNTHESIS AND USE OF (R,S)-7-CHLORO-8-HYDROXY-1[4-(3-AMINOPROPYL)PHENYL]-2,3,4,5-TETRAHYDRO-3-METHYL-1H-3-BENZAZEPINE.**
D. Wray*, S. Wyrick*, J. Petitto* and R.B. Mailman. (Spon: D. Janowsky). Depts. of Psychiatry and Pharmacology, Biol. Sci. Res. Ctr., School of Med., and Dept. of Med. Chem, School of Pharmacy, Univ. of North Carolina, Chapel Hill, NC 27514.

Basic biochemical and molecular biological studies of the dopamine receptor require isolation and purification of important subclasses. To perform such studies, we synthesized (R,S)-7-chloro-8-hydroxy-1[4-(3-aminopropyl)phenyl]-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine [*PropylSCH*], an analog of the drug SCH23390, for use as a possible affinity ligand for the D_1 dopamine receptor. N-methyl-3-chloro-4-methoxyphenethylamine and 4-(2-cyanoethyl)phenacyl bromide were synthesized as starting materials, and key intermediates included N-methyl-N-[4-(2-cyanoethyl)phenacyl]-N-(3-chloro-4-methoxyphenethyl)-amine; N-methyl-N-(3-chloro-4-methoxyphenethyl)-N-[2-(4-(3-aminopropyl)phenyl)-2-hydroxyethyl]amine; and (R,S)-7-chloro-8-methoxy-1-[4-(3-aminopropyl)phenyl]-2,3,4,5-tetrahydro-3-methyl-1H-3-benzazepine.

Competition studies in striatal membranes indicated that the racemate of propylSCH was about one-third the potency of SCH23390 in competing for [^{3}H]-SCH23390 binding sites, with IC_{50}s of 3.6 nM for propylSCH and 1.0 for SCH23390 (n_H equal to 1.00 ± 0.05 for both). A similar ratio of potencies for both compounds was found for inhibiting dopamine-sensitive adenylate cyclase. Neither compound had significant potency in competing for the D_2 dopamine receptor as labeled by [^{3}H]-spiperone. These data indicate that propylSCH, like SCH23390, is a potent and selective D_1 antagonist. The propylSCH was then coupled to cyanogen bromide-activated Sepharose. To verify that the affinity gel retained pharmacological activity, the ability of the Sepharose to compete for [^{3}H]-SCH23390 binding sites in striatal membranes was determined. PropylSCH-linked Sepharose, or soluble SCH23390, competed for [^{3}H]-SCH23390 binding sites, whereas glycine-linked Sepharose had minimal effect. These data indicate that propylSCH-linked Sepharose is capable of binding even crude striatal membranes having SCH23390 recognition sites. The affinity support was then used with crude striatal membranes solubilized with cholate. Initial studies with this soluble membrane preparation indicated that D_1 recognition sites were recoverable after removal of detergent with Biobeads. However, the ability to detect binding activity from the soluble preparation correlated with formation of insoluble membrane vesicles, suggesting that this cholate soluble form may not be active.

Supported by PHS Grant MH40537 and Center Grants HD03110 and MH33127.

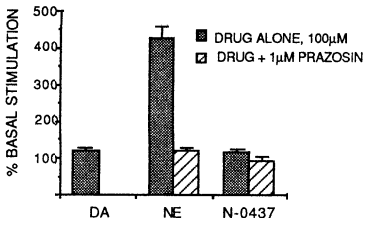
328.9 EFFECT OF THE CA^{2+} ANTAGONIST PN-200-100 ON GBL-ELICITED ENHANCED STRIATAL L-DOPA SYNTHESIS. Y. Namba, E. Meller and M. Goldstein. (SPON: R. Margolis). Department of Psychiatry, New York University Medical Center, New York, NY 10016.

To determine whether presynaptic dopamine (DA) receptors (autoreceptors) which regulate dopa biosynthesis are linked to Ca^{2+} channels, we have investigated the effects of a known Ca^{2+} antagonist, PN-200-100 (PN), on the GBL enhanced striatal L-dopa synthesis. Rats were divided into four treatment groups: (a) NSD 1015 (NSD) alone (100 mg/kg, i.p.); (b) PN (10 mg/kg, s.c.) 25 min before NSD; (c) GBL (750 mg/kg, i.p.) 5 min before NSD; and (d) PN 25 min and GBL 5 min before NSD. All animals were sacrificed 30 min after treatment with the dopa decarboxylase inhibitor (NSD). Treatment of rats with GBL resulted in an enhanced accumulation of L-dopa in the striatum. In animals treated with NSD, pretreatment with PN resulted in a decrease in striatal L-dopa accumulation of approximately 25%. In animals treated with both GBL and NSD, pretreatment with PN reduced the GBL-enhanced L-dopa accumulation by approximately 55-60%. These results show that the increased synthesis of L-dopa which occurs following GBL-induced cessation of dopaminergic impulse flow is inhibited by PN, analogous to the effect produced by DA agonists. This suggests that presynaptic striatal DA receptors, like the D-2 DA receptors of rat anterior pituitary (M. Memo et al., J. Neurochem. 47:1689, 1986) may be functionally linked, in part, to Ca^{2+} channels. However, other mechanisms directly involving Ca^{2+}-dependent protein kinases, cannot be excluded. These studies were supported in part by NIMH Grant 02717, NIH Grant 06801, and the Leon Lowenstein Foundation.

328.10 **DOPAMINE D2 RECEPTOR ACTIVATION FAILS TO STIMULATE PHOSPHATIDYLINOSITOL TURNOVER** J. Black, J.D. Belluzzi, and L. Stein, Dept. of Pharmacology, University of California, Irvine, CA 92717

The dopamine D2 receptor has been implicated in positive reinforcement, both at the behavioral (Gallistel and Davis, 1983) and cellular (Stein and Belluzzi, 1986) levels. To investigate the hypothesis (Stein and Belluzzi, 1986) that phosphatidylinositol (PI) metabolism may underlie DA-mediated positive reinforcement, we attempted to stimulate PI turnover by D2 receptor activation. Previous investigations of the effect of D2 receptor activation on PI metabolism have used D1/D2 agonists, such as DA itself, in conjunction with specific D2 antagonists. These investigations suggest that the D2 receptor does not activate, and even may inhibit, PI turnover; however, the concurrent activation of D1 receptors which interact with D2 receptors in many systems to might have confounded the PI measurements. If so, a specific D2 agonist might reveal an otherwise masked DA-activated increase in PI turnover. The availability of N-0437, a potent and specific D2 receptor agonist, made this test feasible.

The rat hippocampus was used in most experiments because cellular reinforcement has been demonstrated in this tissue, and because the ratio of D2 to D1 receptors in hippocampus is second highest in the brain. The method used was a slight modification of that of Berridge et al., 1982. Briefly, chopped tissue was incubated in oxygenated artificial cerebrospinal fluid, to which was added LiCl, ^{3}H-myo-inositol, and test drugs. Agonist-stimulated PI turnover would result in the appearance of radiolabelled inositol phosphates, which could be collected on an anion exchange column and counted on a liquid scintillation counter. As seen in the Figure, 100μM norepinephrine stimulated PI turnover, an effect antagonized by the α-adrenergic antagonist prazosin but not by the β-antagonist propranolol. This supports findings of other investigators by showing an α-adrenergic stimulation of PI turnover. Although we saw no effect of DA alone at doses up to 5mM, the specific D2 agonist N-0437 at a dose of 100μM resulted in a slight but significant increase (114% of basal, $p \leq 0.025$) in PI turnover. However, addition of 1μM prazosin abolished this increase, though prazosin alone had no effect.



These results indicate that the stimulatory action of N-0437 on PI turnover is mediated via α-adrenergic receptors. Similar results in rat striatum furthermore suggest that our negative findings in hippocampus are not due to low DA receptor density. (Supported by AFOSR grant 84-0325)

PAIN: CENTRAL PATHWAYS IV

329.1 PARCELLATION OF HUMAN THALAMIC NUCLEI BASED ON HISTOCHEMICAL STAINING AND SUBSTANCE P INNERVATION. T. Hirai*, S.H.C. Hendry and E.G. Jones. (SPON: H.D. Schwark). Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Seven human thalami from neurologically normal subjects and from patients suffering from Alzheimer's disease (ages 62 to 78 years), were fixed by immersion in 4%, phosphate-buffered paraformaldehyde and sectioned frozen in horizontal, sagittal or frontal planes. Four out of every five sections were cut at 60 um and one of each was stained for acetylcholinesterase, cytochrome oxidase, with thionin or with a myelin stain. The fifth was cut at 20 um and stained immunocytochemically for tachykinin immunoreactivity, using a rat monoclonal antibody made against Substance P (Sera Labs) and the avidin-biotin method.

Re-evaluation of human thalamic cytoarchitecture indicates a close correlation with the macaque monkey thalamus and nuclei have been renamed accordingly. The subdivisions of the ventral nuclei are particularly clear in man, in acetylcholinesterase (AChE) stained material. The VLa nucleus, corresponding to the nucleus innervated by the globus pallidus, is particularly large and strongly AChE positive. The VLp nucleus, corresponding to the cerebellar relay nucleus and including the so-called Vim nucleus, is AChE negative, while the lemniscal relay nuclei, VPL and VLPM, are strongly positive. VPL in Nissl preparations shows two clear divisions that probably correspond to relays for deep and cutaneous afferents.

The densest concentrations of fiber plexuses showing tachykinin-like immunoreactivity are in the central medial, paracentral, central lateral and limitans nuclei and in a small region postero-inferior to the centre médian nucleus, provisionally identified as the human nucleus submedius. Moderate densities appear in the anteroventral, mediodorsal and reticular nuclei and in parts of the posterior complex. Low densities appear in the pulvinar and ventral anterior nuclei. The major relay nuclei are virtually devoid of immunoreactive fibers.

The major densities of tachykinin immunoreactive fibers are with certain notable exceptions, such as the VLa and lateral geniculate nucleus, coincident with the major densities of acetylcholinesterase staining. These, in turn, tend to correlate negatively with cytochrome oxidase staining patterns.

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329.2 MORPHOLOGY OF GOLGI-IMPREGNATED NEURONS IN THE CAT AND RAT NUCLEUS SUBMEDIUS. V. Miletic and H.-J. Tan*, Dept. of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706.

The nucleus submedius (SM) of the medial thalamus might contain neurons that play an important role in nociceptive information processing. Since little is known about this nucleus, we have used the Golgi impregnation technique to examine the morphology of SM cells.

Cats (n=4) and rats (n=6) were deeply anesthetized, and perfused with buffered 1% paraformaldehyde and 1% glutaraldehyde. Thalami were cut into 2-3mm blocks and processed with the Golgi method (1% osmium tetroxide for 30 min, 5% potassium dichromate for 4-5 days at 4°C with agitation, and 0.5% silver nitrate for 16-24 hrs). Blocks were then cut into coronal or parasagittal sections (100μm), and the sections were dehydrated, mounted, and examined with a light microscope.

Preliminary analysis of the Golgi-impregnated material reveals no clear morphological grouping of the cat and rat SM neurons with respect to soma size (range 7-50μm), area of dendritic spread, or presence and number of dendritic spines. A noticeable difference among SM neurons is seen only with respect to the number of primary dendrites. Most of the impregnated neurons had three primary dendrites emanating from an oval cell body. Two of these dendrites exited the soma at opposite poles, while the third emanated from the midregion. On occasion, this third dendrite branched more extensively than the other two, especially in those neurons located close to the SM borders. In such cases the numerous daughter branches were directed at the center of the SM neuropil. The remainder of the impregnated neurons were either multipolar (4-6 primary dendrites emanating in different directions) or bipolar (two primary dendrites exiting the soma at opposite poles). The multipolar neurons appeared to be somewhat more numerous in the inner half of the nucleus, while the bipolar were frequently found along the SM borders.

The present analysis indicates that, apart from the number of primary dendrites, there are no clearly observable morphological differences among neurons in both the cat and rat SM. It remains to be established whether this morphological similarity suggests a uniformity of function for the SM neurons involved in medial thalamic nociceptive processes.

329.3 NOCICEPTIVE RESPONSES IN NUCLEUS SUBMEDIUS OF THE RAT. J. O. Dostrovsky, G. Guilbaud* and M. Gautron*. Dept. of Physiology, University of Toronto, Toronto, Ontario, and INSERM, u161, 2 rue d'Alesia, Paris 75014, France.

Recent anatomical studies by Craig and others have revealed the existence of a direct projection from the spinal cord to nucleus submedius in medial thalamus of the rat, cat and monkey. In the cat it has been shown anatomically and confirmed electrophysiologically that the projection neurons are located exclusively in lamina I of the spinal and medullary dorsal horns thus suggesting that Sm is involved in pain and/or temperature mechanisms. Most of the neurons in Sm project to ventrolateral orbital cortex. In the present study we have investigated the response characteristics of Sm neurons in the rat.

Experiments were performed on 17 rats anesthetized with 0.5% fluothane in a mixture of 1/3 O_2 2/3 N_2O. Adequate and stable anesthesia and a good brain condition were maintained as assessed on the basis of the EEG. Single unit recordings were obtained from neurons in medial thalamus using glass microelectrodes filled with pontamine dye. Every neuron was tested to determine whether its activity could be altered by innocuous and noxious mechanical and sometimes thermal stimuli applied to various body regions. All recording sites were reconstructed on the basis of dye marks.

Of the 66 neurons recorded in Sm, 29 were excited and 3 inhibited by nociceptive stimulation, whereas the remainder did not respond to any cutaneous stimuli including cold and warm stimuli. The responses could be elicited in most cases by stimuli applied to the tail and bilaterally to the posterior paws. In many cases the neurons responded also to stimulation of the forepaws and face. In almost all cases the responses persisted for the entire duration of the standard 15 second stimulus used and frequently persisted for periods of 5 to 60 seconds after termination of the stimulus. For 8 of the neurons responding to mechanical stimulation, noxious thermal stimulation (46 - 50°C) of the hindlimb or tail was tested and in all but one case excitation was observed. No topographic organization was noted and responsive neurons were found in all parts of the nucleus at all anterior-posterior levels. These results are similar to those we have recently reported for Sm of arthritic rats except that in the former group responses could be obtained also by low intensity mechanical stimuli applied to the inflamed joints and by joint movements.

These findings indicate that Sm may be involved in the processing and relay of pain related sensory information to frontal cortex.

329.4 INTRATHECAL ADMINISTRATION OF PHENTOLAMINE, METHYSERGIDE OR NALOXONE: EFFECTS ON HYPOTENSION, BRADYCARDIA, AND ANTINOCICEPTION INDUCED BY INTRAVENOUS ADMINISTRATION OF D-ALA^2-METHIONINE ENKEPHALINAMIDE IN THE RAT. S.A. Aicher and A. Randich. Department of Psychology, University of Iowa, Iowa City, IA 52242

Two experiments examined the effects of intrathecally administered receptor antagonists on the hypotension, bradycardia, and inhibition of the tail-flick reflex evoked by noxious radiant heat induced by intravenously (IV) administered D-Ala^2-Methionine enkephalinamide (DALA). The receptor antagonists studied were phentolamine, naloxone and methysergide. Awake, restrained rats showed no systematic effects for any of the receptor antagonists on the hypotensive, bradycardic or antinociceptive effects of DALA. However, there was a significant effect of the order of IV administration of the saline vehicle or DALA on these response measures. Specifically, a 5-minute delay between the intrathecal administration of any of these antagonists or saline vehicle and the IV DALA injection, resulted in an attenuation of the antinociception produced by DALA. The arterial blood pressure responses to DALA also were attenuated or reversed by all of the intrathecal injectates, including the saline vehicle with a 5-minute delay. However, at a 20-minute delay between the intrathecal injections and IV administration of DALA, the tail-flick and cardiovascular responses were both similar to those seen in animals without intrathecal catheters and unaffected by the receptor antagonists. Using a single dose (30 µg) of each receptor antagonist and the lightly-anesthetized rat preparation, intrathecal administration of phentolamine produced a significant attenuation of the antinociception induced by IV administration of DALA compared to controls. There were no systematic effects of any of the antagonists on either the arterial blood pressure or the heart rate response to IV administration of DALA in lightly-anesthetized rats.

329.5 **CROSS INTERACTION BETWEEN THE LATERAL RETICULAR NUCLEUS AND THE PERIAQUEDUCTAL GRAY. M.E. Clement and M.M. Behbehani (SPON: A. Mathieu). Department of Physiology and Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 46267-0576.**

Recent experiments have shown that electrical and chemical stimulation of the lateral reticular nucleus(LRN) produces analgesia. There is evidence that lesion of the LRN alters pain perception. Anatomical studies have shown that there is a cross innervation between PAG and LRN.

Adult male rats were used in these experiments. In one series of experiments animals were anesthetized with sodium pentobarbital and after surgery, were maintained under methohexital anesthesia by continuous infusion at a rate of 15mg/Kg/Hr. Under these conditions, heating of the tail produced a reliable tail flick (TF). The second group of animals were maintained under chloral hydrate anesthesia throughout the experiment. Single unit recordings were made from the LRN. The response of the LRN neurons to PAG stimulation, to peripheral stimulation and to norepinephrine applied iontophoretically was measured. In methohexital anesthetized animals, electrical stimulation of LRN produced an increase in TF latency that lasted as long as the stimulus was applied. Injection of 100 to 200 nl of glutamic acid into the LRN produced an increase in the tail flick latency that outlasted the injection time by several minutes.

The activity of 112 cells in 24 animals was recorded. Of these, 30.8% showed a five to ten-fold increase in firing rate and in 48.9% the firing rate was decreased. The majority of these latter cells were completely inhibited for as long as the noxious stimulation was applied. The latency to onset of response of PAG stimulation was variable with a range of 3 to 10 milliseconds and a mean latency of 4.8 msec with a standard deviation of 1.2 msec, with antidromically activated cells showing a latency of 3.9 ms. The response of each cell to 10 seconds 100 Hz electrical stimulation of PAG was recorded. An excitatory response was observed in 47.8% cells. Fourteen cells were excited only during PAG stimulation. Twenty-nine cells responded with an extended excitation with a mean duration of 35.4 seconds. 40.2% of the cells were inhibited by electrical stimulation. Thirty-three cells responded only during PAG stimulation, the majority of these cells were completely inhibited. Only four cells showed an extended inhibition with a mean duration of 16.50 seconds. The response of 20 LRN neurons to iontophoretically applied norepinephrine was measured and compared to their response to peripheral and to PAG stimulation. The most consistent effect of NE was inhibition of the baseline firing rate. There was no significant correlation between the response to PAG stimulation and to NE. The effect of NE could be blocked by phentolamine while yohimbine had a predominantly excitatory effect. It is concluded that 1) the majority of LRN neurons are inhibited by noxious stimulation. 2) There is a fast conducting pathway that connects the LRN region with the PAG that is likely to be monosynaptic. 3) It is possible that analgesia produced by LRN stimulation is mediated through the activation of PAG-rostral ventral medulla- spinal cord pathway.

329.6 CORRELATION OF OPIOID RECEPTOR BINDING WITH ENK AND 5HT IMMUNOCYTOCHEMISTRY IN THE MEDULLARY RAPHE AND RETICULAR NUCLEI. Robert M. Bowker and Roger P. Dilts, Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520

The nucleus raphe magnus and nucleus gigantocellularis are known to be important participants in the descending control of nociception, while the nucleus raphe pallidus and the nucleus raphe obscurus have roles in autonomic and motor control systems. Opiate mechanisms mediate, in part, many of the spinal cord effects produced by these nuclei. The anatomical localization of mu and delta opioid receptors within these cell groups was assessed by quantitative autoradiography, and the resulting distribution correlated with enkephalin-like and serotonin-like immunoreactivity.

Adjacent brainstem sections from guinea pigs and rats were processed for receptor autoradiography, using ^{125}I-Tyr-D-Ala-Gly-N-MePhe-Gly-ol-enkephalin (DAGO) and ^{125}I-D-Ala-D-Leu-enkephalin (DADL) in the presence of 10 nM DAGO to localize mu and delta receptors, respectively (Mankowitz and Goodman, 1982). Other sections were processed for ENK and 5HT immunocytochemistry, using antisera raised to either leucine- or methionine-ENK and to 5HT. The immunocytochemically stained sections were examined and correlated with opioid receptor autoradiography.

Dense DAGO and DADL binding was observed in the NTS, DMV and spinal trigeminal nucleus, consistent with most studies. In addition, moderately intense staining was located within the NRM and the NGC. The specific binding pattern formed a horizontally directed band in the NGC with less binding caudally in the NRO and NRP. The distribution of leucine- and methionine-ENK immunoreactivity was closely correlated with the localization pattern of opioid receptors. In double labeling studies, ENK terminals appeared to make contact with both 5HT and non-5HT neurons in the NRM and NGC, with fewer in the NRO and NRP. These observations provide anatomical evidence for the localization of opioid receptor binding in the medullary raphe and reticular nuclei and a close correlation between the opioid receptors and the ENK- and 5HT-immunoreactive elements. Thus the raphe-spinal and reticulospinal pathways from these nuclei may be modulated, in part, by opioid receptor mechanisms. (Supported by NS22321 and NS24388.)

329.7 MORPHOLOGICAL CHARACTERISTICS OF PHYSIOLOGICALLY IDENTIFIED CELLS IN THE ROSTRAL VENTRAL MEDULLA OF THE RAT. A.M. Williamson, P.T. Ohara and H.L. Fields. Departments of Anatomy and Neurology, University of California, San Francisco, CA 94143.

Within RVM (nucleus raphe magnus and immediately adjacent areas of the medulla) there are neurons whose firing rate increases (on-) decreases (off-) or remains unchanged (neutral) just prior to withdrawal from a noxious stimulus. Since systemic opiate administration elicits specific alterations in the firing of both on- and off-cells, and since electrical stimulation of RVM produces behavioural analgesia, RVM has been postulated as a significant link in a descending system specifically designed to inhibit pain transmission. This study was conducted to determine whether or not electrophysiologically identified cells in RVM exhibit specific morphological differences that might help in understanding the mechanism through which behavioural analgesia is mediated.

The experiments were performed on lightly barbiturate-anesthetized rats. Areas containing on- and off-cells were identified by extracellular recording with metal electrodes, and then the area was searched with high impedance glass electrodes filled with the fluorescent marker, ethidium bromide (1% in 2.0 M K-acetate). Cells were characterized as on-, off- or neutral by the change in their firing pattern immediately preceeding withdrawal from either noxious heat applied to the tail or pinch applied to the paw or ear. At the conclusion of the recording session, the animals were perfused with fixative, and 20 μm thin sections were cut on a freezing vibratome. The sections were viewed with a fluorescent microscope. The filled cells were then identified, photographed and drawn. All cells were found in RVM within 1.0 mM of the midline, and no more than 1.5 mM from the pyramids in the dorsoventral plane. All filled cells were within RVM and were centered on the rostrocaudal plane of the nucleus of the seventh cranial nerve. Off-cell somata have an area of 758 ± 113 μM^2 (mean ± SEM,n=5). On-cell somata are smaller and have an area of 447 ± 145 μM^2 (n=4).

No consistent morphological differences between on- and off-cells were found. Most cells of both classes have triangular cell bodies which give rise to at least three principal dendrites which bifurcate into smaller secondary dendrites within 20-50 μM of the cell body. The orientation of most dendrites is roughly mediolateral, but dorsoventral orientations were seen in two cells.

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329.8 PHYSIOLOGY AND MORPHOLOGY OF PONTOMEDULLARY RAPHE AND RETICULAR NEURONS IN CAT BRAINSTEM: AN INTRACELLULAR HRP STUDY J. F. Whitney, C. E. Poletti and W. E. Foote Depts. of Psychiat. and Neurosurg., Mass. Gen. Hosp. and Harvard Med. School, Boston, Mass. 02114

We have been examining the connections and morphology of pontomedullary raphe and reticular neurons in an attempt to better understand the role these nuclei play in antinociception. One interest involves correlating the morphology and physiology of brainstem neurons located in n. raphe magnus (NRM), n. gigantocellularis (NGC), n. paragigantocellularis (NPGD), and n. magnocellularis (NMC).

Preliminary results indicate that cells with spinal efferents as determined by antidromic invasion from stimulation of the dorsolateral fasiculis exhibited a consistent pattern of dendritic branching. The ratio of dendritic terminal branches to dendritic trunks is less variable for these cells than for neurons not antidromically activated from the spinal cord (Bartlett's test for homogeneity of variance, $X^2(1) = 7.739$, $p < 0.01$). Cells with spinal efferents varied in size (from < 850 μm^2 to < 3,500 μm^2) and location (NGC, NPGD, and NMC). These cells all received rostral input from one or more of the following: periaqueductal grey (PAG), amygdala, and hypothalamus and none were influenced by tooth pulp stimulation.

Other cells were influenced by tooth pulp stimulation with latencies suggesting polysynaptic input. Cells with shorter latencies received excitatory input while the longest latency cell received inhibitory input. These tooth pulp responders exhibited nearly spherical dendritic trees which were found to differ significantly from the other cells (n=14) taken as a group which had more oval shaped dendritic fields that were flattened in the mediolateral direction ($t_{(12)} = 2.327$, $p < 0.05$). All tooth responders were located in the NGC which, as a group (n=7), also had significantly more spherical dendritic trees than the other cells ($t_{(12)} = 6.87$, $p < 0.001$). Tooth responders all received excitatory spinal input and all responded to diverse rostral inputs.

As opposed to NGC cells, cells with oval dendritic fields were found in the NPGD which differed significantly on this measure from the other cells ($t_{(12)} = 2.707$, $p < 0.02$). Unlike NGC, NMC, & NRM cells, NPGD cells did not receive PAG input.

329.9 THE ROLE OF SPINAL CORD MONOAMINES IN THE PRODUCTION OF OPIOID AND NONOPIOID STRESS-INDUCED ANALGESIA. L. A. Yuva* and S. F. Maier. Dept. of Psychology, University of Colorado, Boulder, CO 80309.

Numerous studies have demonstrated that environmental events can activate endogenous pain inhibitory systems within the central nervous system. The antinociceptive response which follows exposure to such environmental events (e.g., electric shocks, cold water, rotation, etc), called "stress-induced analgesia" (SIA), is not produced by a single mechanism. Rather, it is produced by multiple mechanisms whose pattern of activation depends on the nature of the environmental event. These mechanisms are still only poorly understood.

Spinal cord monoamines play a key role in the analgesia produced by opiates (Yaksh, T. L., Pharm. Biochem. Behav, 22:845, 1985), but little is known concerning their importance in SIA. The data which do exist are largely contradictory, possibly because different studies have used SIA paradigms which activate different pain inhibitory systems. The present studies explored the role of spinal cord monoamines using a SIA procedure which produces sequential nonopioid and opioid analgesias in the same subject. Thus we were able to assess the importance of the monoamines with regard to two major classes of SIA simultaneously.

Rats were implanted with intrathecal cannulae in the lumbosacral enlargement. Some rats then received intrathecal (i.t.) administration of 5,7 dihydroxytryptamine (5,7-DHT) to deplete 5-HT and NE from the lumbar spinal cord, others received intraperitoneal (i.p.) desipramine in combination with i.t. 5,7,-DHT to selectively deplete 5-HT, while the remaining rats received i.t. saline as a control. 7 days later all rats were restrained in Plexiglas tubes and received a series of 60 inescapable 5 sec 1.0 mA shocks delivered to the tail via fixed electrodes. Tail-flick to radiant heat was measured after 1, 5, 10, 20, 40 and 60 shocks. Testing was conducted without disturbing the subjects or removing them from the tubes. Half of the animals that had initially received i.t. saline received naltrexone (14 mg/kg) before the shock session, while the remaining controls received i.p. saline.

Naltrexone reduced the analgesia seen after 1 shock, but had no effect on the analgesia occurring after 5, 10, and 20 shocks. It again reduced the analgesia after 40 and 60 shocks, suggesting a pattern of a very brief opioid analgesia, followed by a nonopioid and then another opioid analgesia. 5-HT depletion reduced the analgesia observed after 1 shock, but had no effect on the analgesia after 5, 10, and 20 shocks. It again reduced analgesia after 40 and 60 shocks. In contrast 5-HT+ NE depletion reduced the analgesia seen after all shocks. Thus, 5-HT depletion reduced analgesia at all points at which naltrexone had been effective, but had no effect where naltrexone was without impact. The addition of NE depletion, on the other hand, reduced analgesia at the points at which naltrexone had been ineffective. This pattern suggests that spinal cord 5-HT plays a role in opioid forms of SIA and that NE plays a role in nonopioid SIA.

330.1 PRODUCTION OF AMYLOID-LIKE MATERIAL BY CULTURED MICROGLIA. C. A. Colton, T. Behar*, J. S. Colton*, M. Simon*, and D. L. Gilbert. (SPON: S. L. Pocotte). Dept. of Physiol. Biophys., Georgetown Univ. Med. Sch., Washington, D.C. 20007 & Lab. of Molecular Genetics and Lab. of Biophysics, NINCDS, NIH, Bethesda, MD 20892.

The role of microglia in inflammatory and non-inflammatory pathological processes in the CNS is not clear. Evidence has suggested that microglia share morphological and functional properties with other tissue macrophages. Since Kupffer cells, the resident macrophages in the liver, have been shown to produce amyloid in response to casein and also to endotoxin injection and since microglia are frequently seen at the site of amyloid plaques in the CNS, the ability of microglia to produce amyloid-like material was tested directly using cultured microglia. Primary glial cell cultures were prepared from 2 day old rat cerebral hemispheres. Cultures were grown for 10 to 14 days at 37 C in a 10% CO_2, 90% air environment using Dulbecco's medium (DMEM) containing 10% fetal calf serum. After selective replating, a microglia enriched culture was obtained. Cells were plated on poly d lysine coated cover slips in 24 well culture dishes and allowed to adhere overnight. The cells were then exposed to casein or endotoxin by addition of the agent to the DMEM. After a variable time of exposure, the cells were fixed with formalin and stained with either Congo Red or thioflavin S. After 48 hours of exposure to casein and endotoxin, green yellow birefringence under polarized light was seen within many microglial cells for the Congo Red stained cells and a yellow florescence for the thioflavin S stained cells. Stain was seen only intracellularly and appeared to be in vesicles. No stain was seen in either control cells from the same time period or in cells exposed for 48 hours to opsonized zymosan or arachidonic acid.

330.2 TRUE MICROGLIA PROLIFERATION INDUCED BY BLOCK OF AXOPLASMIC FLOW AND INHIBITED BY ADRIAMYCIN TRANSPORT: A CELL UNRELATED TO THE MACROPHAGE. R.L. Schelper, R. Miyazaki, Univ. Iowa, Iowa City, IA 52242

It has been well documented that true reactive microglia (TRM) are unrelated to monocyte derived brain macrophages (MΦ). Unlike TRM, brain MΦ have monocyte esterases, react with anti-macrophage antibody and specific lectins, are labeled by non-reusable DNA precursors, and have Fc receptors. Brain MΦ appear only after direct traumatic injury. TRM appear after injury to a neuronal cell process and are seen in a nerve nucleus after axotomy or around neurons adjacent to areas of direct trauma. The neuron may perceive that it has been injured when it no longer receives a retrogradely transported trophic factor from the innervated structure. To test this hypothesis we blocked axonal transport with colchicine and monitored TRM proliferation by thymidine uptake. Hypoglossal or sciatic nerves of mice were exposed to saline or to colchicine (60 μM) for 30 minutes. They were subsequently given three intraperitoneal injections of ^{3}H-thymidine (2 μCi/gm body wt) per day and were killed 2 days after colchicine treatment. Carnoy's fixed, paraffin embedded sections were cut and coated with Kodak NTB2 emulsion. Saline treated animals showed no evidence of cellular proliferation in the spinal cord or hypoglossal nucleus. Colchicine treated animals showed a TRM response identical to that seen in animals after hypoglossal or sciatic axotomy. Thus, colchicine disruption of axonal transport appears to play a role in the TRM response, supporting the hypothesis that loss of a peripherally produced trophic factor is the signal to the neuron that it has been injured.

Because TRM are so intimately associated with neurons injured by axotomy or colchicine, we reasoned that the neurons must release a factor that stimulates the TRM response. In an attempt to block local TRM proliferation, we injected the tongue with 80-160 μgm Adriamycin (ADM). ADM, which is autofluorescent and carried by retrograde transport, blocks both DNA-RNA transcription and DNA replication by becoming intercalated between the DNA strands. ADM leaks out of neurons and is taken up by glia. In our study ADM was detected by fluorescence as early as 4 hours after injection in cell nuclei of neurons and glia of the hypoglossal nucleus, but not elsewhere. One day after ADM injection the hypoglossal nerve was cut and mice were given ^{3}H-thymidine for 2 days before being killed. No labeled cells were found after axotomy in ADM treated mice. Controls injected with saline showed the usual TRM response. Thus, inhibition of local DNA transcription and replication by ADM blocks the TRM response. This is probably a direct effect of ADM on the TRM but might also be explained by an inhibition of neuronal synthesis of a TRM mitogenic/chemotactic factor.

330.3 SECRETION OF SUPEROXIDE BY CNS MICROGLIA. D. L. Gilbert and C. A. Colton. (SPON: B. G. Szaro). Lab. of Biophysics, NINCDS, NIH, Bethesda, MD 20892 and Dept. of Physiol. and Biophys., Georgetown Univ. Med. Sch., Washington, DC 20007.

Microglia have been implicated in both physiological and patholgical processes in the brain. Since microglai share many of the same surface antigens as blood macrophages, they have been considered to be resident CNS macrophages. Thus, it is possible that microglia can kill invading organisms by secreting oxygen radicals. To test this possibility, superoxide secretion of microglia in tissue culture was studied. Primary glial cell cultures were prepared from 2 day old rat cerebral cortices and grown in Dulbecco's medium (DMEM) with 10% fetal calf serum at 37 C in a gas mixture containing 10% CO_2 and 90% air. Selective replating of the cells after 10 to 14 days of growth produced two cell fractions; one enriched in microglia and the remaining adherent fraction, rich in astrocytes. Both fractions were assayed for superoxide production using a microcytochrome C reduction assay. Specificity for superoxide was provided by comparing the amount of cytochrome C reduction in the presence and absence of superoxide dismutase (SOD), the enzyme which degrades the superoxide radical ion.

Superoxide production was shown to increase over 2 hours at a rate of 3.7 nmoles/mg protein per minute in the microglia enriched fraction when stimulated with opsonized zymosan. In contrast, no superoxide production was seen in the astrocyte fraction when stimulated with opsonized zymosan. Also, no superoxide production was seen in either fraction in non-stimulated conditions. This production of superoxide was dependent on the dose of the opsonized zymosan and on the cell density. In addition the microglia were responsive to stimulation by phorbol myristate acetate in a dose dependent fashion. This ability to secrete free radicals may implicate the microglia in pathological states.

330.4 CALCIUM CHANNEL AND SUPEROXIDE SECRETION IN MICROGLIA. M. Jia*, C. A. Colton, and D. L. Gilbert. Lab. of Biophysics, NINCDS, NIH, Bethesda, MD 20892 and Dept. of Physiol. Biophys., Georgetown Univ. Med. Sch., Washington, D.C. 20007.

CNS microglia have been shown to be similar functionally and anatomically to blood and other tissue macrophages. These cells do secrete the superoxide radical ion. Since calcium has been implicated in the superoxide secretory mechanism of macrophages, we decided to determine if calcium has any influence on superoxide secretion by CNS microglia. Cultured microglia cells were examined for 1) calcium dependent channels and 2) the effect of calcium in the external medium on superoxide secretion.

A primary culture of microglia was obtained from 2 day old rat cerebral hemispheres. The cells were grown at 37 C in a 10% CO_2, 90% air environment using Dulbecco's modified essential medium (DMEM) containing 10% fetal calf serum. After 10 to 14 days, a microglia enriched culture was obtained by using the differential adherence properties of microglia and astrocytes. Microglia were then seeded into either 96 well culture dishes for the superoxide assay and to 15 cm^2 petri dishes for patch clamping.

Superoxide secretion, as measured by a microcytochrome C reduction assay, in the presence and absence of superoxide dismutase, was increased within 30 minutes when calcium concentration of the medium was increased from 2.5 mM to 20 mM. Addition of the calcium ionophore, A23187, to the media produced a similar increase in superoxide production.

Single calcium channel currents were observed from cell attached patch recordings. The bath medium contained 140 mM KCl, 1 mM $CaCl_2$, 1.8 mM $MgCl_2$, and 10 mM HEPES buffered solution to pH 7.35. The pipette was filled with 110 mM $BaCl_2$ and 10 mM HEPES buffered to pH 7.35. A non-inactivating inward current was noticed at resting and at hyperpolarized membrane potentials (i.e., -60 mV to -100 mV). The single channel conductance is about 10 pS with the barium in the pipette. This channel has the same functional properties as the B type calcium channel previously described (Rosenberg, R. L. and Tsien, R. W., Biophys. J. 51:29a, 1987). Using these results, we estimated that the calcium concentration in the cell could possibly be doubled in 10 minutes when the cell is exposed to 20 mM calcium. This increase in calcium concentration could be a factor in the superoxide secretion.

330.5 EFFECTS OF ANTICONVULSANTS ON CALCIUM, CALMODULIN DEPENDENT PROTEIN KINASE IN PRIMARY ASTROCYTE CULTURES. E. Babcock-Atkinson*, L.O.B. Norenberg*, M.D. Norenberg, and J.T. Neary. Laboratory of Neuropathology, Vet. Admin. Med. Center and Univ. of Miami School of Medicine, Miami, FL 33101.

Calcium-regulated processes have been implicated in epileptogenesis; for example, calcium, calmodulin-dependent protein phosphorylation is altered in kindling (Wasterlain and Farber: Proc Natl Acad Sci USA, 81: 1253, 1984), and anticonvulsants such as diazepam and phenytoin inhibit rat brain calcium, calmodulin-dependent protein kinase (Ca/CaMK) (DeLorenzo et al: Science 213: 546, 1981). To evaluate the potential contribution of astrocytes to calcium-dependent mechanisms related to epilepsy, we have investigated the effect of anti-convulsants on Ca/CaMK activity in astrocytes in primary culture.

Astrocytes were derived from neonatal rat cortices and were at least 95% pure (J Neuropath Exp Neurol 44: 397, 1985). Cells from 3 to 6 week old cultures were homogenized in calcium-free buffer containing protease inhibitors. Supernatant and membrane fractions were prepared by centrifugation at 23,000 x g for 30 min at 4°C. Membranes were resuspended in homogenizing buffer supplemented with 0.1% Triton X-100. Ca/CaMK activity was measured by phosphorylation of (a) endogenous substrates by SDS gel electrophoresis, autoradiography, and densitometry and (b) casein by a slight modification of the method of Kuret and Schulman (Biochemistry 23: 5495, 1984).

We found that diazepam inhibited phosphorylation of several endogenous substrates for Ca/CaMK, including two major phosphoproteins (59 and 53 kDa) present in supernatant and membrane fractions. Little to no inhibition was observed with phenytoin or valproic acid (50-500 µM). Preliminary results indicated that 50% inhibition in these crude preparations was attained at about 100 µM diazepam. Since astrocyte cultures treated with dibutyryl cyclic AMP (dBcAMP) have some properties of reactive astrocytes (Fedoroff, et al: J Neurosci Res, 12: 15, 1984) which are frequently found in epileptic foci, we investigated the effect of anticonvulsants on Ca/CaMK activity in cultures treated with dBcAMP. These cells showed a 4-fold increase in calmodulin-dependent phosphorylation in the major phosphoprotein (59 kDa) present in the supernatant fraction. This band was particularly sensitive to inhibition by diazepam, but not by phenytoin or valproic acid.

Our results indicate that diazepam is capable of inhibiting Ca/CaMK activity in astrocytes. These findings suggest a possible site of diazepam action and a potential mechanism for a role of astrocytes in epileptogenesis.

330.6 PHOSPHORYLATION OF AN ACIDIC 80 kDa PROTEIN BY PROTEIN KINASE-C IN ASTROCYTES. P.L. Mobley and B.C. Harrison*. Dept. of Pharmacology, Univ. of Texas Health Science Center at San Antonio, San Antonio, TX 78284.

Studies indicate that a calcium/phospholipid dependent protein kinase (protein kinase-C) is present in astrocytes and activation of this kinase by the tumor promoter phorbol myristate acetate (PMA) can induce morphological changes in these cells (Brain Res. 398:366-369). Because of the important role this kinase may play in cell function, studies were conducted to identify phospho-protein substrates in astrocytes for this kinase.

Astrocyte cultures were prepared from the neocortex of newborn Sprague-Dawley rats. Cells were grown to confluency, then contaminating cells were removed, and astrocytes were replated (30,000/sq.cm.). After cells again reached confluency, they were labeled in phosphate-free medium containing approximately 0.5 mCi/ml of ^{32}P-orthophosphate. Cells were incubated with ^{32}P for 2 hours then treated as indicated. Following treatment the medium was removed and cells were then dissolved in 1% SDS and subjected to 2-D gel electrophoresis. From the resulting gels autoradiograms were prepared and the amount of ^{32}P incorporation into various phosphoproteins estimated.

Using this technique several apparent phosphoproteins have been identified in astrocytes which appear to be phosphorylated by protein kinase-C. The most prominent of these is an acidic protein (pI value 4.0-4.5) with a molecular weight of approximately 80 kDa. The ^{32}P content of this protein was increased by a 15 minute treatment with 300 nM PMA. In contrast, treatment of astrocytes with 2 mM dibutyryl cyclic AMP for 15 minutes did not increase the ^{32}P content of this protein although this treatment did increase the ^{32}P content of other phosphoproteins. The effects of PMA on the ^{32}P content of the 80 kDa protein appear to be mediated by protein kinase-C since no effects were observed with PMA in cells depleted of protein kinase-C by pretreatment with PMA 24 hrs earlier.

While the role of this 80 kDa protein in astrocyte function is unknown, it is likely that this phosphoprotein is identical to an acidic protein of similar molecular weight identified in several other tissues and which appears to be a substrate only for protein kinase-C. Therefore the phosphorylation of this protein may serve as a useful marker for the activation of protein kinase-C in astrocyte cultures.

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330.7 PHORBOL ESTER DOWN-REGULATES PROTEIN KINASE C ACTIVITY IN PRIMARY CEREBELLAR NEURONAL AND ASTROCYTE CULTURES. R.A. Philibert*, K.L. Rogers and G.R. Dutton, Dept. of Pharmacology, College of Medicine, University of Iowa, Iowa City, IA 52242

We have studied the effects of chronic treatment of primary rat cerebellar neuronal and astrocyte cultures with phorbol 12, 13-dibutyrate (PDBU). Neuronal and astrocyte cell cultures were prepared from 7-9 day old rat cerebella according to the method of Dutton et al. [J. Neurosci. Methods, (1981) 3: 421-427] and grown in poly-d-lysine coated T-flasks. At 7-9 and 16-18 days in vitro for neuronal and glial cultures, respectively, studies using appropriate antibodies revealed that neuronal cultures consisted of 92-95% granule cells, 5-7% inhibitory interneurons and <1% astrocytes, while astrocyte cultures consisted of approximately 95% type I astrocytes, <1% oligodendrocytes, 4% macrophages, fibroblasts, smooth muscle and endothelial cells. Cultures were incubated with PDBU or vehicle (0.2% DMSO) for 18 hours, and then homogenized in a Tris-based buffer. Homogenates were applied to a DEAE-cellulose column and Protein Kinase C (PKC) was eluted with a 0-300 mM NaCl gradient. PKC activity was assayed by determining the rate of transfer of ^{32}P from [γ^{32}P]-ATP to histone by the method of McArdle and Conn [Mol. Pharm. (1986) 29: 570-576].

After 18 hours, PKC down-regulation in neuronal cultures was dose-dependent and produced nearly complete loss of enzyme activity at 1 µM PDBU (EC_{50} approximately 5 nM). Time-course studies showed that the down-regulation of neuronal PKC activity reached backround levels after 6 hours of incubation with 1 µM PDBU. Following removal of of the phorbol ester by repeated washing, PKC activity recovered significantly within only 6 hours. After 18 hours of incubation with PDBU, down-regulation of astrocyte PKC activity was also dose-dependent, with complete loss of activity at 1 µM PDBU (EC_{50} approximately 15 nM).

In other studies with neuronal cultures, preliminary results indicate that a 12.5 minute preincubation with 0.1 µM PDBU attenuates the release of the neurotransmitters normally seen in response to stimulation with 50 µM kainic acid.

These results demonstrate that PKC activity is present in cultures of neurons and astrocytes from the cerebellum, and that this enzyme can be down-regulated by phorbol ester in a way which is reversible and perhaps related to neurotransmitter release.

This work was supported by NS20632 and American Heart 84-520. RAP and KLR are with the Neuroscience Program at the University of Iowa.

330.8 FORSKOLIN AND PHORBOL ESTERS MODULATE THE SAME K^+ CONDUCTANCE IN CULTURED OLIGODENDROCYTES. B. Soliven,* S. Szuchet,* B.G.W. Arnason, D.J. Nelson.* (SPON: P. Hoffmann) Dept. of Neurology, The Univ. of Chicago, Chicago, IL 60637.

Cultured ovine oligodendrocytes (OLG) express a number of voltage-dependent potassium currents as they attach and develop processes. At 24-48 hours following plating, a potassium outward current can be identified that represents a composite response of a rapidly inactivating component and a slowly or noninactivating component. After 4-7 days in culture, OLG also develop an inward rectifier current. We studied the effects of forskolin and 4-phorbol-12 myristate -13 acetate (PMA), compounds known to modulate the myelinogenic metabolism in these cells, on OLG outward currents. PMA, an activator of protein kinase C (PK-C), has been shown to enhance myelin basic protein phosphorylation while forskolin acting on adenylate cyclase increasing cAMP, inhibits it. Both forskolin and PMA increase the phosphorylation of 2' 3' cyclic nucleotide phosphodiesterase, an OLG/myelin protein. We have found that forskolin decreased the steady state outward current at 120 mV by 10% at 1 nM, and by 72% at 25 uM (n=6), from a holding potential of -80 mV. The time course of inactivation of the peak currents was decreased affecting both the fast and slow time constants. There was no significant change in the steady state parameters of current activation and inactivation. A reduction in the inward rectifier current was also observed. The effect of forskolin was attenuated when the adenylate cyclase inhibitor adenosine (2mM) was present in the intracellular/pipette filling solution. The results of PMA experiments (n=9) were similar to those obtained with forskolin. Whereas the amplitude of the currents in the presence of PMA was reduced by 30% at 1.5 nM and 60-70% at 600 nM, the decay phase of the peak currents was less affected. The PMA effect could still be seen when the intracellular Ca++ was reduced to 10^{-9}M with 5 mM BAPTA, but was inhibited partially when the cells were preexposed to 10 uM sphingosine, a PK-C inhibitor. We postulate that the potassium currents in OLG could be physiologically modulated by two distinct second messenger systems, perhaps converging at the level of a common phosphorylating enzyme or regulatory protein.

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330.9 PHORBOL ESTER INDUCED SPONTANEOUS AND RHYTHMIC OSCILLATIONS IN ASTROCYTES IN VITRO B.A. MacVicar, S.A. Crichton,* D.M. Burnard, and F.W. Tse*. Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Glial cells of the mammalian central nervous have complex functions which are difficult to decipher because of the intimate intertwining of glial cells with neurons. Recent electrophysiological studies on pure glial preparations in primary tissue culture have identified voltage activated conductances to calcium, sodium, potassium and chloride, as well as calcium activated potassium conductances. To investigate the properties of astrocytes that have not been cultured we have developed an essentially neuron free preparation of CNS astrocytes in the kainic acid (KA) lesioned hippocampal slice. Following KA injection into the lateral ventricles, glial cells proliferated and neurons degenerated in the CA3 region thus allowing electrophysiological analysis of glial properties without neurons present. Histological examination of the hippocampus indicated that the CA3 region after KA injection was a gliotic area free of neurons as has been previously reported by many other investigators. With this preparation we have examined the effect of activating protein kinase C in astrocytes with a phorbol ester, TPA (12-O-tetradecanoyl-phorbol-13-acetate). Intracellular impalements were obtained from glial cells in KA lesioned hippocampal slices. Following TPA application (50nM) we observed that 60% of the cells (n=26) showed rhythmic oscillations which were 5-10 mV in amplitude and were associated with large changes in whole cell input resistance. An inactive phorbol ester, 4-α-PMA, did not induce oscillations. These oscillations were characterized by the following: rhythmicity, spontaneity, increased resistance during the depolarizing phase, sudden transitions from a high to a low resistance state, a waxing and waning of the phenomenon, reversability of the wave form around membrane resting potential, and lastly voltage sensitivity (whereby depolarization can decrease the frequency of oscillation). We postulate that the oscillations are due to sudden changes in potassium conductance that are evoked by oscillating intracellular calcium levels. These rhythmic oscillations in astrocytes could have profound effects in generating neuronal rhythmic activity in widespread areas of the CNS and perhaps also seizures.

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330.10 CARBACHOL STIMULATES RHYTHMIC OSCILLATIONS IN ASTROCYTES S.A. Crichton* and B.A. MacVicar, (SPON: R. Berdan). University of Calgary, Calgary, Alberta, Canada T2N 4N1

Protein kinase C activation by phorbol esters induces spontaneous and rhythmic oscillations in astrocytes (see MacVicar et al.this meeting). These oscillations appear to be due to rhythmic changes in potassium permeability of astrocytes. Muscarinic receptors have been reported in glial cells in culture and in glioma cell lines. Activation of these receptors causes increased inositol phospholipid metabolism. This should, therefore, lead to protein kinase C activation. We have tested the effects of muscarinic activation in astrocytes in kainate (KA) lesioned hippocampal slices to see if the effects are similar to phorbol ester activation of protein kinase C.

Intracellular recordings were obtained from astrocytes in CA3 and CA4 region of KA lesioned hippocampi. The average resting potential was -76mV and the average input resistance was 10.5MΩ. These properties are similar to cells which we have stained with lucifer yellow and have shown to be astrocytes. Neurones were never impaled in the CA3 region of lesioned hippocampi. Following perfusion of carbachol (50-100μM), input resistance increased by an average of 9.35MΩ and a gradual depolarization, reversible in wash, was seen. In 8 out of 15 cells rhythmic changes in input resistance and membrane potential were observed 4 - 10 minutes following carbachol application. The behavior of these oscillations was quite variable in these cells, sometimes the rhythmicity lasted up to 30 minutes and sometimes for only a few minutes. The characteristics of these oscillations appeared to be the same as those induced by phorbol ester application. The depolarizing phase was associated with an increased input resistance and the sudden hyperpolarizing phase was associated with a decreased input resistance. The wave form reversed polarity around the estimated equilibrium potential for potassium. To ensure that these effects were mediated through a muscarinic receptor, carbachol was applied in the presence of atropine (a muscarinic antagonist). Atropine (10μM) blocked the depolarizing phase and the change in input resistance induced by carbachol; oscillations were not observed. Therefore, muscarinic activation in astrocytes induces an increase in input resistance and oscillations of membrane potential. Possibly this is due to activation of the phosphatidyl inositol pathway because activation of protein kinase C by phorbol esters also induces spontaneous and rhythmic oscillations. These rhythmic oscillations could have profound effects in generating neuronal rhythmic activity in widespread areas of the CNS and may play a role in generating the theta rhythm of the EEG.

330.11 PENTYLENETETRAZOL HAS DIRECT ACTIONS ON ASTROCYTES. D.M. Burnard and B.A. MacVicar. Department of Medical Physiology, Univ. of Calgary, Calgary, Alberta, T2N 4N1.

The electrophysiological properties of astrocytes are currently being investigated in a relatively neuron-free preparation, the kainic acid lesioned hippocampal slice. Recent work indicates that activation of protein kinase C in astrocytes with a phorbol ester results in spontaneous and rhythmic oscillations of membrane potential. The depolarizing phase of these oscillations is accompanied by an increase in input resistance (MacVicar et al., this meeting). Similar oscillations have been observed in astrocytes exposed to carbachol (Crichton and MacVicar, this meeting). It is hypothesized that these oscillations are due to rhythmic changes in the potassium permeability of astrocytes, which may be related to oscillating intracellular calcium concentrations. Such oscillations may cause rhythmic increases in extracellular potassium and thus rhythmically excite surrounding neurons. This could be a basic mechanism for seizure discharge. We have therefore investigated the action of a convulsant, pentylenetetrazol (PTZ), on the electrophysiological properties of astrocytes. PTZ causes bursting activity in neurons, profound displacements of intracellular calcium, and protein kinase activation.

Male Sprague Dawley rats (150 - 200 g.) received a bilateral intracerebroventricular injection of 1.0 μg kainic acid a minimum of two weeks prior to hippocampal slice preparation. Intracellular recordings of glia were reliably obtained in the intensely gliotic CA3/CA4 region of the hippocampus. Astrocytes were identified by their high resting membrane potentials, low input resistances and the absence of action potentials. Lucifer yellow (8%) injections confirmed that recordings were obtained from astrocytes, not neurons. PTZ (50 mM) was perfused into the bath for 5 to 15 min and changes in glial input resistance and membrane potential were recorded intracellularly.

The average resting membrane potential of astrocytes was 82.1 ± 2.7 mV. In the presence of PTZ (50 mM), glial membrane potential depolarized (3-9 mV) and in 60% of the astrocytes studied, spontaneous oscillations in input resistance and membrane potential were observed. There were both low and high frequency oscillations, and the increases in input resistance (25 - 50%) were typically associated with membrane depolarization.

These findings suggest that PTZ induces oscillations of membrane potential and input resistance in some glial cells. The mechanism underlying these oscillations remains to be determined, however it may be similar to the mechanism underlying phorbol ester induced oscillations. We are currently investigating whether or not these oscillations can be blocked by phenytoin, a widely used anticonvulsant drug.

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330.12 ISOPROTERENOL ENHANCES CALCIUM CURRENT IN CULTURED ASTROCYTES F.W. Tse,* and B.A. MacVicar (SPON: Fred Quandt). Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Astrocytes in tissue culture have voltage activated calcium channels and calcium activated potassium channels as well as voltage activated sodium, chloride and potassium currents. Receptors for several neurotransmitters such as norepinephrine, GABA, excitory amino acids and adenosine have also been found in glial cells and in some cases have been shown to regulate glial cAMP levels. In other cell types properties of the calcium channels are altered by neurotransmitters via cAMP. Therefore we have examined the effects of cAMP and isoproterenol (a beta-noradrenergic agonist which is known to increase cAMP in glial cells) on barium spikes and calcium currents in cultured astrocytes. Primary cultures of astrocytes were prepared from neonatal rats as described by MacVicar (Science 226: 133). Two microelectrodes (20 to 100MΩ) were used to impale the astrocytes to perform two electrode current and voltage clamping. Cells were impaled while superfused with artificial cerebral spinal fluid. Then a solution containing barium (10mM) TEA (10mM) 4-aminopyridine (0.2mM) and apamin (10nM) was superfused to enhance calcium currents and decrease potassium currents. In this solution injection of depolarizing current in some cells evoked barium spikes. Two electrode voltage clamping indicated that these cells had an inward current that was not blocked by TTX but was blocked by cobalt, cadmium or nifedipine. This inward current was reduced by lowering [Ba^{++}] in the solution; therefore, it is a barium current entering through calcium channels. In two electrode voltage clamp this current could be stable for up to two hours. Addition of isoproterenol (100μM) increased the barium current. This increase in barium current was mimicked by 0.5 mM dibutyryl cAMP (a membrane permeable form of cAMP). Therefore increasing cAMP intracellularly either by the addition of a cAMP analog or isoproterenol enhances calcium currents in cultured astrocytes. This agrees with a previous report (Chun et al.Soc. Neurosci. Abs. 12: (1986) 1346) that calcium currents could only be observed in cultured astrocytes that had been treated with cAMP. The function of this calcium current is unknown, although we have evidence that the morphological differentiation of astrocytes induced by cAMP or isoproterenol in tissue culture can be blocked by blocking calcium entry.

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330.13 COMPARATIVE STUDIES OF BETA-ADRENERGIC RECEPTOR IN THE NEURON AND ASTROCYTE GLIA OF CEREBELLAR GRANULE CELLS. Y.Watanabe[1,2,3], T.Shibuya[1,2], S.Culp[3], C.Rabe[3] and B.Tabakoff[1,3] 1) Dept. of Biomed. Sci., Univ. of Ill. Sch. of Med. at Rockford, 1601 Parkview Ave., Rockford, IL 61107 2) Dept. of Pharmacol., Tokyo Medical College, Japan 3) Lab of Physiol. and Pharmacol., NIAAA, 12501 Washington Ave., Rockville, MD 20852

Primary culture of granule cells, prepared from cerebellum of 8 day old neonatal rats, were utilized in the characterization of Beta-adrenergic receptors on neurons and glia. The following assays were performed in cells cultured for 8 days in vitro (DIV): cyclic AMP (cAMP) radioimmunoassay; immunochemistry for determining the number of neurons and glia; radioreceptor assay for determining the characteristics of Beta-receptor. Isoproterenol (Iso) administration produced a dose dependent increase in the cAMP levels with a dose as low as 1 nM resulting in a significant change. The time course for stimulation of cyclase by isoproterenol was dose-dependent, with maximal accumulation within 5 min and this rate of formation was maintained for 30 min. Accumulation of Iso-induced cAMP was completely blocked by Beta-adrenergic antagonists but not Alpha-adrenergic antagonists. The binding to Beta-receptors of the 8DIV granule cell was characterized with ^{125}I-iodo-cyanopindolol (^{125}ICYP). For this membrane preparation, the affinity (K_D) and maximum binding sites (B_{max}) were 90.4 ± 9.4 pM and 32.0 ± 1.8 fmol/mg protein, respectively. The above results, like those of previous reports, are not sufficient to localize the binding sites to neurons. There is a slight contamination of this culture with glia (95.8 ± 1.0% neurons and 4.2 ± 0.6% glia). To determine if Beta-receptors exist on glia, a second set of cultures was prepared in which cytosine arabinoside, which prevents replication of non-neuronal cell, was not included in the culture media on 2DIV. The resulting glia rich culture was 49.9 ± 1.6% neurons and 50.1 ± 1.6% glia. ^{125}ICYP binding to membranes from this culture exhibit a K_D of 64.5 ± 13.8 pM and a B_{max} of 66.3 ± 6.7 fmol/mg protein. The glia rich 8DIV culture possessed approximately 2 times as many sites as the neuron rich 8DIV culture, reflecting a doubling of the total cell population when glia are allowed to replicate. Furthermore, Iso increased cAMP levels in the glia rich culture suggesting functional receptor sites. In summary, Beta-receptor is localized in both neuron and astroglia on 8DIV cerebellar granule cells. Moreover, the Beta-receptor appears to be more prevalent in glia than in neuron.

330.14 **GLIA CULTURED FROM DIFFERENT BRAIN REGIONS ARE HETEROGENEOUS WITH RESPECT TO ADRENERGIC RECEPTOR DENSITY.** *L. Lyandvert*, L. Iacovitti, P. Ernsberger, and D. J. Reis,* (SPON: D.H. Park). Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

It is generally believed that glia represent a homogeneous group of cells exhibiting no distinguishing features from one brain region to another. In this study, we examined the possibility that glia are regionally specialized with respect to their density of neurotransmitter receptor sites.

Mixed glial cell cultures were generated from a variety of brain regions from 1-2 day old rats (McCarthy & DeVellis, *J. Cyc. Nucl. Res.* 4:15-26 1978). Cells were trypsin-dissociated, plated onto plastic dishes at a density of 4 x 10^5 cells/cm^2, fed media containing 15% fetal calf serum, and maintained in a humidified atmosphere of 97% air and 3% CO_2. The complete absence of immunoreactivity for neuron-specific enolase (NSE) and the presence of immunoreactivity for glial fibrillary acidic protein in the vast majority of cells (> 95%), indicated that the cultures contained nearly pure populations of glial astrocytes. Two to three weeks after plating, the cells were harvested, homogenized in a teflon-glass homogenizer, and membranes (P_2 fraction) were prepared and washed for radioligand binding assays for α_2- and β-adrenergic and muscarinic acetylcholine receptors (Ernsberger & U'Prichard, *Life Sci.* 38:1557, 1986). α_2- and β-adrenergic and muscarinic receptors were assayed by incubating membranes with ^{3}H-*p*-aminoclonidine (1 nM), ^{3}H-dihydroalprenolol (0.4 nM), or ^{3}H-quinuclidinyl benzylate (0.4 nM), respectively, for 20-60 min at 25° C. Nonspecific binding was defined in the presence of either guanabenz (10 μM), (±)-propranolol (1 μM), or atropine (1 μM). Muscarinic receptors could not be detected in glial membranes, indicating that not all neurotransmitter receptors are present in glia. Shown below is specific adrenergic receptor binding to glial membranes from each region expressed as sites per cell (n=3 to 11):

	Cortex	Caudate	Midbrain	Sup. Col.	Hypothal.	Hippocampus
α_2	14±1	5±1	8±2	17±2	12±1	8±1
β	60±6	24±3	37±6	16±4	16±4	28±2

The number of receptors detected per cell varied 3-fold from the highest to lowest regions for both adrenergic subtypes. It is unlikely that this variation is due to contamination with neurons, since a) the cultures are devoid of cells containing NSE immunoreactivity and b) the regional distribution of receptors in glia was unrelated to the distribution in whole tissue as previously determined by receptor autoradiography (rank-order correlation = -0.54 for α_2 and 0.29 for β-receptors). There was no significant tendency for regions enriched in one adrenergic subtype to be enriched in the other (r = 0.53 for the correlation between α_2 and β-receptor density, p > 0.10). We conclude that (a) muscarinic receptors appear to be absent in cultured glia, and (b) adrenergic receptors in glia are regionally distributed. Therefore, the anatomical mapping of receptors may reflect variations in glial as well as neuronal function since glia may also be heterogeneous with respect to their expression of neurotransmitter receptors. (Supported by NIH grant 18974, N.Y. Heart Assoc. Postdoctoral Fellowship to P.E.)

330.15 GLIAL CELL PROLIFERATION AND ION CHANNELS: A POSSIBLE LINK Donald G. Puro. Bascom Palmer Eye Institute, University of Miami, Miami, FL 33101.

A variety of pathophysiological conditions in the adult CNS are associated with the proliferation of glial cells. For example, in the mammalian retina, glial cell proliferation may occur under abnormal conditions such as inflammation, ischemia and diabetes. Since a breakdown of the retina-blood barrier is a feature often found in retinal disorders associated with glial cell proliferation, an hypothesis is that certain plasma-derived molecules are mitogenic for retinal glial cells. At present, the regulation of glial cell proliferation is poorly understood. In this report, I began to examine the possibility that the activity of certain ion channels play a role in the response of retinal glial cells to mitogens.

Experiments were performed on glial cells dissociated from adult rat retinas and maintained in cell culture. The cells stained positively by immunocytochemistry with antibodies specific for glial fibrillary acidic protein and with Muller cells of the rat retina. Patch clamp studies of these glial cells previously demonstrated a diversity of ion channels including a calcium-activated potassium channel with a high conductance (124pS, SD=37) and a sensitivity to tetraethylammonium. In this study, the activity of this class of potassium channel was assessed prior to and after exposure to a conditioned medium that is obtained from spleen cells and is mitogenic for cultured retinal glial cells. Within one hour of exposure to the conditioned medium, ion channels having outward currents and conductances >100pS at 32° demonstrated an increase in the percent of time that they were opened at various depolarizing potentials. Another indication of a possible link between the activity of potassium channels and the action of mitogens was suggested by the finding that tetraethylammonium (20mM) virtually eliminated the proliferative effect of the conditioned medium on retinal glial cells. These experiments are consistent with the notion that the activity of certain potassium channels may play a role in the proliferative response of retinal glial cells to mitogens produced by the immune system in the course of intraocular inflammation.

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330.16 **A CABLE MODEL OF THE ELECTRICAL PROPERTIES OF RETINAL MULLER CELLS.** R. L. Winslow*, R. F. Miller, and P. A. Coleman* (SPON: C. Blazynski). Inst. Biomed. Comp. and Dept. Opth., Washington Univ. Sch. Med., St. Louis, MO, 63130.

The membrane resistance of retinal (Muller) glial cells is highly non-uniform, with roughly 95% of the cell membrane conductance localized to the endfoot region (Newman, J. Neurosci. 5(8):2225). The charging curves of isolated Muller cells also exhibit an exceptionally fast rise-time (Newman, J. Neurosci. 5(8):2225). We have attempted to relate these two observations by formulating a simple cable model of the Muller cell. In this model, the distal end, cell body, and stalk is represented by an equivalent cylinder. The highly localized conductance of the the endfoot region is modeled by terminating the cylinder with a resistor. We have derived an expression specifying membrane potential in response to a current pulse injected into the cylinder. Simulation results show that a current pulse injected at the distal end produces changes in voltage at the site of current injection with a rise time which can be significantly faster than that of a semi-infinite cable. Decreases in the electrotonic length of the cylinder or increases of the value of the terminating conductance produce monotonic decreases in the rise time of model charging curves.

The model is completely specified by three parameters: A) the electrotonic length L of the cylinder; B) the percentage P of input conductance accounted for by the endfoot; C) the membrane time constant τ. We have derived an algorithm for generating estimates of these parameters given an experimentally measured charging curve and estimated values of the exponents of the first two terms of the series representation of this response. Performance of the algorithm has been tested using simulated data. We have used the algorithm to estimate parameter values that yield best fits to charging curves obtained from both dissociated tiger salamander (*Ambystoma tigrinum mavortium*) Muller cells as well as Muller cells in the intact retina eyecup preparation. Estimated parameter values obtained from measurements in dissociated cells are $L \approx .38$, $\tau \approx .9$msec, and $P \approx 95\%$. Muller cell charging curves obtained using measurements from the eyecup preparation exhibit rise-times which are clearly slower than those recorded from dissociated cells. Parameter values estimated using these data are $L \approx 2$, $\tau \approx 1.0$ msec, and $P \approx 98\%$. These two sets of data support the finding that a significant fraction of Muller cell membrane conductance is localized to the proximal face of the endfoot region. These data differ significantly in the rise time of the charging curves, and in the estimated values of electrotonic length. These differences may result from an alteration of the physical dimensions of Muller cells due to the dissociation procedure. We consistently noted that dissociated Muller cells become less elongated and more spheroidal after dissociation, a distortion which may tend to decrease L. Simulations have shown that a decrease in L results in charging curves with faster rise time.

An extreme non-uniformity of membrane conductance may be common to all astrocytes, and may play an important role in the spatial buffering of extracellular K^+. The development of simple procedures for estimating the degree of this non-uniformity should have important implications for the analysis of glial function in a variety of normal and abnormal states.

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330.17 ELECTROPHYSIOLOGY AND MORPHOLOGY OF EPENDYMAL CELLS AND TANYCYTES IN RAT HYPOTHALAMUS. C.R. Jarvis and R.D. Andrew. Anatomy Dept., Queen's University, Kingston, Ontario K7L 3N6.

The ependyma lines the ventricular system of the vertebrate brain and spinal cord. Although its embryology and morphology have been studied extensively, little is known of its physiological properties, particularly in mammals. Tanycytes are modified ependymal cells that are found predominently lining the floor of the third ventricle, overlying the median eminence (ME). Their processes accompany and enwrap neuroendocrine axons that course from hypothalamic nuclei to terminals in the ME but the significance of this interaction is not yet understood.

Intracellular recording and injection techniques were used to study ependymal cells and tanycytes of the rat in the hypothalamic slice preparation after differentiating their respective regions morphologically. At rest, the mean membrane potential $\pm$ SD for common ependyma was 79.9 $\pm$ 1.40 mV (n=30) and for tanycytes 79.5 $\pm$ 1.77 mV (n=46). From the Nernst equation, these values indicate that K^+ is the primary ion determining Vm, considering $[K^+]_o$=6.24 mM. Input resistances (Rin) were very low (<< 1 Mohm). Single cell injection of Lucifer Yellow, a fluorescent dye of low molecular weight, revealed dye coupling among 2-70 ependymal cells or 5-48 tanycytes. In both freeze-fractured replicas and thin sections, large numbers of gap junctions were found between adjacent ependymal cells or between adjacent tanycytes. The observations of numerous gap junctions, extensive dye coupling, and low input resistance, demonstrated that both ependymal cell and tanycyte populations in mammals are strongly coupled networks. Perhaps for this reason, attempts to uncouple these cells using sodium propionate, chlorpromazine or CO_2 were unsuccessful.

Electrical stimulation of the arcuate nucleus did not elicit any synaptic response in impaled tanycytes, so that the functional significance of synaptoid contacts between neuroendocrine neurons and the postsynaptic tanycytes is not yet apparent.

Ependymal cells and tanycytes demonstrated a near Nernstian response to changes in extracellular $[K^+]$ between 3 and 20 mM. This finding, as well as their high negative resting potential, low Rin, electrical inexcitability, and extensive coupling demonstrate that ependymal cells possess numerous glial characteristics and therefore may have similar functions. The high K^+ permeability of tanycyte cell bodies suggests that their processes may buffer the extracellular [K+] by taking up K+ released from adjacent neurons and shunting it to the ventricular space.

REGENERATION: PNS

331.1 LOCALIZATION AND AXONAL TRANSPORT OF B50 (GAP43) IN REGENERATING RAT PERIPHERAL NERVES: ASSOCIATION WITH OUTGROWING AXONS AND SCHWANN CELLS. M.A.Bisby, H. Zwiers* and W. Tetzlaff* (SPON: H.B. Sarnat). Dept. Med. Physiology, Univ. of Calgary, Calgary, Alta, T2N 4N1, Canada.

The synaptic phosphoprotein B50 has been shown, by several laboratories, to be identical to growth-associated protein (GAP) 43, whose synthesis and axonal transport is greatly increased in regenerating peripheral axons. We have used an affinity-purified rabbit antibody to rat brain B50 to investigate the localization and axonal transport of B50/GAP43 in regenerating peripheral nerve.

Nerve segments were processed for one-dimensional SDS-PAGE and Western blots were probed with antibodies to B50, then labelled with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulins. The greatest amount of B50-like immunoreactivity occurred within newly-regenerated axons, approximately midway between the site of injury and the point of maximum outgrowth.

Immunocytochemistry, using rhodamine-conjugated goat anti-rabbit immunoglobulins as secondary antibody, revealed intra-axonal B50-like immunoreactivity in the nerve proximal to the injury, as well as in newly regenerated axons close to the point of maximum outgrowth. However, in the zone of greatest immunoreactivity, label was associated not primarily with axons but with apparent Schwann Cell profiles of the bands of Büngner, enclosing neurofilament-containing axons.

Conventional ligature techniques were used to measure anterograde and retrograde transport of B50-like immunoreactivity in regenerating sciatic nerve proximal to the injury. Only about 1/6 of the B50 appeared mobile, and 3-4 times as much was transported in the anterograde direction as in the retrograde direction. Anterograde velocity was 6.3 mm/hr, consistent with previous data for fast transport of GAP43.

As expected, B50-like immunoreactivity was increased in regenerating nerve. The association of immunoreactivity with structures other than axons was unexpected, and raises the possibility that B50/GAP43 can be secreted from regenerating axons.

Acknowledgments: Supported by MRC of Canada. HZ is a scholar and WT a fellow of AHFMR.

331.2 TIME DEPENDENT CHANGE IN ACTIVITY OF RAT MEDULLARY RESPIRATORY NEURONS REGENERATING AXONS INTO PERIPHERAL NERVE GRAFTS. P. Gauthier* and M. Rasminsky. Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Quebec, Canada H3G 1A4.

Autologous segments of peroneal nerve were implanted into the medulla oblongata of young adult rats. To investigate activity of medullary respiratory neurons regenerating axons into these grafts, unitary recording from single fibers was performed on small strands teased from the grafts in anesthetized, curarized and artificially ventilated rats. Central respiratory rhythm was monitored by recording phrenic nerve activity.

Spontaneous respiratory related activity was observed in teased fibers in 5 of 9 grafts recorded 2 to 5 months after graft implantation. All but one of 42 respiratory related units were identified as efferent inspiratory or expiratory units because of their persistent respiratory related activity during arrest of artificial ventilation. Medullary respiratory neurons include both intrinsic neurons having their axonal projections entirely within the CNS and cranial motoneurons projecting their axons towards the periphery. Patterns of spontaneous activity of some recorded units were characteristic of intrinsic brainstem respiratory neurons rather than of cranial motoneurons. The persistence of activity with deepening level of anesthesia seen in most units was also characteristic of intrinsic brainstem respiratory neurons. In the 2 to 5-month grafts, the integrity of the input connections to the neurons that had regenerated axons was manifested not only by normal patterns of activity but also by the responsiveness of firing patterns to lung hyperinflation and by the interruption of inspiratory discharge of graft units by vagal stimulation (inspiratory off-switch effect).

Ten grafts were studied 9 to 11 months after implantation. Five were blind-ended, as in the 2 to 5-month grafts; the distal ends of five other grafts were implanted into the left C4 ventral horn. No spontaneous respiratory activity was found in fibers teased from any of the 9 to 11-month grafts inserted into the brainstem.

These results suggest that there may be only a limited period of time following graft implantation during which a peripheral nerve graft can exert a supportive effect on function and/or survival of the CNS neurons innervating the graft with regenerated axons. In our small preliminary sample of bridge grafts, recordings from the left phrenic nerve revealed no physiologic evidence of functional connection between electrically stimulated graft fibers and phrenic motoneurons. It remains to be established whether interaction between appropriately placed terminals of fibers regenerating in the grafts and target neurons will have a stabilizing effect on the function and/or survival of neurons regenerating axons into peripheral nerve grafts.

331.3 CHOLINERGIC NEURONES IN THE VAGAL AFFERENT FIBRES. J.P. Ternaux*, B. Palouzier*, M. Falempin*, C. Simon*, P. Portalier* and M-C. Chamoin-Barrit* (SPON: J.P. VEDEL). INSERM-U.6 and CNRS-UA634, 280 Bd Sainte-Marguerite, 13009 Marseille, France.

In various mammalian species the anastomosis of vagal afferent fibres, cut above the nodose ganglion, with the fibres of the peripheral end of the spinal nerve is followed, several months after, by a functional reinnervation of the sterno-cleido-mastoïd muscle (SCM). This heterogeneous suture allows, in chronic sheep and rabbit, the characterization of various interoceptors located in the upper part of the digestive tract, by simply recording the EMG activities of the reinnervated SCM muscle.

Since muscular fibres are reinnervated by vagal afferent neurones, it can be assumed that some of these fibres contain acetylcholine (ACh) or may be that some afferent neurones are able to express cholinergic potentialities when sutured with the spinal nerve.

Various biochemical results indicate the presence of an ACh neuronal system in the vagal afferent fibres of both rat and rabbit. In the nodose ganglion, which contains all the cell bodies of the vagal afferent fibres, choline acetyl transferase (ChAc) as well as acetylcholine esterase (AChE) activities can be detected. An uptake of choline is also measured and seems to be related to a high affinity transport of the precursor. Finally, ACh endogenous content as well as release can be detected in the nodose ganglion. Release from cell bodies is sodium- and calcium-dependent and indicates that afferent messages can be controlled at nodose level.

Presence of a cholinergic pool in afferent vagal fibres is also confirmed by the immunohistochemical determination of ChAc in some cell bodies of the nodose ganglion.

In the sutured nodose ganglion of the rabbit, ChAc activity as well as ACh content decrease until four months. Five months after the suture when electrophysiological activities can be recorded from the SCM muscle, the amount of ACh detected in the sutured ganglion is higher compared to four months. In the non sutured ganglion the endogenous ACh is first enhanced and then decreases suggesting the involvement of a compensatory mechanism.

331.4 $pp60^{c\text{-}src}$ PROTEIN-TYROSINE KINASE IS STRONGLY INDUCED IN REGENERATING SCIATIC NERVE. J.M. Le Beau and G. Walter*, Department of Pathology, University of California, San Diego, La Jolla, California 92093.

$pp60^{c\text{-}src}$, the cellular homolog of the transforming protein of the Rous sarcoma virus oncogene, $pp60^{v\text{-}src}$, is a phosphoprotein with a high tyrosine kinase activity in the nervous system. Recent findings have implicated the expression of $pp60^{c\text{-}src}$ with CNS neuronal differentiation and function. In the present study we asked whether $pp60^{c\text{-}src}$ was associated with Schwann cell and axonal differentiation during peripheral nerve regeneration. To examine this, a model of regeneration was used in which the proximal and distal stumps of a severed rat sciatic nerve were sutured into a 14 mm long silicone tube. Regeneration across the 10 mm gap was accomplished by 35-42 days following nerve-transection. Segments of regenerated nerve as well as other associated tissues were extracted at various times following nerve transection and analyzed for c-src activity by in vitro kinase assays. An induction of c-src kinase activity occurred at 7 days following nerve transection in segments of regenerated nerve. This activity increased with post-transectional time, reaching a level that was 10-15 fold greater than control nerve at 21-28 days following nerve transection. Significant changes in kinase activity were not observed in other tissues associated with regenerated sciatic nerve (i.e. spinal cord and dorsal root ganglia). Western blotting experiments showed results similar to those of in vitro kinase assays suggesting that the altered expression of $pp60^{c\text{-}src}$ was due to an increase in protein synthesis rather than specific activity. Since the increased expression of $pp60^{c\text{-}src}$ in regenerated nerve segments coincided with both axonal sprouting and myelination, c-src may play a role in these events during nerve regeneration. Cellular and sub-cellular localization of $pp60^{c\text{-}src}$ in regenerated nerve segments are currently in progress.

331.5 IMMOBILIZED FIBROBLAST GROWTH FACTOR AFFECTS PERIPHERAL NERVE REGENERATION IN A SILICONE CHAMBER MODEL. N. Danielsen*, H.L. Vahlsing*, B. Pettmann*, M. Manthorpe and S. Varon (SPON: H. Muller). Dept. of Biology, Univ. California San Diego, La Jolla, CA 92093.

The silicone chamber model allows the investigation of the cellular and molecular events underlying successful regeneration of a peripheral nerve. We report here a modification of the standard silicone chamber into a two-compartment model which also allows the investigation of the effects on nerve regeneration of initially immobilized growth factors. Two opposing longitudinal grooves were cut into the interior surface of a silicone tube (1.8 mm I.D., 14 mm long). A strip of nitrocellulose paper was then inserted into the grooves, thus dividing the chamber into two compartments. Before insertion, the nitrocellulose strip was soaked overnight in saline (control) or saline containing purified basic Fibroblast Growth Factor (FGF). The chambers were used to entubate a 10 mm gap between the proximal and distal stumps of the rat sciatic nerve as done in previous studies. The rats were sacrificed 16 days after chamber implantation and the nerve regenerates were processed for light microscopy. Macroscopic observations revealed that in both control (n=8) and FGF (n=4) chambers, regenerates formed on each side of the nitrocellulose partition and adjacent to it. The migration of the cellular elements (perineurial-like cells, vasculature and Schwann cells) into control chambers was restricted to 1-3 mm from the proximal and distal nerve stumps. At mid-chamber level (5 mm) control chambers still displayed "blood-lakes" within a fibrin matrix. In contrast, FGF-containing chambers showed a vascularized cellular structure across the gap in 3 out of 4 chambers. Statistically significant differences were observed between control and FGF chambers in the spatial progress of migrating perineurial-like cells, vasculature and Schwann cells, while the differences in axonal elongation were not significant in this limited series. Thus, initially immobilized FGF promotes both the vascularization of the nerve regenerate and the migration of perineurial-like and Schwann cells into the regenerate.
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331.6 MOTONEURON--MUSCLE UNIT MISMATCHES IN REINNERVATED SOLEUS MUSCLE. GW Sypert, RC Foehring & JB Munson Univ of Florida, Gainesville.

When a fast muscle is cross-innervated by a nerve formerly innervating a slow muscle, the fast muscle fully acquires slow-muscle properties. In the reverse cross, however, the formerly slow muscle partly retains slow properties (1). In normal or self-reinnervated MG motor units (2), and in LG muscle units cross-innervated by MG motoneurons (3), a close correspondence exists between electrical properties of motoneurons and contractile properties of their innervated muscle units (5). When MG motoneurons cross-innervate soleus muscle [a slow muscle which resists conversion by fast motoneurons (1)], most motor units consist of either slow motoneurons (electrical properties) innervating slow muscle units (contractile properties) or fast motoneurons innervating fast muscle units (as above) (3). In a few cases, however (6 of 44), there are apparent mismatches; i.e. fast motoneurons innervating slow muscle units.

We further investigated this apparent anomoly in 4 cats by self-anastomosing the LG-soleus nerve and analyzing the properties of reinnervated soleus motor units 6 mos later. Of 69 motoneurons studied, 47 innervated LG and 22, soleus. These percentages (68% and 32%) mirror the 2:1 proportions of alpha motoneurons in each muscle and suggest that, as in the rat (4), neither muscle has an advantage in recapturing motor axons, despite the fact that axons destined for soleus must first traverse LG. Analysis of contractile properties of motor units in soleus showed 16 slow-twitch and 3 fast-twitch units (the latter all type FR; 3 others not fully analyzed). As with cross-innervated MG-->soleus (3), the motor units of 5 of the 16 slow-twitch units had motoneurons with "fast" properties, in particular AHP half-decay time <30 ms (5). Other properties of these motor units (rheobase, input resistance, conduction velocity, twitch time-to-peak) were also consistent with their being former (probably LG) fast motoneurons now innervating soleus muscle units classified as slow on the basis of their contractile properties (cf 2).

We conclude that regenerating LG and soleus motor axons express no preference to regenerate through endoneurial tubes of their former type or muscle, nor to reinnervate their former muscle (cf 4). Some fast motoneurons respecify the contractile properties of their innervated soleus muscle units, but others do not. Enduring motoneuron-muscle unit mismatches are the result.

(1) Buller et al. J Physiol 150: 399-414, 1960
(2) Foehring et al. J Neurophysiol 55: 931-946, 1986
(3) Foehring et al. J Neurophysiol 57: 1227-1245, 1987
(4) Gillespie et al. J Physiol 372: 485-500, 1986
(5) Zengel et al. J Neurophysiol 53: 1323-1344, 1985
Support: NS15913 (NINCDS); RERDS 822-124 & MRS 821-103 (VA).

331.7 IMPAIRMENT OF SCIATIC NERVE REGENERATION BY PROTEASE INHIBITOR TREATMENT: INHIBITION OF SCHWANN CELL MIGRATION. N. Kalderon, J.P. Kirk*, and A. Juhasz*. The Rockefeller Univ. New York, NY 10021.

The Schwann cell is an essential component in support of the repair process of an injured nerve. The present study is focused on the elucidation of one of the biochemical processes which is elaborated by the Schwann cell and is instrumental in successful nerve regeneration. Schwann cells, the proliferating populations, express high levels of plasminogen activator activity (Kalderon, PNAS, 81:7216, 1984). During nerve regeneration the Schwann cells seem to precede the regenerating axons. The temporal sequence of events in peripheral nerve regeneration in a model system, through a 10mm silicone chamber, has been established: (a) within one week postsurgery, an acellular fibrin bridge is formed between the nerve stumps; and (b) in the second week, extensive migration within this bridge of Schwann cells and other cells takes place, while the regenerating axons lag by 1-2 days behind the Schwann cells (Williams et al., J. Comp. Neurol., 218:460, 1983). The experiments described in this report examine the idea that Schwann cells utilize proteolysis to invade, paving the way for the regenerating axons. For this purpose we are studying the effects of protease inhibitors on rat sciatic nerve regeneration through a 10mm silicone chamber and herein describe the effects of the plasmin inhibitor, E-aminocaproic acid. The sciatic nerve was cut and the stumps were sutured to a silicone tubing which was filled with a physiological salt solution. The inhibitor was injected into the chamber (final concentration of 0.5 mg/ml) at seven days postsurgery, at which time a massive cell migration was about to occur, and its effect on nerve regeneration was examined a week later. Samples were processed for cryostat sectioning, sectioned through their horizontal axis and then were analysed in parallel for: cell content, regenerating axons, morphology, etc. The effect of the inhibitor was unambiguously clear: it inhibited the cell migration into the chamber. Analysis with thionin staining revealed that while cells filled up the entire bridge between the nerve stumps in the control samples, cell migration was impaired by the presence of the inhibitor leaving a gap of 2-5mm without any cells. Analysis of the extent of axonal elongation by immunocytochemistry with antibodies against neurofilaments revealed that the neurites advanced for 7-8mm in the control, and only for 2-4mm in the presence of the inhibitor. It is concluded that in the process of nerve regeneration, Schwann cells utilize proteolytic activity to invade/migrate, since inhibition of this activity prevented cell migration; accordingly, axonal regrowth was delayed.

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331.8 **EARLY DIFFERENCES IN PNS AXONAL REGENERATION WITHIN TUBULAR PROSTHESES FILLED WITH LAMININ VS. COLLAGEN MATRICES.** I.J. Kljavin*, R. Madison, and R.L. Sidman. (Spon:Brooke Seckel). Depts. of Neuropathology and Neuroscience, Harvard Medical School and Children's Hospital, Boston, MA. 02115

Entubulation repair experiments have shown that modification of the local environment within the tube can change the rate of axonal regeneration. At 2 to 4 weeks post-section more axons cross a 4mm nerve gap within laminin-containing than collagen containing tubes. The present study examines cellular relationships in the first 12 days post-section that may account for the differences in axonal regeneration noted at later times in the laminin and collagen matrices.

One sciatic nerve in adult male C57BL/6J mice was transected at midthigh level. Proximal and distal nerve stumps were sutured into the ends of a 6mm long Tygon tube, maintaining a 4mm gap between stumps. The tube lumens were filled either with a laminin-rich gel (gift of Drs. Kleinman and Martin, NIH), or a collagen matrix (Vitrogen-100, Flow Laboratories). Three animals from each group were killed at 1,3,5,7,10 and 12 days after implantation, tissues processed for light and electron microscopy, and examined in longitudinal sections.

Up to 3 days post-implantation, both laminin-rich and collagen samples showed a distinct cellular border, composed mostly of RBC's and macrophages, between the stumps and matrix. No cells entered either matrix until 5 days, at which time axonal sprouts were still confined to the stump. By 7-12 days, putative Schwann cells, fibroblasts, and endothelial cells had migrated even further into the matrices, and the first morphological differences in outgrowth within laminin and collagen were seen. Outgrowth from the proximal and distal stumps extended up to 1.0mm in laminin and up to 0.5mm in collagen at 7 days. The cellular density was less in the laminin-rich matrix (11 cells/0.1mm), compared to collagen (25 cells/0.1mm). This difference became more obvious at 10 and 12 days, when cells extended across the entire gap. The extent of axonal growth was independent of nonneuronal cell density, and matched the distribution of putative Schwann cells furthest from the proximal stump. Thus, axons typically had extended further from the proximal stump in the laminin samples. Reconstructions from serial sections revealed that in the laminin matrix, some axonal portions were free from cell contacts, (naked), whereas in the collagen samples the complete axonal surface was associated with Schwann cells. Axonal numbers appeared similar in both matrices.

The greater length of axons in the laminin-rich matrices thus appeared related to the faster outgrowth of supporting cells, probably Schwann cells, but did not correlate with the concentration of nonneuronal cells.

Supported by NIH grant NS22404

331.9 ANTIBODIES TO THE NEURAL CELL ADHESION MOLECULE DISRUPT CELL INTERACTIONS IN REGENERATING ISCHIATIC NERVES. L.G. Remsen,* G.M. Strain, A.A. Smith,* and J.K. Daniloff, Veterinary Anatomy & Fine Structure, and Physiology, Pharmacology & Toxicology, LSU School of Veterinary Medicine, Baton Rouge, LA 70803

The neural cell adhesion molecule (N-CAM) mediates homophilic binding between neurons; although also present on Schwann cells, its function there is unknown. Previous studies suggested that modulation of N-CAM is critical for regrowth of nerve fibers (Daniloff et al., 1986, J. Cell Biol. 103:929). The aim of the present study was to elucidate the contributions of N-CAM to regeneration by using antibodies to N-CAM to disrupt it.

Monoclonal antibodies (Ab) were raised against major polypeptide components of rat and chicken N-CAM. Ab or their Fab' fragments were concentrated (100 ug/ml) into a solid collagen gel (Vitrogen; Collagen Corp) within pieces of inert, sterile silastic tubing. Control tubes contained nonspecific Ab isolated from nonimmune mice, or Ab to Thy-1. A 4-mm section of one ischiatic nerve was removed from adult rats and chickens. Gel containing tubes were then surgically attached to the nerve stumps with epineurial sutures. After 10 or 30 days, muscle and somatosensory evoked potentials were recorded to assess the return of function. Animals were then killed and their nerves were prepared for light and electron microscopy and immunoblot analysis.

In short-term survivors, Ab disrupted interactions between both neural and nonneural cells within the tubes and produced abnormal gaps between them. At 30 days after implantation, regenerating fibers and nonneural cells had grown out and around the tubes to join the distal stumps. No such growth was observed in controls. Minimal responses to stimulation returned in some experimental animals after 30 days, although no significant differences were observed at that survival time. Physiological data recorded from long-term survivors (45 and 90 days) were used to compare the return of function in all experimental conditions. The number of nerve cells/unit area was measured in sections of nerves labeled with Ab to neurofilament protein. Relative number and position of Schwann cells and fibroblasts were analyzed in sections labeled with Ab to S100 protein and fibronectin, respectively. Results are discussed relative to potential contributions of N-CAM to regeneration of the peripheral nervous system.

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331.10 CYTOSKELETAL CHANGES IN AXOTOMIZED HAMSTER CORTICOSPINAL NEURONS: SIMILARITIES AND DIFFERENCES TO THE METABOLIC RESPONSE EXHIBITED BY PERIPHERAL NEURONS. M.M. Oblinger, M. Vanefsky* and J. Pickett*. Dept. Biol. Chemistry & Structure, Chicago Medical School, North Chicago, IL 60064.

Aspects of the axotomy response in peripheral neurons have been well characterized but little is known about the molecular response of central mammalian neurons to injury. Among the prominent changes that ensue after axon injury in the peripheral nervous system are a decrease in the synthesis and axonal transport of neurofilament (NF) proteins and a corresponding increase in tubulin (TUB) and actin (Oblinger and Lasek, 1985). Recent studies using specific cDNA probes have revealed that mRNA levels for major cytoskeletal proteins in peripheral neurons change after axotomy (Hoffman et al., 1987). In this study we have examined whether the axotomy response of a class of CNS neurons is similar to that exhibited by mammalian PNS neurons. Corticospinal neurons were studied because these long projection neurons are easily axotomized in the spinal cord and because slow transport and other aspects of the cytoskeleton in normal axons has been well characterized (Oblinger, 1987).

Hemisections of the upper cervical spinal cord (C4) were made in adult male Golden hamsters. In one series of experiments, animals were sacrificed 1d to several weeks later and the brains and spinal cords were removed and frozen. Samples of motor cortex, corticospinal tracts and spinal cord on the lesioned and normal side were homogenized in phosphate buffer, assayed for total protein, diluted with an equal volume of 2% SDS, 16M urea, 4% BME .2M Tris, pH 6.8 and subjected to SDS-PAGE. Immunoblotting of this material using various monoclonal antibodies (phosphorylated and nonphosphorylated NF proteins, beta tubulin, microtubule associated proteins) revealed that marked changes in the levels of cytoskeletal proteins resulted after injury. These changes included a reduction in NF proteins that became quite prominent 5-14 days after injury. The magnitude of change in tubulin levels in injured corticospinal axons was much less marked. Preliminary results obtained on corticospinal neurons in the motor cortex using NF and TUB specific probes are consistent with the changes observed in the axons by immunoblotting. Thus, the axotomy response of these CNS neurons is both similar to (NF) and different from (TUB) the response of PNS neurons to injury.

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331.11 BYPASSING SPINAL CORD LESIONS USING PERIPHERAL NERVE GRAFTS.
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Following spinal cord lesion, voluntary function below the lesion is permanently lost. Many attempts to "bridge" these lesions, spinal cord to spinal cord, have met with limited success. In this pilot study, we have successfully "bypassed" a spinal cord lesion by anastomosing a peripheral nerve above the lesion to a peripheral nerve below the lesion.

In Group A (sham surgery, N-12), the femoral and tibial nerves, innervated by L3-4 and L4-6, respectively, were exposed on one side, but left intact. In three other groups, these two nerves were transected. In Group B (fem-tib BP, N=12), the distal tibial nerve was anastomosed to the proximal femoral nerve by tunnelling it under the adductor muscles. In Group C (tibial reanast., N=12), the tibial nerve was reanastomosed to itself. In Group D (cut tibial, N=12), the nerves were left divided. Periodically, the rats were tested for recovery of lower hind limb motor function using the twitch-tension method (i.e., transcutaneously stimulating the spinal cord and recording the strength of foot flexor contrastion via a transducer attached to the middle toe).

After 4-6 months, motor recovery was 99, 72, 54, and 31% for Groups A-D, respectively ($p<.05$ for all combinations). HRP studies confirmed that innervation to the operated lower hind limb of Group B was higher in the spinal cord (L3-4 level) than in Groups A and C (L4-6) and absent in Group D (except in two animals where some reinnervation occurred because of close approximation of the nerve stumps). Furthermore, after an L4 spinal transection, motor function remained only in the operated side of Group B and was absent in the unoperated side of Group B and both sides of the remaining groups; thereby "bypassing" the spinal lesion to retain function by re-routing the peripheral nerves.

In the hope of some day being able to return useful function to spinal patients via peripheral nerve bypassing, further studies using more proximal peripheral nerves along with nerve grafts for length are necessary.

331.12 FIBRIN GLUE VS BIORESORBABLE TUBING VS CONVENTIONAL ANASTOMOSIS IN PERIPHERAL NERVE REPAIR: A FUNCTIONAL COMPARISON.
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Many techniques are available for peripheral nerve repair, but few have shown clear functional superiority. The results of nerve reanastomosis using fibrin glue have been variable, most likely because of the lack of consistency of the glue. Nerve guide tubes have been used to span gaps; but are they better than conventional repair when length is not a problem?

In 18 adult male rats, the tibial nerves were exposed bilaterally at mid-thigh and, via random assignment, either crushed (CRUSH, N=9) or divided and reanastomosed using: conventional epineural repair with two 10-0 sutures (SUTURE, N=9); a two-component fibrin seal (GLUE, N=9); or a 1 mm internal diameter, 6 mm length, bioresorbable nerve guide tube with a 1-2 mm nerve gap (TUBE, N=9). Recovery of tibial nerve motor function was assessed at weekly intervals by the twitch tension method (i.e., percutaneously and supramaximally stimulating the sciatic nerve and recording the tension developed by the digital flexors via a transducer attached to the middle toe). By transecting only the tibial nerve and leaving the sural and peroneal branches of the sciatic nerve intact, toe chewing was not a problem.

After 28 days post-op, the CRUSH group was at its preoperative function, while the remaining three groups had little motor recovery ($p<.001$). Motor recovery began in these groups at around 35 days post-op. By 56 days post-op, recovery had plateaued. There was no significant difference in the mean motor recovery indices (EXP/CONTR amplitudes X 100%) among the three repair groups: SUTURE 63% (5, S.E.M.), GLUE 60% (5) and TUBE 63% (5). Histological and HRP studies to confirm these results will follow.

In conclusion, although nerve guide tubes and fibrin glue technically may facilitate nerve repair, we presently cannot offer proof that they will improve recovery of motor function over conventional anastomosis alone.

331.13 ANALYSIS OF PROTEINS MODIFIED BY AMINO ACID ADDITION DURING REGENERATION OF THE RAT SCIATIC NERVE.
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Rat sciatic nerves were crushed and allowed to regenerate for 13 days. Nerve segments containing the leading edge of regenerating fibers were isolated, homogenized, centrifuged at 150k xg, and the supernatant was passed through a Sephacryl S-200 column. The void volume fraction was then incubated with a mixture of 3H amino acids (ICN) for 20' at 37° C after which samples were immediately frozen and stored. In one fraction of the stored sample proteins were precipitated with TCA and unincorporated amino acids were removed by successive washes in TCA. The radioactive precipitated product was hydrolyzed in 6N HCl and a portion of the hydrolyzate was applied to an amino acid analyzer. Radioactivity which eluted from the analyzer comigrated with threonine/ serine (5%), proline/glycine (11%), alanine (6%), valine (6%), leucine/isoleucine (30%), phenylalanine (3%), lysine (20%), and arginine (19%) standards. Less than one percent of the total radioactivity was associated with aspartic and glutamic acid, and tyrosine and histidine.

Another portion of the stored sample was applied to a 10% SDS polyacrylamide slab gel. Following electrophoresis, the gel was cut into 1 mm sections and radioactivity was removed from the gel by incubation overnight in H_2O_2. Radioactivity was found associated with molecular weight markers of approximately 88,82,66,53,32 and 17 kD.

A third portion of the stored sample has been applied to an HPLC gel filtration column and labeled product is being separated and collected according to molecular size for further analysis.

These experiments show that specific amino acids are incorporated posttranslationally into proteins of regenerating sciatic nerves and that they label a specific subset of the total nerve proteins. The labeled proteins are similar to those labeled at 6 days post crush suggesting that similar proteins are targets for modification at different points in nerve regeneration. Supported by grants NS19148 and EY06728 from NIH.

331.14 PIEZOELECTRIC GUIDANCE CHANNELS ENHANCE REGENERATION IN THE MOUSE SCIATIC NERVE AFTER AXOTOMY
R. F. Valentini *, P. Aebischer *, P. Dario *, C. Domenici *, V. Guenard *, S.R. Winn *, P. M. Galletti * (SPON: J. T. McIlwain). Artificial Organ Laboratory, Brown University, Providence, RI 02912 and Centro "E. Piaggio", University of Pisa, 56100 Pisa.

Synthetic tubular guidance channels have been used experimentally to support the regeneration of transected peripheral nerves. We have previously reported that the permeability characteristics of the guidance channel play a major role in the regeneration process. Since in vitro neurite outgrowth has been shown to be influenced by electrical activity it is possible that piezoelectric guidance channels, which generate transient electrical charges upon slight mechanical deformation, may enhance peripheral nerve regeneration.

Polyvinylidene fluoride (PVDF), a biocompatible polymer, displays one of the highest piezoelectric activities of any synthetic polymer known. Tubes of PVDF can be rendered piezoelectric by stretching them along their longitudinal axis and then poling them under a high intensity electrical field. The sign of the generated charge can be controlled by the poling procedure. Minute mechanical deformations, such as those induced by natural movements of an experimental animal, lead to the generation of transient electrical charges. The tubes used in this study generated a positive charge on the order of 350 picoCoulomb on their luminal surface for a 1 mm vertical deflection along their longitudinal length. After resecting 4 mm of mouse sciatic nerve, the proximal and distal nerve stumps were secured 4 mm apart within a 6 mm long, 0.85 mm ID channel. Positively poled PVDF channels were compared to unpoled, but stretched PVDF channels. All tubes were cleaned and sterilized identically prior to implantation. Cohorts of 5 animals were implanted for each tube type for periods of 4 and 12 weeks. Upon retrieval, all tubes contained a regenerated nerve cable bridging the proximal and distal nerve stumps. The regenerated cables were round, free from attachment to the inner wall and surrounded by an acellular gel. Transverse sections taken at the midpoint of the guidance channel revealed that nerves regenerated in positively poled channels contained a significantly higher number of myelinated axons at both 4 weeks (2,179 ± 177 versus 1,456 ± 398) and 12 weeks (3,183 ± 224 versus 2,163 ± 210) than those regenerated in unpoled channels.

These results indicate that a guidance channel which generates transient positive charges on its luminal surface significantly promotes peripheral nerve regeneration.

331.15 BLIND-ENDED PERMSELECTIVE GUIDANCE CHANNELS ALLOW PERIPHERAL NERVE REGENERATION IN THE ABSENCE OF A DISTAL NERVE STUMP

P. Aebischer *, V. Guenard *, S.R. Winn *, R.F. Valentini *, P.M. Galletti *
(SPON: K. M. Chapman). Artificial Organ Laboratory, Brown University, Providence, RI 02912

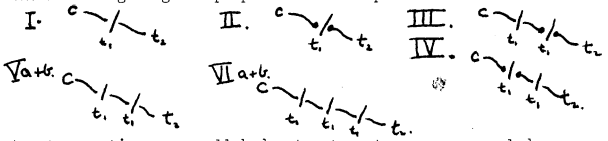
Guidance channels serve as tools for elucidating the cellular and molecular processes underlying peripheral nerve regeneration. The importance of the distal nerve stump in promoting regeneration in synthetic, impermeable tubes has been stressed by several authors. When the distal end of an impermeable channel is plugged and the distal nerve stump avulsed, no regeneration from the proximal nerve stump occurs. We have previously reported that permselective guidance channels support the regeneration of nerves with thinner epineurium, fewer blood vessels and more myelinated axons than impermeable channels. In order to determine whether a channel which allows controlled exchange across its wall will allow regeneration in the absence of a distal stump, the following procedures were performed. In this series of experiments only the proximal stump of a transected mouse sciatic nerve was positioned within a 6 mm long channel; the distal nerve was fully resected and discarded. In some animal the distal channel end was left open and in others it was capped with a polymer plug. Permselective acrylic copolymer (AC) channels were compared to impermeable silicone elastomer (SE) channels. Cohorts of 3 animals were implanted with capped and uncapped AC and SE channels for 8 weeks. All capped AC channels contained large regenerated nerve cables which extended to the distal end of the channel. The nerve cables contained over 1,000 myelinated axons with no significant decrease up to the cap level. Two of 3 uncapped AC channels contained small regenerated cables extending from the proximal stump to the open distal end but contained fewer axons than those regenerated in capped AC channels. The capped SE channels contained only fine threads of connective tissue extending for less than 2 mm and not containing any myelinated axons. Two uncapped SE channels contained small 2-3 mm long tissue cables containing fewer than 100 myelinated axons.

These results suggest that metabolic support and growth factors from the external wound environment and/or the proximal nerve segment are capable of supporting nerve regeneration. The enhanced regeneration observed in capped versus uncapped AC channels may be due to increased maintenance of growth factors within the regenerating environment.

331.16 POSSIBLE SOURCES OF INFLUENCE ON EARLY RECONNECTION OF TRANSECTED RAT SCIATIC NERVE. K. Harman, J.C. de la Torre, Dept. of Anatomy, Univ. of Ottawa Health Sciences, Ottawa K1H 8M5

Successful mammalian peripheral nerve regeneration depends upon a number of factors which have recently been the focus of investigation by many researchers. Neurites have been stimulated to extend by different substrates, exogenously applied substances and electrical stimulation. It has been found that regenerative growth across a nerve gap begins with a fibrin matrix connecting the proximal and distal stumps. Schwann and epineurial cells then migrate in from both stumps forming a cellular bridge, succeeded by axonal extension into the region (Williams et. al., '84). A study was performed to examine possible sources of influence upon the establishment of the acellular (fibrin) bridge after transection of the sciatic nerve of Long-Evans hooded rats.

Using a polyethylene tube to surround the transected nerve, the following surgical preparations were performed:



t_1 = transection c = cell body t_2 = target = sealed

In all preparations, open ends of the catheter were plugged with petroleum jelly. In an attempt to exclude the external environment more completely, another triple transection (VIb) was performed, sealing the open ends with plastic. At nine days, under anaesthesia, the nerves were removed, measured for growth, frozen or fixed for histology, and then the rats were killed.

It was found that the two nerve stumps reconnected with a fibrin matrix, containing a small number of cells. This matrix spanned the gap averaging 3.5 mm. Reconnection was observed in 63%, 78%, 100%, 60% and 0%, of I, Va, Vb, VIa and VIb respectively. When the cell bodies' and/or target's influence is removed, there is still reconnection.

These results suggest that initial proximal/distal reconnection after transection is a locally mediated event.

(Supported by Am. Paralysis Society & Canadian Paraplegic Society).

331.17 REGENERATION OF SPINAL MOTOR AXONS INTO CUTANEOUS SENSORY PATHWAYS. Paul B. Farel. Dept. of Physiology, Univ. of N. Carolina Sch. of Med., Chapel Hill, NC 27514.

In the bullfrog (Rana catesbeiana), axons of spinal motoneurons regenerate to their appropriate hindlimb regions only during the first third of larval life. Regeneration of motor axons is not specific during the balance of larval life or in postmetamorphic frogs (J. Comp. Neurol. 254:125-132, 1986). Although regenerating motor axons in older animals cannot select the pathway leading to the muscle they previously innervated, they nevertheless may be capable of distinguishing between purely sensory (cutaneous) and motor nerve branches. A mechanism that could place regenerating motor axons at a competitive disadvantage in reinnervating purely sensory nerve branches has recently been identified. Taniuchi et al. (PNAS 83:4094-4098, 1986) found that denervated Schwann cells express low-affinity receptors for NGF which, they suggest, may make NGF available for the support and guidance of regenerating axons of NGF-sensitive (e.g., sensory) neurons.

The ability of motoneurons to regenerate their axons into a purely sensory nerve branch was examined in juvenile bullfrogs. In 6 experimental frogs, the three spinal nerves that innervate the hindlimb were transected on one side slightly rostral to the sciatic plexus. After 6-8 weeks recovery, the medial cutaneous femoral nerve (MCFN, which innervates skin of the posterior thigh) was dissected free and prepared for electrical stimulation through suction electrodes. The spinal cord was removed and responses were recorded from the cut distal stumps of dorsal and ventral roots. Stimulation of the MCFN elicited responses in recordings from dorsal roots, but never from ventral roots, in 13 control frogs and on the unoperated side of experimental frogs. However, on the operated side, MCFN stimulation produced ventral root responses in 5 of 6 preparations. These ventral root responses were similar in size to those recorded from dorsal roots, indicating no significant selectivity of the pathway for sensory axons.

HRP was placed on the MCFN in 10 additional frogs (4 unoperated, 6 experimental). No retrogradely labeled motoneurons were found in the spinal cords of unoperated frogs; however, labeled motoneurons were found in the spinal cords of 3 of the 6 previously operated frogs.

These results show that regenerating motor axons are not excluded from purely sensory pathways. Further, the similarity in the size of responses recorded from dorsal and ventral roots in experimental frogs indicates that a mechanism favoring reinnervation of previously sensory pathways by sensory neurons is likely not sufficiently selective to be of significant importance during regeneration.

Supported by NIH grant NS16030 with facilities support by NIH grant NS14899.

331.18 DISTRIBUTION OF ALBUMIN-LIKE IMMUNOREACTIVITY IN RAT SCIATIC NERVE AFTER NERVE CRUSH INJURY. M. Mata, J. Staple*, and D. Fink, Neurology Research Laboratory, University of Michigan and VA Medical Center, Ann Arbor, MI 48105.

In order to better understand the role of local factors in the response of peripheral nerve to crush injury, we studied the distribution of albumin-like immunoreactivity (A-LI) in the rat sciatic nerve from 1 day to 8 weeks after a crush using electron microscopic immunocytochemistry.

Male Sprague Dawley rats 200-250 grams were anesthetized with choral hydrate, the sciatic nerve exposed in the gluteal region, and crushed 1 min with a jewelers forceps. At 1 day, 1 week, 2 weeks, 4 weeks or 8 weeks after injury, the animals were sacrificed by perfusion through the heart with 75-100 mM phosphate buffer, followed by 0.5% glutaraldehyde and 4% paraformaldehyde. The sciatic nerve was removed and embedded in LR white. Ultrathin sections were exposed to goat anti-rat serum albumin antibody at dilutions of 1:1500 or 1:2000 for 1.5-2 hrs, followed by rabbit anti-goat IgG bound to 15 nm colloidal gold (1:10) for 1 hr and then stained with uranyl acetate. The specificity of the anti-RSA antibody was tested by immunodiffusion; it precipitated RSA but not bovine serum albumin, ovalbumin or phosphorylase A. Controls for the immunohistochemical reaction included deleting the anti-RSA antibody and blocking the anti-RSA antibody with RSA.

In the nerve distal to the crush degenerating areas demonstrated intraaxonal A-LI early. These axonal contents subsequently disappeared. By 1 week most of the Schwann cells in the distal nerve also demonstrated A-LI. As regenerating sprouts entered the distal nerve, those Schwann cells in contact with sprouts lost their A-LI, while those cells not in contact with axons retained immunoreactivity up to 8 weeks after injury.

Proximal to the nerve crush many axons showed intra-axonal A-LI from 1 to 2 weeks after injury, despite appearing normal ultrastructurally. This immunoreactivity diminished as the distance from the crush site increased. Many Schwann cells proximal to the crush also showed A-LI from 1 to 4 weeks after injury, some more proximal than the most proximal distribution of axonal A-LI.

Recent studies have emphasized the role of the microenvironment at the tip of regenerating axons in determining the success or failure of regeneration, and the complex interaction of regenerating axons with Schwann cells in controlling Schwann cell protein synthesis and secretion. The results of this study describe a previously unknown aspect of this interaction which may be important in the process of peripheral nerve regeneration.

Supported by VA Merit Review Grants to Dr. Mata and Dr. Fink.

331.19 **INSULINLIKE GROWTH FACTOR-II GENE EXPRESSION IN MUSCLE: RELATIONSHIP TO SYNAPSE ELIMINATION AND NERVE REGENERATION.** D. N. Ishii. Physiology Department, Colorado State University, Fort Collins, CO 80523.

During development individual immature muscle fibers become innervated by axons from several motor neurons (Redfern, J. Physiol. 209: 701, 1970). Superfluous synapses are later eliminated so that a mature muscle fiber retains innervation from only a single motor neuron. Denervated and immature muscles share a biochemical "state" receptive to innervation, whereas mature muscle is unreceptive. The potential involvement of insulinlike growth factor-II (IGF-II) is suggested, in part because it can induce neurites and support neuron survival, and IGF-II mRNA is present in muscle and the CNS (discussed in Recio-Pinto et al, J. Neurosci. 6: 1211, 1986; Ishii and Mill, Curr. Topics Membrane Transport, in press, 1987. Is the level of IGF-II gene expression correlated with synapse elimination and the muscle state receptive to innervation? This hypothesis was tested. The expression of the IGF-II gene was studied in calf muscles from rat littermates. Purified RNA was analyzed by the Northern blot method using rat cDNA clone 27, which contains the IGF-II coding region (Soares et al., J. Mol. Biol. 192: 737, 1986). Transcript levels were high in muscles from pre and early postnatal rats. A profound decline in transcript abundance began in the second week of life and followed exactly the time course for the elimination of multiple synapses in muscle (Brown et al., J. Physiol. 261: 387, 1976). Transcript levels were very low in rats older than 15-days. Following unilateral transection of the sciatic nerve, IGF-II mRNA levels transiently continued the developmental decline for 2-4 days, then became elevated in muscles from the operated but not the unoperated side. Sham operation was without effect. Following bilateral transection, transcripts were elevated in muscles from both sides in 24-day-old rats (10 days postoperative). The differences had a high level of significance: 6.84 $\pm$ 3.04, N = 7 intact muscles; 14.65 $\pm$ 3.83, N = 9 denervated muscles (means $\pm$ S.D., relative areas under the curve of the densitometric scans from the autoradiograms of Northern blots). These results suggest a) the abundant IGF-II transcripts in immature muscles may predispose to polyinnervation, b) the decline in transcript abundance may modulate the elimination of superfluous synapses, c) IGF-II gene expression in muscle is regulated by innervation, and d) the up regulation of IGF-II gene activity in denervated muscle may contribute to nerve regeneration. (Supported in part by grants ROl NS 24787 from the NINCDS and ROlNS 24327 from the NIDDKD).

331.20 SENSITIVITY OF REGENERATED SCIATIC NERVE TO 4-AMINOPYRIDINE AND TETRAETHYLAMMONIUM FOLLOWING INJURY AT BIRTH AND LATE MATURATION. C.M.Bowe AND C.H.Yu* Dept. of Pediatrics and Section of Neurobiology, Brown University, Providence, RI 02912

Morphological and physiological properties of regenerated mammalian nerves differ following lesions at various postnatal ages. The enhanced sensitivity to potassium channel blockade by 4-AP reported for regenerated nerves following injury during adulthood is less evident in nerves lesioned between 2-3 weeks of age (Bowe, Devel Brain Res, in press) suggesting that ongoing maturational processes influence regeneration. The recent identification of the presence of a second, pharmacologically distinct tetraethylammonium (TEA)-sensitive potassium channel on immature mammalian axons prompted this examination of the physiological properties of regenerated rat sciatic nerve in the presence of 4-AP and TEA.

Sciatic crush lesions were performed on 1 day and 10 week old rats. Following a recovery period of 9-16 months, contralateral control and regenerated nerves were excised and studied in vitro during superfusion with a physiological solution (NS) and NS containing 4-AP (.5-1.0 mM), TEA (2.5-10 mM), or 4-AP+TEA. Compound action potential (CAP) waveform characteristics, recovery cycle properties, and ability to follow trains of stimuli at fast frequencies were compared for control and the two lesion-age groups. Control and regenerated nerve recordings were comparable during superfusion with NS with the exception of prolonged latency to peak amplitude noted for both groups of regenerated nerves. Minimal changes were observed for control nerves during 4-AP, but application of TEA resulted in an appreciable CAP amplitude decrement and delayed recovery of conditioned CAP amplitude during paired stimulation. More marked sensitivity to 4-AP was observed for all regenerated nerves associated with the development of a prominent, delayed negativity and prolongation of recovery cycles during paired stimulation. Distinctive CAP waveform alterations, previously described following adult lesions, were noted only for the older lesion-age group. Application of TEA or TEA+4-AP to regenerated nerves resulted in significant amplitude reduction and loss of 4-AP effects on waveform. Recovery cycles were prolonged for all regenerated nerves with most marked delays observed in nerves from the younger lesion-age group.

Physiological effects of both 4-AP and TEA differ for normal and regenerated nerves. Furthermore, the relative magnitude of sensitivity to 4-AP and TEA varies for regenerated nerves following lesions at different maturational ages.

331.21 MOTOR NERVE TERMINALS CAN PRODUCE SYNAPTIC ACETYLCHOLINESTERASE AT NEUROMUSCULAR JUNCTIONS. L. Anglister. Dept. of Anatomy and Embryology, Hebrew Univ.-Hadassah Med. Sch., Jerusalem, Israel.

Acetylcholinesterase (AChE) in skeletal muscle is concentrated at neuromuscular junctions where it is found in the synaptic cleft between muscle and nerve. This raises the question of whether the synaptic enzyme is produced by muscle, nerve or both. Studies on denervated and regenerating muscles show that myofibers can produce synaptic AChE (Anglister & McMahan, J. Cell Biol. 101:735, 1985). The motor nerve may play an indirect role: By inducing activity in the myofibers it triggers them to produce synaptic AChE (Weinberg & Hall, Dev. Biol. 68:631, 1978). The aim of the following study was to determine whether or not some of the AChE that is made and transported by the motor nerve contributes directly to synaptic cleft enzyme.

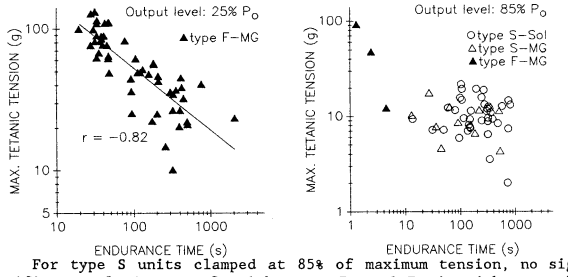
The experiments were carried out on operated cutaneus pectoris (CP) muscles of frog in which muscle fibers had been removed from the basal lamina sheaths, while leaving intact motor axons, nerve terminals and synaptic basal lamina sheaths. In these preparations nerve terminals persist at almost all the synaptic sites on the sheaths for a year in the absence of myofibers and show no discernible differences in structural features from normal (Yao & McMahan, Soc. Neurosci. Abs. 10:1085, 1984). At the time of operation muscles were exposed to DFP or MSF, which block irreversibly and inactivate all AChE activity detected by either histochemical or biochemical assays. Five weeks later the preparations were examined for the appearance of newly formed AChE. Histochemical staining revealed an arborized pattern, characteristic of neuromuscular junctions, which co-localized with the staining for nerve terminals. AChE reaction product was concentrated adjacent to the nerve terminals, obscuring synaptic basal lamina. No staining appeared at denervated sites. Since all original AChE had been inactivated and myofibers had been removed, the newly formed AChE at synaptic sites must have been produced by the persisting axon terminals. These results demonstrate that motor nerves are capable of producing some of the synaptic AChE at the neuromuscular junctions.

(Sponsored by grants from the Charles H. Revson Foundation and from Israel-US Binational Science Foundation).

332.1 ENDURANCE OF SLOW-TWITCH MOTOR UNITS AT SUB-MAXIMAL CONTRACTIONS OF CONSTANT TENSION. C. B. Webb*, T. C. Cope and B. R. Botterman. Dept. of Cell Biology and Anatomy, Univ. Texas Health Sci. Ctr., Dallas, TX 75235.

Recent work from our laboratory has shown that among type F motor units there is a significant relationship between maximum tetanic tension (P_o) and the length of time (endurance time, E_t) that a unit can maintain tension at 25% of maximum (left panel, below). Whether a similar relationship exists for type S units is not known. For the few type S units studied at this level (n=5), the pattern of activation indicated that the contraction would be maintained well in excess of our arbitrary limit of 3000s. Because of the impracticality of studying type S units for this length of time, we chose instead to test for the relationship at a higher output level.

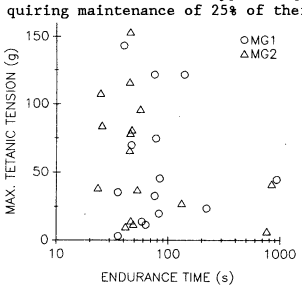
In 6 cats, motor axons to either soleus (SOL; n=38) or medial gastrocnemius (MG; n=13) muscles were isolated from ventral root filaments. Motor-unit tension was 'clamped' at 85% of maximum by altering the activation rate of a unit's motor axon through computer feedback control.



For type S units clamped at 85% of maximum tension, no significant relation was found between P_o and E_t in either muscle (right panel, above). This was true despite the fact that the absolute range for E_t was comparable to that found for type F units at 25% of maximum tension. For both type F and S groups, the relative range for P_o was similar as well (~10-fold). The lack of a relation for type S units may simply be due to the higher output level studied. Alternatively, there may be fundamental differences between type S and F units with regard to the P_o-E_t relation. In the latter case, a recruitment scheme based on tension would result in a random activation of units with respect to endurance. (NIH grants NS17683 to B.R.B and NS21023 to T.C.C.)

332.2 TENSION AND ENDURANCE ARE UNCOUPLED FOR MOTOR UNITS IN SELF REINNERVATED MUSCLE T.C. Cope, C.B. Webb* and B.R. Botterman Dept. of Cell Biology and Anatomy, Univ. of Texas Hlth. Sci. Ctr., Dallas, TX 75235.

Fast-twitch motor units in reinnervated muscle regain their normal relative differences in both tension and fatigue when grouped by type. Our recent finding that tension and endurance are precisely ranked in normal muscles enabled us to determine the recoverability of this relationship after reinnervation irrespective of unit type. Isometric contractile properties of motor units in self-reinnervated medial gastrocnemius (MG) muscles were studied more than 2 years after nerve-section in 2 adult cats. Single motor axons were functionally isolated and electrically stimulated in ventral root filaments for characterization of each unit's type and performance under conditions requiring maintenance of 25% of their maximum tension (P_o) achieved by modulating motor axon stimulation rate through computer feedback control. Under this condition, fast-twitch units in normal muscles, including MG, have a continuous inverse relation between P_o and the duration that the sustained contraction can be maintained, defined as endurance time (E_t; see Webb, Cope and Botterman in this volume).



This figure plots P_o vs. log E_t for 30 reconstituted type F units in reinnervated MG muscles. The range in E_t for these units (24-937s) overlapped with that for essentially all normal type F MG units (n=52; 20-767s), meaning that it can recover in reinnervated muscle. With regard to P_o, we confirm other studies in showing that approximately normal values were recovered, although the range was greater than normal. In face of the recovery of these parameters, the figure shows that for neither pooled nor individual samples were P_o and E_t related after reinnervation, as they are normally. Expressed quantitatively, Spearman rank order correlation coefficients for P_o vs. E_t were 0.13 and -0.34 for MG1 and MG2, respectively. These values were not significant ($p>0.1$) and were less than half those found for type F units sampled in normal MG muscles (smallest value -0.83). We conclude that two important functional properties of motor units, namely tension and endurance, cannot act synergistically after self-reinnervation. (Supported by NIH grants NS21023 to TCC and NS17683 to BRB)

332.3 FORCE HYSTERESIS OF SINGLE MOTOR UNITS DURING FREQUENCY-VARYING STIMULATION S.A. Binder-Macleod and H.P. Clamann. Dept. of Physiology, Medical College of Virginia, Richmond, VA 23298.

The force output of single motor units in cat soleus muscle was studied in response to stimulus trains of continuously varying frequency. Although a sigmoid relation between frequency and force is well known in whole muscles and single motor units, this relationship is determined during stimulus trains of constant frequency and fails to explain a number of features of rate coding.

Single soleus motor units were isolated in cats deeply anesthetized with sodium pentobarbital. Each unit was stimulated with a continuous train whose frequency varied linearly from 0 to 20% above the unit's fusion frequency and back to 0. The period of this cycle was 1, 2.5, 5, 10 or 20 seconds, and was repeated 2 to 5 times.

As we previously reported for medial gastroc-nemius (MG) motor units (Clamann and Binder-Macleod, Neuroscience Abstracts, 1985) a marked hysteresis was seen in all motor units during frequency-varying stimulation. That is, a motor unit produced more force at any given stimulus frequency if frequency was decreasing than if frequency was increasing. The MG data suggested that motor unit slowing and potentiation could be contributing to the hysteresis. A time delay in force production and a catch-like property may also be contributing. The contribution of these last two factors should depend on the rate of change of frequency (cycle period).

Because soleus units showed no potentiation or contractile slowing during stimulation, these factors could not contribute to the hysteresis seen here. The use of longer cycle periods did decrease the hysteresis. Therefore, the soleus unit hysteresis appear to depend on the rate of change of frequency. Mathematical modelling suggests that a time delay in force production and a catch-like property may contribute to the hysteresis.

The force-frequency relation during decreasing frequency was quite stable. In voluntary or reflex movement, CNS control of force output can be made predictable by the use of a discharge pattern in which frequency is initially high and then decreases. Such a discharge pattern is typical of many movements in intact animals and man.

332.4 FIRING RATE CHANGES STUDIED USING CLOSED-LOOP CONTROL OF ISOLATED SINGLE MOTOR UNITS IN RAT EDL. G.P. Rovai* and T.G. Sandercock*. (SPON: E. Polley). Bioengineering Dept., Univ. of Ill. at Chicago, Chicago, IL 60680.

Research on human adductor pollicis muscle has shown that when a subject sustains a maximal voluntary contraction a fall in muscle tension is accompanied by a fall in motor unit firing frequency (Bigland-Ritchie, et al., J. Physiol. London. 340:335-346,1983). Artificial stimulation of the muscle at higher frequencies fails to produce more tension. It has been hypothesized that as a motor unit fatigues its contractile properties change so full tension can be achieved with lower firing rates. Because of the difficulty in recording motor unit contractile properties in humans we chose to study isolated motor units in the extensor digitorum longus(EDL) muscle of rats. The goal of the study was to test the hypothesis that during a prolonged contraction of rat EDL motor units there is a decline in the stimulating frequency needed to maintain maximum tetanic tension(Po).

Rats were anesthetized with sodium pentobarbital. The hindlimb was mounted in a rigid frame, and the EDL tendon was cut and attached to a force transducer. Single motor units were isolated and stimulated through the ventral roots. Two computer-controlled stimulus paradigms were developed to maintain tension at a constant percentage of Po by modulating the stimulation frequency. The paradigms are described:

1) Tension control. Tension was used as the feedback variable and the stimulating frequency was adjusted to maintain tension at the desired setpoint. We used a setpoint of 80% of Po. To compensate for muscle fatigue, Po was periodically remeasured, and the setpoint was adjusted accordingly.

2) Ripple control. This control strategy used a percentage of ripple for the setpoint. Ripple is defined as the amount of fluctuation in the unfused portion of tetanus. The stimulation frequency was modulated to maintain ripple at 5% of the average tension. Preliminary experiments showed that in rat EDL this control paradigm kept tension above 80% of Po without the need to remeasure Po.

Both paradigms showed that even though Po fell over time, Po could be achieved using a lower activation rate. Most of the change in the firing rate was observed in the first 20 seconds of the experiment. Results from 10 motor units studied with the tension control paradigm showed the average stimulation frequency decreased by 20.4 Hz during the first 20 seconds. An average decrease of 14.9 Hz in 20 seconds was determined from 6 motor units studied with the ripple control strategy.

332.5 THE DEPENDENCE OF THE EMG-FORCE RELATIONSHIPS ON THE MUSCLES CONTROL STRATEGY AND PREDOMINANT FIBER TYPE. M. Solomonow, R. Baratta*, B. Zhou*, H. Shoji* and R. D'Ambrosia*. Bioengineering Laboratory, Louisiana State University Medical Center, New Orleans, LA 70112.

The impact of various action potentials firing rate and motor unit recruitment control strategies on the EMG-Force relationships of a predominant fast (m. gastrocnemius) and slow (soleus) twitch muscles was determined with a recently developed electrical nerve stimulation system (Zhou, B. et al, IEEE trans. BME, 34: 128-139, 1987). The system consisted of dual stimuli delivered to the muscle nerve of nine adult cats via two electrodes. The first electrode induced action potential of increasing rate while the second electrode allowed motor units to be concurrently recruited according to their size. The simultaneous stimuli could be calibrated to recruit all the motor units any time during the firing rate increase phase such that control strategies similar to that known to exist in various skeletal muscles could be duplicated.

It was shown, for the soleus muscle, that strategies which recruited all the motor units by the time 30% or 40% of the maximal muscle force was reached and firing rate complimented the generation of the final force segment, the EMG-Force relationships were linear. Progressive increase in non-linearity was evident as total recruitment was allowed to occur from 50% and up to 70% of the maximal force. No statistically significant difference ($p < .05$) was observed in the relationships for strategies employing recruitment ranges from 70% to 100% MVC.

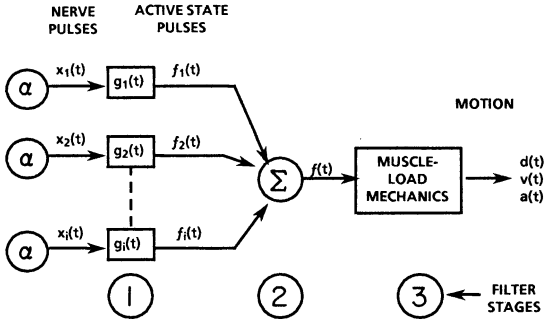
Linear relationships were evident in the m. gastroc for a strategy of 50% recruitment range, with progressive increase in non-linearity for strategies of 60% to 80% recruitment range.

It was concluded that the EMG-Force relationships are strongly dependent on the control strategy employed by the muscle as well as on its motor unit architecture. The fact that a specific muscle responded physiologically to a wide range of control strategies is importantly noted.

332.6 THREE STAGES OF LOW-PASS FILTERING BY A MUSCLE-LOAD SYSTEM. R. N. Stiles, Department of Physiology and Biophysics, University of Tennessee, Memphis, Memphis, TN 38163.

Low-pass filtering is a major step in frequency demodulation of pulse trains (Bayly, *IEEE Trans. BME*: 15, 1968). Also, motor unit (MU) fibers of skeletal muscle are normally stimulated by frequency-modulated pulse trains, and a muscle-load system behaves as a low-pass filter. How the total low-pass filter effect is divided between the active state process and muscle-load mechanics is unclear. Also unclear is the *in situ* contribution of a third stage of low-pass filtering, spatial summation of asynchronous MU pulses. Of particular interest is the possibility that the cut-off frequency of each filter is under CNS control. These three stages are illustrated below.

The frequency response of a muscle-load system (treated as a single MU) can be obtained using a constant low-rate (2-4 pps), constant-amplitude pulse-train stimulus. In this study, frequency response plots were obtained for hamster (and rat) gastrocnemius-plantaris lever-preload systems, using both an accelerometer to detect load kinematics and spectral analysis of the acceleration record. Mechanical measurements from these same systems indicate that the third-order low-pass behavior of a typical frequency response could be attributed primarily to muscle-load mechanics. These results suggest that the equivalent width (treated as a rectangular pulse) of the active state pulse was less than about 10 ms. This value compares well with certain estimates of the duration of the active state pulse.



332.7 **PHYSIOLOGICAL AND HISTOCHEMICAL CHARACTERIZATION OF THE RECTUS FEMORIS MUSCLE IN THE CAT.** B.G. Samojla, P.J. Rebeta*, L.L. Glenn. *Department of Physiology, Ohio College of Podiatric Medicine, Cleveland, Ohio, 44106-3082*

Although neuromuscular compartments have been identified in various muscles of the hindlimb of the cat, none have been described in the quadriceps. It was the purpose of this study to identify and characterize (physiologically and histochemically) the muscle compartments that comprise the rectus femoris m. (RF) in the cat.

Innervation patterns, fiber angles, lengths, and volumes were determined from fixed animals. It can be difficult to define a region within a muscle using glycogen depletion and PAS stain, so a modified method to enhance the contrast between depleted and non-depleted regions was used. Nicotinic acid (NA) was injected i.v., followed by stimulation of first order nerve branches at 40 Hz/333 ms/second at 15 minutes; epinepherine was injected i.v. followed by an additional 5 minutes of stimulation. The muscle was excised and frozen in isobutane cooled by liquid nitrogen. Every 3 mm, a 10 μm section was taken and stained for glycogen with PAS. Fiber types were determined on additional sections stained for ATPase (acid and alkaline) and NADH-TR. Contraction properties were studied using standard procedures that were uniform for each compartment identified by dissection and glycogen depletion.

RF is a bipennate muscle which is innervated by three, occasionally four, primary nerve branches from the femoral nerve; however, only three compartments are consistent in their morphology. The proximal half of RF is divided into a lateral (PL) and a medial (PM) compartment, and the distal (D) half is itself a single compartment. The percentages of muscle fibers (SO : FOG : FI : FG) within the PL compartment was 3.9 : 39.1 : 2.0 : 49.8; PM was 7.4 : 32.7 : 1.1 : 53.2; D was 28.2 : 31.4 : 4.3 : 31.9. Glycogen depleted muscles using NA, when compared to control slides, showed identical staining patterns and intensities for ATPase and NADH-TR. The fiber angles (22.6°) and lengths (15.5 mm) were constant throughout the muscle. The physiological cross-sections follow: whole muscle, 5.4 cm^2; compartments PL and PM, 2.7 cm^2; D 5.0 cm^2. The maximum tetanic tensions were 33.9 N (PM), 31.1 N (PL), and 89.2 N (D). Summation coefficients (SC) were 0.283 (PM), 0.199 (PL), 0.313 (D). Specific tensions were 11.5 N/cm2 (PL), 12.6 N/cm2 (PM), 17.8 N/cm2 (D).

RF is composed of three compartments defined by innervation patterns and glycogen depletion. The two proximal compartments are similar in all respects, appearing like mirror images with respect to the central RF tendon. The distal compartment differs from the proximal by having a larger cross sectional area, MTT, specific tension, and more even proportion of muscle fiber types. These proximal-to-distal differences can be associated with the function of RF during locomotion. The proximal compartments may be the major contributor to the muscles ability to flex the hip. While, the distal compartment by virtue of the larger cross sectional area and may be responsible for tension production during deceleration and antigravity functions. [Supported by Ohio Board of Regents Research Challenge Program]

332.8 **MUSCLE FIBER LENGTHS WITHIN SINGLE MOTOR UNITS IN THE CAT TIBIALIS ANTERIOR MUSCLES.** **M. Qunjian, R.R. Roy, E. Eldred, and V.R. Edgerton. Dept. of Kinesiology and Brain Research Institute, UCLA, Los Angeles, CA 90024.**

The length of a muscle fiber, the number of sarcomeres arranged in series, relates closely to the maximum shortening velocity potential of a fiber. Based on whole muscle acid digestion and maceration techniques for determining muscle fiber lengths, there appears to be conflicting results regarding the range in lengths of fibers in cat skeletal muscles. In this study, the lengths of fibers belonging to a single motor unit (MU) and located within a single fascicle were measured in 4 tibialis anterior muscles of adult cats. To identify fibers of a MU, ventral root filaments were split until a single MU response was obtained. After having measured routine contractile properties, the muscle fibers were depleted of glycogen by repetitive stimulation (*e.g.*, 75 Hz trains each lasting ~ 100 ms and each train repeated at 5 Hz) of the split ventral root. A periodic acid-Schiff (PAS) reaction was used to stain for glycogen. In order to maintain the physiological optimal muscle length during quick freezing, the whole muscle was secured at its physiological resting length. The frozen muscle was cut into ~ 18-20 blocks of 4.0-8.0 mm segments. Every block of each of the 4 muscles was sectioned serially into 20 μm slices. Approximately 10-20 fibers within a fascicle that were depleted of glycogen were traced throughout their lengths by examining each 20 μm thick section. The results from these 4 units are summarized in the following table:

MOTOR UNIT NUMBER	1	2	3	4
Physiological Type	Fast Fatigable	Fast Intermediate	Fast Fatigable	Fast Fatigue Resistant
ATPase Type	Fast	Fast	Fast	Fast
Contraction Time (ms)	22	20	25	21
Tetanic Tension (g)	21.6	16.0	40.3	10.1
Mean Fiber Length (mm)	8.86	19.83	48.04	36.07
SD	± 0.08	± 3.51	± 7.31	± 8.42
Number of Fibers	12	20	11	8
Muscle + Tendon Lengths (mm)	N/A	106	114	125
Fiber Length: Muscle Length (%)	N/A	19	42	29
Location of MU in Muscle (% of Muscle Length)	0-10	0-30	20-70	50-80
No. of Fibers Tapering				
Proximal End of Fibers	0	0	9	3
Distal End of Fibers	0	0	4	3
Body Weight (kg)	2.49	3.60	4.50	3.20

These results illustrate that fiber lengths can vary widely from one MU to another. Further, it appears that the fiber lengths of a MU within the same fascicle are similar. In one case, one fiber (MU No. 4) appeared to divide into two fibers. This fiber's total length was 22.8 mm and appeared to split 7.63 mm from its most proximal end. The cross-sectional area of a number of fibers changed progressively towards their ends (tapered). No tapering occurred in the proximal ends of fibers of MUs 1 and 2 which were located in the most proximal part of the muscle. However, some tapering was apparent in some or all of the fibers of each of the 3 units located more distally in the muscle. This progressive tapering usually occurred over a distance of about 2 mm.

This work was supported by NIH Grant 16333.

332.9 CHRONIC IMMOBILIZATION-INDUCED CHANGES IN CAT MUSCLE-UNIT POPULATIONS. G.A. Robinson, R.M. Enoka, C.R. Kirby* and D.G. Stuart. Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85724.

The properties of individual tibialis posterior muscle units were evaluated after a 3-week period of hindlimb immobilization in 6 adult male cats (3.2-4.5 kg). All cats were conditioned for 3 weeks prior to immobilization in a large communal environment. Splinting the right hindlimb (with the ankle extended and the knee flexed) reduced the mobility of the limb and produced an average decrease of 19% in test-muscle weights (n = 5) relative to contralateral muscles. Muscle units were characterized into one of four types (FF, FI, FR, or S) based on conduction velocity (CV), twitch contraction time (CT), a progressive fatigue index (PFI), tetanic force (Po), and a test for sag as described previously for normal, tibialis posterior muscle units (McDonagh et al., J. Neurophysiol., 44: 696, 1980). Immobilization results were then compared to these normal data. In general, both nonfatigable (FR + S) and fatigable (FF + FI) units were altered by immobilization. Specifically, type FR and S units had PFI values significantly greater than those found in normals, whereas values for Po and CT were unchanged. Fatigable units exhibited a trend for slower CVs than in normals, which was significant for type FI units. The population distribution of different unit types was also changed from normal. The population of fatigable units was reduced by one half whereas the proportion of nonfatigable units remained unchanged. Furthermore, of the 102 units studied, 21 could not be classified into one of the four types. These units had CVs consistent with other fast units but produced tetanic forces below the resolution of the measurement system (0.5 mN). The absence of such units in the sampling of normal muscle, combined with the reduced populations of type FF and FI units after immobilization, suggests that these altered units (AU) may represent type FF and FI units which have undergone preferential and severe atrophy.

MUSCLE-UNIT POPULATIONS

Type	% Distribution	
	Normal	Immobilized
FF	30.1	14.7*
FI	8.7	3.9
FR	19.4	29.4
S	41.8	31.4
AU	---	20.6*

*p < 0.05

Supported by USPHS grants NS 20544 and HL 07249.

332.10 MUSCLE FIBRES SHORTEN WHEN THE WHOLE MUSCLE IS BEING STRETCHED IN THE 'YIELD' PHASE OF THE FREELY WALKING CAT. R.I. Griffiths* and J.A. Hoffer. (SPON: F.T. Hambrecht), Depts. Medical Physiology and Clinical Neurosciences, Univ. of Calgary, Calgary, Alberta, T2N 4N1, Canada.

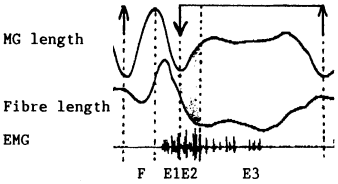
During the E2 phase of the step cycle, the ankle extensor muscles are stretched by the weight of the animal. By measuring force and length changes in the medial head of gastrocnemius muscle (MG) in the wallaby, and by subtracting expected length changes in the tendon (calculated from isolated tendon measurements), Griffiths (Ph.D. thesis 1984) predicted that the muscle fibres would actually shorten during this period.

In the cat MG the tendon is about five times longer than the muscle fibres, and this represents a significant series elasticity. Using the ultrasound transit-time technique, Griffiths (J. Neurosci. Meth. in press) measured 25% shortening of the muscle fibres in 'isometric' tetanic contractions. This occurred due to stretch of the compliant tendon.

MG muscle fibre length has now been measured directly in the conscious freely walking cat (See Figure). MG EMG begins prior to footfall (↓) and increases during the E1 phase of the step cycle. It results in shortening of the muscle fibres, and since there is no load during E1, the whole muscle also shortens. When the foot is placed on the ground (E2 phase), **the whole muscle is stretched, but the active muscle fibres continue to shorten.**

The lengthening of the whole muscle is due to the compliance of the tendon, which is stretched from both ends, and to any decrease occurring in the pennation angle of the muscle fibres. This compliance may act as a shock absorber to prevent sudden, possibly damaging jerks to the muscle fibres. The tendon stretch will contribute to the storage of elastic energy and will also provide for a greater range of movement in the muscle than would be available from the muscle fibres alone.

Since whole muscle length and muscle fibre (and therefore muscle spindle) length are not always correlated, spindle discharge patterns must be analized with respect to the muscle fibre length rather than whole muscle length. For example, the lack of stretch of the muscle fibres during E2 implies that previously observed increases in muscle spindle firing must be controlled by fusimotor activation and to a greater extent than was previously thought (eg. Prochazka et al., 1976, J. Neurophysiol. 39: 1090).



Funded by the Muscular Dystrophy Association of Canada and the Alberta Heritage Foundation for Medical Research.

332.11 QUADRICEPS CONTRACTILITY CHANGES WITH BRIEF MAXIMAL EFFORTS. A.V. Sirin and A.E. Patla, Neural Control Lab, Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, N2L 3G1.

This study was conducted to determine the effect of maximal voluntary contraction (MVC) on the contractility of individual as well as the overall quadriceps muscle group. The subjects (n=11, 10 female, 1 male) were seated on the experimental bench with the right leg in full extension and tightly coupled with the force transducer. Copper plate electrodes 2 cm in diameter were used for stimulating vastus lateralis (VL), rectus femoris (RF), and vastus medialis (VM). Furthermore, large plate electrodes were placed on the quadriceps group (Q) just below the hip joint (anode), and just above the knee joint (cathode) for whole muscle group stimulation. The stimulation equipment consisted of Grass Instruments S44 stimulator, isolation unit, and constant current unit. Square wave pulses of 5 msec duration were used for cutaneous stimulation. The voltage was adjusted such that the stimulus was as high as possible without the excitation spreading into adjacent muscles. This was verified through manual palpation. This was further confirmed by viewing the distinct twitch profile for each muscle on the oscilloscope prior to testing. The stimulus intensity was held constant throughout the experiment.

Individual muscle and whole muscle group submaximal stimulations were done prior to and immediately following a ten second MVC. Force records were converted to digital format (sampling rate 100 Hz) and stored onto floppy disks for later analysis.

Table 1: Peak Twitch Torque (N.m)

	Pre	Post	P
VL	1.2 (± .5)	1.6 (± .7)	< .01
RF	1.7 (± .7)	1.8 (± .7)	NS
VM	2.2 (± 1.1)	2.8 (± 1.8)	< .05
Q	10.9 (± 1.9)	13.3 (± 3.0)	< .005

These peak twitch results indicate that although the quadriceps group as a whole exhibits a 22% potentiation in the peak twitch torque, the contribution to this potentiation appears to be from the vastii muscles and not from the rectus femoris. Twitch potentiation is highly correlated with increased phophorylation of myosin light chains in type II fibres. In fact three types of phosphorylated light chains were reported in the VL which increased their phosphorylation level following a similar protocol (Stuart D. Msc. thesis, University of Waterloo, 1986). The fibre type of the VL is generally used to estimate the fibre type of the quadriceps group. However, the current results indirectly indicate that this may not be appropriate since RF does not appear to undergo potentiation and therefore may be much lower in type II content than either VL or VM. Supported by NSERC grant (A#0070).

332.12 MOTOR UNIT FIRING RATE CHANGES DURING FATIGUE INDUCED BY SUBMAXIMAL, INTERMITTENT, ISOMETRIC, VOLUNTARY CONTRACTIONS OF QUADRICEPS. B. Bigland-Ritchie, F. Furbush*, D. Karrmann*, J. Woods* and C. Thomas, J.B. Pierce Foundation Lab. & Quinnipiac College, New Haven, CT 06519.

Motor unit discharge rates were examined in the quadriceps muscle using an intermittent, submaximal voluntary contraction protocol described previously (Bigland-Ritchie, B. et al., J. Appl. Physiol. 61: 421, 1986). It involved repeated 6s contractions at 50% of the maximum voluntary contraction (MVC) force followed by 4s rest until the maximum force generating capacity had declined by 50%. At this point in time (Tlim), the original target force was now the best force the subject could generate. During all of the target force contractions up to and at Tlim, discharge rates were recorded from single muscle fibers using tungsten microelectrodes. They were compared with those recorded during brief non-fatigued MVCs and target force contractions. Changes in force generating capacity were assessed using a "fatigue" test interjected every minute during the contraction protocol. It included both tetanic and single shock stimulation and an MVC during the last three seconds of a target force contraction.

The time taken for a 50% MVC force loss (Tlim) averaged 7.7 ± 2.1 (SE) min. During this time, the decline in voluntary force was paralleled by a proportional decline in the response to tetanic muscle stimulation. Initially, the mean target force firing rates were approximately 50% of the mean MVC firing rates. At Tlim, MVC firing rates had declined by 30% on average while those recorded during target force contractions had increased so that they were not significantly different from fatigued MVC rates. Surface integrated EMG (IEMG) activity followed similar trends (i.e. target force contraction IEMG increased to match the maximal IEMG). These results suggest that a decline in mean maximal motor unit firing rates is apparent whether fatigue is induced by intermittent, submaximal contractions or by sustained, maximal efforts as reported in our earlier work. However, a decrease in contractile speed remains to be documented for this intermittent form of fatiguing exercise. This work was supported by NIH grants NS 14756 and HL 30026.

332.13 CORRELATION ANALYSIS OF MULTIUNIT MOTOR UNIT TRAINS TO REVEAL COMMON INPUTS TO DIFFERENT MOTONEURON POOLS IN MAN. RK Powers, S Vanden Noven, D Bourbonnais, J Gemperline* and WZ Rymer. Dept. Physiol., Northwestern Univ. Med. Sch. and Sensory-Motor Performance Prog., Rehabilitation Inst. of Chicago, Chicago, IL 60611.

In order to assess the prevalence of common inputs to different motoneuron pools, we are investigating synchrony in the discharge of paired groups of motoneurons (Sears and Stagg, 1976, J.Physiol. 263:357-381), based on multiunit motor unit records from different upper limb muscles. Short-term synchronization of the discharge from different motoneurons, manifested as a narrow, central peak in the crosscorrelation histogram, has been attributed to the joint arrival of unitary EPSPs from branches of the same presynaptic fiber (Sears and Stagg, 1976). Such synchronization has been found previously in humans for pairs of single motor units from the same muscle (e.g., Datta and Stephens, 1980, J.Physiol. 308:19-20P). However, if shared inputs make up a relatively small proportion of the total input to different motoneuron pools, then the detection of synchrony will be more difficult, requiring the examination of a large number of pairs of single motor units as well as long periods of simultaneous discharge. Alternatively, the chance of detecting synchrony should be improved be examining simultaneous activity in paired groups of motoneurons.

Motor unit activity was recorded from two synergist muscles via needle electrodes while subjects performed steady, low level, isometric contractions. The motor unit recordings were amplified (1- 10K), bandpass filtered (0.5 - 10Khz) and recorded on magnetic tape for off-line analysis. Using window discriminators, the recorded motor unit spikes were converted to a series of TTL pulses to trigger programmable clock-counters which measured the interspike intervals with a resolution of 0.1 msec. Cross-correlation histograms (lags of -100 to 100 msec, bin width 1 or 2 msec) were calculated from these two interspike interval records. The amplitude and statistical significance of peaks in the histogram were determined as described by Sears and Stagg (1976).

Preliminary results support the existence of common inputs to different elbow flexor muscles. Crosscorrelation histograms were constructed by correlating the discharge of single motor units in the brachioradialis muscle with multiple motor units in either the brachialis or the long head of the biceps. Although these histograms were generally flat, significant central peaks of 12 - 20 msec duration were obtained in several instances where large numbers of spikes were analyzed (giving mean bin counts of at least 200). In these examples, spike-triggered averages from the single unit in brachioradialis into the raw, multiunit record from the other muscle showed that remote pickup from the triggering motor unit was insignificant. However, under certain recording conditions these remote potentials could lead to spurious peaks in the cross-correlogram. We are currently applying this technique to other synergist muscles of the upper limb in both normal and spastic, hemiparetic subjects.

Supported by R&T Center Grant #20, NIH grant 2-R01-NS19331-04 and MRC fellowship grant (DB).

332.14 EFFECTS OF SAMPLING RATE AND RECTIFICATION ON THE POWER SPECTRUM OF THE ELECTROMYOGRAM N. L. Suresh*, G. C. Agarwal, G. L. Gottlieb, Dept. of Physiology, Rush Medical College, Chicago IL 60612.

The purpose of this study was to investigate the effects of sampling rate and rectification on the power spectrum of the electromyogram (EMG).

The EMG from the biceps of three normal subjects was recorded from three channels (ch 1 - raw, ch 2 - full-wave rectified, ch 3 - full wave rectified and filtered) while the subject sat comfortably in a chair next to an elbow device with the elbow flexed 90 degrees. A DC torque motor was used to put out a constant torque against which the subject was asked to contract for 60 seconds. A computer was utilized to digitize the data at a 1000 Hz sampling rate (on each channel), which was then stored for later analysis. Filtering was done through a third order Paynter filter with a 10 ms averaging time. For analysis, data was sampled through a computer program by retaining every nth point (n = 1,2,4,8) yielding 1000,500,250 and 125 Hz sampling rates respectively. The power spectrum was calculated by using blocks of 1024 points (with the first and last 10% cosine tapered).

The power spectrum of the raw EMG, sampled at 1000 Hz, contained a peak spanning the range from 50 - 145 Hz after which the magnitude rapidly decreased. The results indicate that 500 Hz is the lowest rate at which the raw and full wave rectified signal can be sampled, while retaining the correct frequency information. For the full-wave rectified and filtered signal, 250 Hz is allowable.

Rectification of the EMG considerably altered the frequency spectrum, producing a major peak to the left (0 - 50 Hz) and a relative peak to the right(80 - 150 Hz) of that in the raw signal spectrum (sampled at 500 and 1000 Hz). The rectified and raw signal spectra did look identical beyond 170 Hz, although there is little energy in the original signal at these frequencies. Digital rectification of the raw signal (1000 Hz) produced a spectrum almost identical to that of the analog rectified signal in the low frequency region but differed from it in the higher frequency portion.

332.15 CONDUCTION VELOCITY OF MOTOR UNIT ACTION POTENTIALS IN HUMAN ANTERIOR TIBIAL MUSCLE AS A NEW SIZE PRINCIPLE PARAMETER. S. Andreassen* and L. Arendt-Nielsen*,(SPON: R.B. Stein) Dept. of Medical Informatics, Aalborg University, Strandvejen 19, DK-9000 Aalborg, Denmark.

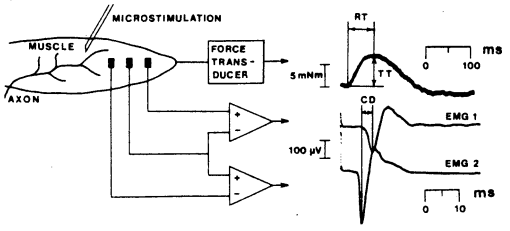


Fig. 1

Single motor units in human anterior tibial muscle were stimulated intramuscularly by a bipolar needle electrode.

The contractile properties of the motor units were obtained by averaging the torque developed around the ankle joint. Twitch torques (TT) ranged from less than 10^{-3} Newton·meter (Nm) to $16 \cdot 10^{-3}$ Nm, with a mean of $5.7 \cdot 10^{-3}$ Nm. Rise times (RT) ranged from 47-80 ms with a mean of 61 ms. The correlation coefficient R between rise times and twitch torques was -0.81 (N = 27, fig. 2a.).

The conduction velocity of motor unit action potentials was calculated from the conduction delay (CD) and the distance between the surface electrodes (see fig. 1). Conduction velocities ranged from 2.6-5.3 m/s with a mean of 3.7 m/s.

For individual motor units conduction velocity was highly correlated to twitch torque (R = 0.87, N = 29, fig 2b.) and to rise time (R = -0.75, N = 27, fig 2c.). This suggests that conduction velocity of motor unit action potentials is a member of the family of interrelated "size principle parameters".

This material has been submitted for publication in J. Physiol.

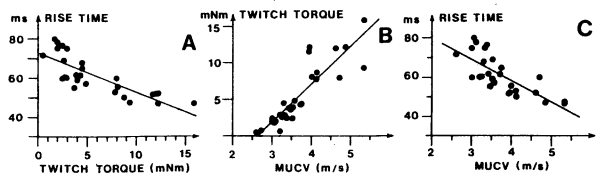


Fig. 2

332.16 A FAST, ACCURATE, ON-LINE SYSTEM FOR DISCRIMINATING ACTION POTENTIALS, BASED ON A TEMPLATE MATCHING ALGORITHM. T.S. Miles*, N. Smith*, K.S. Türker* and M.A. Nordstrom* (SPON: A. MACKAY-SIM). Department of Physiology, University of Adelaide, AUSTRALIA 5001.

In a multi-unit recording in nerves or muscles, the action potentials of individual neurones are recognized by their distinctive waveforms. However, most attempts to discriminate individual units are based on simply the amplitude of the waveform, or its amplitude at a particular time. A new system is now described, based on the ubiquitous IBM-compatible personal computer, which uses a sophisticated template-matching algorithm to discriminate simultaneously up to 3 units in real time. The system consists of a special-purpose printed circuit board which is plugged into an expansion socket in the computer, together with the operating software. The incoming multi-unit signal is digitized by an 8-bit analog-to-digital converter at up to 90 kHz sampling rate, and is stored by direct memory access. When an action potential exceeds an adjustable voltage threshold, a specifiable number of samples is collected and compared with stored templates of the waveforms of 3 unit potentials. The templates themselves are records of the unit potentials previously selected by the operator. When the waveform of an action potential in the incoming signal matches one of the templates, on analog acceptance pulse is output through one of three digital-to-analog ports. At the same time, the waveform of that action potential is displayed briefly on the computer screen alongside the image of the template to which it was matched to check the reliability of the discrimination. The instantanous firing frequencies of each unit are also displayed on the screen, and are available analog outputs from the circuit board. To deal with the situation in which a unit potential changes slowly in amplitude or waveform over time, the templates can be made adaptive; i.e., progressive changes in the unit potential are reflected by similar changes in the template. This is achieved by a continuous, weighted averaging of the incoming matched waveforms and the template. Waveforms which fail to be matched to a template are stored in memory and can be viewed one-at-a-time after a run. Inspection of the "rejected" waveforms is helpful in verifying the appropriateness of the discrimination settings. The reject file also contains the superimposed waveforms of pairs of units which occasionally fire simultaneously. To optimize the speed of the system, the program is written entirely in assembler language, utilizing scaled, fixed-point arithmetic. The user is able to vary all important operating parameters through a simply-learned set of keyboard commands. In practice, the system has been found to discriminate reliably between pairs of units which, because of similar amplitude action potentials, could not be separated by conventional window discriminators.

333.1 EFFECTS OF TEMPERATURE AND COLD ACCLIMATION ON NEUROMUSCULAR PROPERTIES OF CHELAE OF DECAPOD CRUSTACEAN SPECIES FROM TEMPERATE AND TROPICAL POPULATIONS. J.A. Blundon* (SPON: P.J. Stephens). Department of Zoology, Univ. MD, College Park, MD 20742.

This study tests the hypothesis that populations within a latitudinally dispersed species that experience different ranges of environmental temperatures will have different physiological responses to changes in temperature. Excitatory postsynaptic potential (EPSP) amplitude, summation, and facilitation as well as muscle stress and muscle fiber cable properties were measured in the chela closer neuromuscular system of temperate and tropical stone crabs Menippe mercenaria and blue crabs Callinectes sapidus. Northern populations of both crab species experience year round temperatures from < 5°C to 30°C, while the southern populations are exposed to a narrower range of temperatures as low as 15 to 20°C and as high as 30°C. No differences were found in neuromuscular performance of the northern and southern populations of both species when tested at summer temperatures common to both (30°C). Both northern and southern blue crabs were capable of acclimating from 30°C to 8°C in 4 weeks and showed similar levels of muscle stress at the two temperature extremes. Cold acclimated blue crabs from both populations had higher specific muscle fiber membrane resistance at 8°C than warm acclimated crabs. Neither stone crab population was capable of acclimating to 8°C over a 7 week period in the laboratory. However, when tropical stone crabs were transported to temperate waters in the summer and retested in the winter (at 8 °C), they showed similar neuromuscular properties as winter temperate crabs. There was a significant difference in specific muscle fiber membrane resistance between stone crabs naturally acclimated to 8°C and those that failed to acclimate in the laboratory. In all crabs, muscle performance was enhanced at cold temperatures due to increased muscle fiber membrane resistance which resulted in a broadening of EPSPs which enhanced EPSP summation and muscle fiber depolarization. The difference in acclimation ability of blue crabs and stone crabs is reflected in their natural environment. Blue crabs live in estuarine environments where large short term temperature fluctuations are much more common than in the marine subtidal environment of stone crabs.

333.2 DYNAMICS OF MUSCLE STIFFNESS IN A CRUSTACEAN SLOW MUSCLE. William D. Chapple, Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06268.

Although information has long been available about the dynamic properties of vertebrate muscle, little is known about the variations in dynamic stiffness in crustacean slow muscles with stretch amplitude and level of activation. Hoyle (1983) has suggested that decay after stretch has a time constant composed of two exponential terms, fast and slow.

To examine this in more detail, a decapod slow muscle, the right superficial ventral muscle of the fourth abdominal segment of the hermit crab, Pagurus pollicarus, was subjected to ramp stretches (36 mm/s, 3.2 muscle lengths/s) with amplitudes ranging from 0.5 to 1.6 mm. (4% to 14% of optimal length). Decays from peak force, sampled at 1KHz, were fit with a non linear least squares routine to an equation of the form $A_0 + A_1e^{-B_1t} + A_2e^{-B_2t}$. A third exponential term did not improve the fit of the equation. The slow time constant (B_1 = 0.214 s + 6%, n=18) did not vary with stretch length or level of isometric force prior to stretch. The fast time constant (B_2 = 0.013 s + 21%,n=18) decreased slightly (0.011s to 0.016s) but significantly ($p < 0.01$) with increasing isometric force but did not vary as a function of stretch length. All three coefficients (A_0,A_1,A_2) increased as a function of stretch length multiplied by the level of isometric force. At one stretch amplitude, A_1 was about 25% of A_0, but A_2 ranged from 50% of A_0 in passive muscle to 30% of A_0 in muscles stretched at higher levels (0.15 N) of isometric force.

Calculation of the parameters for active stiffness (coefficients of activated muscle minus the coefficients for passive muscle) suggests that muscle activation (over the ranges produced by the stretch reflex) does not alter substantially the dynamics of muscle stiffness, but multiplies it by a constant determined by the level of activation.

Supported by NSF grant BNS-8505694

333.3 EFFECTS OF EXTERNAL Ca^{2+} ON CAFFEINE AND THEOPHYLLINE CONTRACTURES IN TONIC MUSCLE FIBERS OF THE FROG. J. Muñiz*, J. Dueñas* & M. Huerta. Centro Universitario de Investigaciones Biomédicas, Universidad de Colima, Apdo. Postal 199, Colima, Col. 28000 México.

To further investigate the role of extracellular Ca^{2+} on contractile activation in tonic muscle fibers, we have made a study of the ionic dependence of caffeine and theophylline contractures. Tension was isometrically recorded from bundles of cruralis muscle of Rana pipiens. Fibers of the cruralis muscle were current-clamped. The membrane potential corresponds to the values measured immediately after the insertion of the micropipette. Tonic muscle fibers were identified according to their passive electrical properties. Experiments were done at room temperature (20-22°C). Control solution was (mM): NaCl 117.5, KCl 2.5, $CaCl_2$ 1.8. pH was adjusted to 7.4 with imidazole-chloride. Caffeine and theophylline were added from concentrated aqueous solution. To reduce possible changes of the surface potential, $CaCl_2$ was replaced by $MgCl_2$ (3mM) or $NiCl_2$ (0.5mM). Contractures were elicited when caffeine or theophylline (0.5 - 14mM) was added to the control solution. The tension was sustained and the muscle fibers relaxed after caffeine or theophylline withdrawal. In both, the threshold concentration to elicited tension was about 0.5mM. With larger concentration (1-14mM) tension clearly rose to a peak and then relaxed to a low sustained level and it reached the maximum at about 14mM. The membrane potential of tonic muscle fibers was not modified by caffeine or theophylline. The membrane potential in control solution was -74 ± 8 mV (20), with caffeine (4mM) and theophylline (4mM) added they were -76± 9 mV (11) and -75 ± 6 mV (5) respectively.

We found that caffeine and theophylline contractures were highly Ca^{2+} -dependent. When external Ca^{2+} was omitted from the contracture fluid the caffeine and theophylline (0.5 , 1, 6mM) elicited a very small tension. This effect was reversible. The results suggest that external Ca^{2+} has to be continuously present to maintain the tension during caffeine or theophylline contracture.

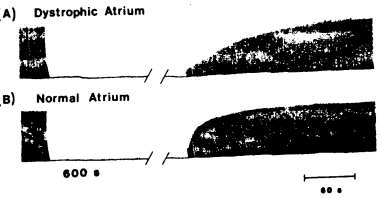
Supported by R.J.ZEVADA 11/86 and SEP-SESIC of México, grants 84-01-0126/8 and 84-01-01/26/10

333.4 MECHANICAL RESTITUTION IN NORMAL AND CARDIOMYOPATHIC HAMSTER HEARTS. S.E. Howlett and T. Gordon. Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Cardiomyopathy (CM) in the hamster may be secondary to an increase in intracellular calcium (Ca) which may arise from defective Ca sequestration by the sarcoplasmic reticulum (SR). Ca distribution in the heart is dynamic, cycling between uptake, release and exchange compartments. The amount of Ca in these compartments may be systematically varied in isolated, stimulated (0.2 Hz, 1 Hz) left atria (2.5 mM Ca) and papillary muscles (1.0 mM Ca) from normal and CM hamsters by comparing the effects of interposing varying test intervals on steady state muscle force (mechanical restitution). The force produced by the heart is a function of the amount of Ca released by the SR release compartment. At short test intervals (≈ 1 s) there was a small peak in the force-interval curve which reflects Ca movement from SR uptake to release compartments; this was similar in normal and CM muscles. At longer test intervals (≈ 25 s) a large peak, related to Ca movement from exchange to release compartments, was also similar in normal and CM tissues. When stimulation was interrupted for very long intervals (>10 min) there was a marked decrease in force in both normal and CM muscles which reflects Ca loss from the release compartment. Normal and CM hearts differed only in that the rate of recovery of force following long test intervals was greatly slowed in CM atria (Figure 1). The mechanical restitution data shows that 1) the dynamic Ca movement between uptake, release and exchange compartments is similar in normal and CM hearts and 2) the recovery of force is slowed following long test intervals and is consistent with less Ca in the uptake compartment. The slowed recovery of force is consistent with 1) less Ca influx, which is unlikely as there is no change in the number of Ca channels in normal and CM hearts (Howlett & Gordon, Biochem. Pharmacol., in press), 2) reduced Ca sequestration by the SR or 3) a decrease in the Ca storage capacity of the SR and may account for Ca overload in CM hearts.

Supported by AHF & AHFMR.

Figure 1



333.5 ACTIVATION OF SINGLE MUSCLE FIBERS REVEALED BY 2-DEOXYGLUCOSE-6-PHOSPHATE (2DG6P). D.A. Gordon, R.M. Enoka, D.G. Stuart, B.J. Norris*, P.M. Nemeth and O.H. Lowry*. Depts. of Neurology and of Anatomy and Neurobiology and of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110. Dept. of Physiology, Arizona Cen. for Health Sci., Univ. of Arizona, Tucson, AZ 85724.

In skeletal muscle cells, carbohydrate substrates for energy metabolism arise from endogenous glycogen breakdown and exogenous glucose uptake. Both of these processed are operative during muscle fiber activation. In the past, depletion of glycogen has been used as an indicator of activity in single fibers, however, the adequacy of this technique has recently been questioned (J. Neurosci. 6:892, 1986). In the present work, the relation between glycogen depletion and glucose uptake was investigated in fibers of cat tibialis anterior muscle activated in situ by electrical stimulation of single motor axons. 2-deoxyglucose, which has the same intercellular transport mechanism as glucose, was intraveneously administered and provided the substrate for the cellular accumulation of 2DG6P. Following activation, the muscle was removed and single fibers were examined for qualitative glycogen depletion using the glycogen-specific periodic acid Schiff reagent (PAS), and for quantitative levels of 2DG6P. In muscles exhibiting the typical motor unit mosaic pattern of PAS-negative fibers, glycogen depletion and increased 2DG6P coexisted, with few exceptions. The 2DG6P was about 20 times higher in PAS-negative fibers compared to the PAS-positive, presumably inactive, control fibers. In contrast, there was a dissociation between glycogen depletion and 2DG6P in fibers which did not exhibit the typical motor unit depletion pattern, in particular, where entire fascicles contained PAS-negative fibers. In these fascicles, none of the glycogen depleted fibers had elevated levels of 2DG6P. This suggests that there may be differential activation of the glycogen breakdown and glucose uptake processes. Local ischemia can induce glycogen breakdown in muscle fibers, but not increase glucose uptake. Thus, glycogen depletion is not always a reliabale indicator of muscle fiber activation. In such instances, the uptake of glucose, as measured by the accumulation of 2DG6P, can be used to verify acute activity of muscle cells.

333.6 GLYCOSPHINGOLIPID SYNTHESIS IN VITRO BY FUSION COMPETENT (C-2) AND FUSION DEFECTIVE (BC3H1) MURINE MUSCLE CELLS. K.C. Leskawa* and E.L. Hogan (SPON: D.A. Cotanche). Department of Neurology, The Medical University of South Carolina, Charleston, SC 29425.

We have previously reported that ganglioside synthesis by rat L6 muscle cells in culture transiently doubles when myoblasts achieve contact and begin fusing, while neutral glycosphingolipid (GSL) synthesis gradually decreases as myotubes form (Leskawa et al., Soc. Neurosci., 1986). Such a transient increase in the synthesis of either gangliosides, neutral GSLs or both has also been observed with the murine G-7 and G-8 lines and primary cultures of chick embryo pectoral muscle (Leskawa et al., Int. Soc. Neurochem., 1987). Here, we report a comparison of a murine muscle cell line, C-2, and a nonfusing varient, BC3H-1.

Both cell types were cultivated for 9 days in complete medium, and harvested at days 2, 3, 4, 7 and 9, after addition of [3H]-galactose 12 hr prior. Gangliosides and neutral GSLs were purified by extraction, saponification, and column chromatography on silicic acid, C18 and DEAE-Sephadex. Both cell types reached confluency at day 3, when C-2 cells began fusing (over 75% total nuclei within myotubes by day 7). Creatine kinase activity gradually rose with time in culture in both cell types.

Labeling of gangliosides by C-2 cells increased significantly on day 3 (approximately twice day 2 values) and then decreased. Neutral GSL labeling decreased during this period, with day 3 values being one-half those at day 2, and then remained constant. The decrease was greatest with nLcOse4Cer, which comprised 83% of labeled neutral GSLs on day 2, but 51-31% days 3-9. Labeling of both gangliosides and neutral GSLs by BC3H1 cells during this period did not vary significantly. BC3H1 cells synthesized LacCer, GbOse3Cer and a GSL tentatively identified as the Forssman hapten, with no changes during time in culture. GM3 was the major ganglioside synthesized by both cell types, with little change in ganglioside distribution with time in culture.

These results support our observations of a transient increase in GSL synthesis during myogenesis, and suggest that this response is lacking in fusion-defective myoblasts. While BC3H1 cells do not fuse to form myotubes, others have shown that they aquire many other biochemical aspects of differentiated muscle, including shifts in creatine kinase isoenzymes, expression of acetylcholine and insulin receptors and N-CAM. Therefore, the present results further suggest that increased synthesis of glycolipids may be integral to development of the morphological phenotype, i.e., the fusion of myoblast membranes to form multinucleated myotubes.

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333.7 CHONDROITIN SULFATE COMPONENT IN MEMBRANES (EXTERNAL LAMINAE) SURROUNDING INDIVIDUAL AVIAN INTRAFUSAL FIBERS. A Maier and R. Mayne. Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

Serial cross sections of muscle spindles from eight-week old chick tibialis anterior muscle spindles were incubated with monoclonal antibodies against O-sulfated (IB5), 4-sulfated (2B6) and 6-sulfated (3B3) chondroitin sulfate. These antibodies have been shown to combine with a residue of the glycosaminoglycan portion of the proteoglycan after it has been incubated with chondroitinase, and they do not react with the intact molecule (Couchman et al., Nature 307:650-652, 1984). Other sections were incubated with a monoclonal antibody (CS-56) (Avnur and Geiger, Cell 38:811-822, 1984) which reacts with intact, undigested chondroitin sulfate chains. The location of the immunohistochemical staining was verified by inspecting sections with phase contrast microscopy. Chondroitinase did not affect the immunohistochemical appearance of heparan sulfate proteoglycan. In the muscle spindle the presence of immunohistochemical reaction product was essentially limited to the external (basal) lamina that is associated with intrafusal fibers. No staining was apparent in the periaxial space or the outer spindle capsule. The antibody against the O-sulfated compound (IB5) produced no reaction product, even after pretreatment with chondroitinase. At the equator after incubation with 2B6 or 3B3 staining was limited to a narrow band. It coincided in location with the external lamina that is interposed between sensory terminals and the collagenous sheath that covers the sensory terminals. Staining around entire intrafusal fibers and along the external surface of the collagenous sheath was apparent at the equator after incubation with CS-56. At the polar region 2B6 gave a very weak reaction or no reaction at all, but 3B3 and CS-56 produced a staining pattern around extrafusal and intrafusal fibers that was reminiscent of the location of the external lamina. Results of this study show that subspecies of chondroitin sulfate in which the position of the sulfate group differs may be selectively distributed along excitable cells. With this arrangement the negatively charged chondroitin sulfate conceivably could impart specific electrical properties to intrafusal fibers. In this context it should be noted that at the equator the location of the 4-sulfated and 6-sulfated variants is limited to that portion of the external lamina which overlies the sensory terminals, the sites where the afferent signals originate. Alternately, proteoglycans may act to attach collagen to basal (external) laminae (Koda et al., J. Biol. Chem. 260:8157-8162, 1985). This view is supported by the presence of chondroitin sulfate immediately adjacent to the collagenous sheath.

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333.8 CLASSIFICATION OF FIBER TYPES IN TURTLE NECK MUSCLE. R.J. Callister, R. Callister, and E.H. Peterson. Dept. Zoological & Biomedical Sciences and College of Osteopathic Medicine, Ohio University, Athens, Ohio, 45701.

As part of a study aimed at understanding the control of head movement in a turtle, Pseudemys scripta we have characterized its neck muscle fiber types using histochemical markers which are known to have functional significance. We reacted serial sections with markers for contraction speed (Myosin ATPase), oxidative metabolism (NADH-diaphorase) and two glycolysis associated markers (alpha-glycerophosphate dehydrogenase [GPDH] and glycogen phosphorylase A [GPA]). We used microphotometry to quantify the intensity (optical density) of the reaction deposit in each fiber, and we subjected the resulting optical density measurements to a cluster analysis to assign fibers to groups (fiber types). For comparison we processed turtle hindlimb muscle ambiens and rat sternomastoid simultaneously.

Our results suggest that in turtle muscle, as in mammals, slow (Type I) and fast (Type II) contracting fibers can be distinguished by reversal of the routine ATPase staining pattern (generated at pH 9.4; or with preincubation at pH 10.4) after acid preincubation, but the pH at which reversal occurs is higher (pH 4.5) than in mammals (pH 4.2). In both neck and limb turtle muscle an intermediate staining fiber type (IIb) can be demonstrated following preincubation at pH 4.7; this is higher than the pH needed to demonstrate intermediate fibers in rat (pH 4.5). Furthermore, intermediate fibers demonstrated by ATPase staining differ in the two species: turtle IIb fibers have higher oxidative capacity and GPA activity than IIa fibers (fibers with low myosin ATPase activity after acid preincubation), but in rat muscle this condition is reversed.

Cluster analysis results suggest that turtle (like rat) muscle can be grouped into three fiber classes: slow oxidative (SO), fast oxidative glycolytic (FOG) and fast glycolytic (FG). A discriminant function analysis of the groups generated by the cluster analysis suggests that a fiber classification based on both ATPase and metabolic markers yields more accurate group assignments than a scheme based on ATPase staining alone, and that these two classification schemes yield somewhat different group assignments. This analysis also revealed that the histochemical markers which best differentiate muscle fiber types differ in turtle and rat.

Our results suggest that turtle muscle can usefully be grouped into three fiber types (SO, FOG, FG), but these types differ from like-named fiber types in rat muscle both in the pH lability of their myosins and in their metabolic profiles.

333.9 NEONATAL SOLEUS MUSCLE FIBERS ARE HETEROGENEOUS IN THEIR MYOSIN HEAVY AND LIGHT CHAIN COMPOSITIONS. H. L. Sweeney*, L. A. Sutton* and W. J. Thompson. Depts. of Zoology and Physical & Health Education, University of Texas, Austin, TX 78712.

Myofibrillar ATPase histochemistry of neonatal rat soleus suggests the muscle contains two types of fibers 16-18 days after birth. By use of glycogen depletion it has been shown that single motor units in these muscles are comprised mostly of one or the other of these two fiber types (Nature 309: 709), suggesting that the muscle contains two distinct types of motor units. Measurements of the contractile characteristics of single units in these muscles showed, however, that these units had a wide range of unloaded velocities of shortening, distributed apparently unimodally (Soc. Neurosci. Abs. 11: 1287). Since the unloaded shortening velocities were determined using the "slack test" (J. Physiol. 291: 143) and since the slack test gives the velocity of shortening of the fastest contracting fibers in each unit, this result was interpreted to mean that motor units in the muscle are comprised of a non-random assortment of fibers. A random innervation would be expected to result in units each of which should contain some of the fastest fibers present in the muscle. However, this result also suggests that myofibrillar ATPase histochemistry underestimates the diversity of fiber types present in the muscle. We have attempted to test this prediction by examining the myosin heavy and light chain composition of single muscle fibers in 16-18 day old rat soleus muscles.

Single fibers were mechanically teased from 16-18 day old muscles treated overnight with a skinning solution (Tissue & Cell 11: 143). The proteins in each fiber were extracted in 2 µl of SDS sample buffer and analyzed on SDS polyacrylamide gels. Myosin heavy chains (MHC's) and light chains (MLC's) were detected following silver-staining. Electrophoresis on 5% gels (Biochem. Biophys. Res. Comm. 116: 793) resolves two clear bands representing the MHC's in these muscles: the faster migrating band moves to the same position in the gel as adult slow MHC whereas the slower migrating band moves to the same position as adult fast MHC. Since additional bands corresponding to embryonic and neonatal MHC's do not clearly resolve as extra bands on these gels, we cannot positively identify the MHC's comprising the bands. Nevertheless, more than three patterns of MHC's are seen in individual fibers: either of the two bands by itself or both of the bands in varying relative intensities. Electrophoresis on 13% gels resolves at least two patterns of myosin light chains in the individual fibers. The first pattern consists of $LC1_S$, $LC2_S$, and $LC2_F$. The second pattern consists of the three LC's in the first pattern together with two additional LC's: $LC1_F$ and a fifth LC of mobility intermediate between that of $LC1_S$ and $LC1_F$. Each of these two patterns of light chains was observed in fibers having each pattern of MHC. This heterogeneity of myosin among single fibers is consistent with the previously observed broad distribution of velocities of unloaded shortening measured in single motor units. Thus, the histochemically derived notion of only two fiber types existing at this developmental stage is an oversimplification.

333.10 CORRELATION OF FIBER SIZE AND NECROSIS IN SKELETAL MUSCLE OF C57BL/6J DY2J/DY2J DYSTROPHIC MICE. H.L. Davis, G. Desypris and S.L. Lui*, Department of Anatomy, and School of Physical and Occupational Therapy, McGill University, Montreal, Canada, H3A 2B2; and Department of Physiology, University of Ottawa, Ottawa, Canada, K1H 5A3.

The muscular dystrophies have long been considered as inherited diseases of muscle (myogenic theory) because of the widespread changes in the muscle, however the basic biochemical defect has yet to be elucidated. The currently popular myogenic theory is that of a sarcolemmal abnormality, and it has been proposed by G. Karpati and colleagues that muscle fibers must obtain a certain diameter before the dystrophic phenotype (necrosis) is expressed, possibly due to factors related to contraction-related strains per unit surface area. The considerable evidence which supports this theory has been obtained from studies on human (Duchenne), hamster and MDX murine dystrophic muscle, in which it was shown that fibers with large diameters were severely affected while those of smaller caliber (e.g. developing, denervated while young) did not show signs of necrosis. The present study examined the relationship between fiber size and severity of necrosis in C57BL/6J dy2j/dy2j male dystrophic mice.

The extensor carpi radialis longus (ECRL) and brevis (ECRB) muscles from normal (+/+) and dystrophic (dy2j/dy2j) mice at 4 wk or approximately 1 yr of age were used for this study (n=5 per age group). Cross-sections from the mid-belly regions of the muscles were stained with haematoxylin and eosin, for activity of myofibrillar ATPase, and by immunohistochemistry for localization of specific isoforms of heavy chain myosin. The two muscles were found to have similar fiber type composition and there were no differences between mean cross-sectional areas of type II fibers (stained for activity of ATPase) of ECRL and ECRB muscles of either normal or dystrophic mice in either age group. Dystrophic ECRL muscles, however did exhibit significantly more necrosis, as determined by percentage of total fibers with centronucleation, than ECRB muscles ($\bar{x} \pm$ SEM = 28 + 2% and 18 $\pm$ 2 % respectively for 1 yr old animals). Since the ECRL and the ECRB muscles occupy a similar environment and work together functionally, the results from the present investigation do not support the hypothesis that phenotypic expression of the dystrophic gene is dependent on the caliber of the skeletal muscle fibers in dy2j/dy2j mice.

This work was supported by a grant from the Medical Research Council of Canada to H.L.D. The antibodies were a generous gift of Dr. S. Schiaffino, Padua, Italy.

333.11 HISTOPATHOLOGY OF SKELETAL MUSCLES IS SIMILAR IN AGED DYSTROPHIC CHICKENS AND HUMAN DUCHENNE DYSTROPHY. R.K. Entrikin, R.T. Abresch*, D.B. Larson*, R.B. Sharman*, and W.M. Fowler*. Dept. of Physical Medicine & Rehab., Univ. of Calif., Davis, CA 95616.

Although hereditary muscular dystrophy of the chicken (HMDC) has been widely used as an animal model of human Duchenne muscular dystrophy (DMD), its validity has been questioned due in part to less severe histopathological changes in comparison to DMD muscle. Since previous histological analyses of muscles in HMDC have involved relatively young animals (usually about 8 weeks of age), we have tested the hypothesis that progression of the dystrophy might lead to more severe changes in older, adult birds.

Histopathology was analyzed qualitatively and quantitatively (computer-based image analysis system) in pectoralis major muscles from 2 genetic lines of normal (03 & 412) and dystrophic (433 & 413) chickens at early (6 weeks) and advanced (18 months) stages of the disease. At 18 months muscles from both lines of dystrophic chickens exhibited severe histopathological changes that were strikingly similar to those observed in human biopsy slides. In fact, 3 "blinded" clinicians could not distinguish between hematoxylin-eosin stained cross-sections of dystrophic chicken and human DMD muscles, except for internal nuclei typical of avian muscles. Both HMDC and DMD muscles exhibited a significant increase in fiber size variability, proliferation of connective tissue, phagocytosis, and degeneration. Avian sections also revealed extensive splitting of large muscle fibers, one possible origin of the many small fibers in late stages of avian dystrophy. Fiber size (cross-sectional area in square microns) and percent connective tissue in muscles from one normal and one dystrophic line of chickens are shown below (N: number of muscles; values: mean $\pm$ SEM):

	AGE	LINE	N	FIBER SIZE	CONNECTIVE TISSUE
Normal	6 Wk	03	4	332 $\pm$ 16	31% $\pm$ 3%
Normal	18 Mo	03	8	1447 $\pm$ 64	22% $\pm$ 1%
Dystrophic	6 Wk	433	6	424 $\pm$ 20	34% $\pm$ 2%
Dystrophic	18 Mo	433	10	896 $\pm$ 79	73% $\pm$ 3%

In addition to the histopathological similarities between HMDC and DMD, recent reports have shown that a single drug that improved muscle function in avian dystrophy has now produced positive effects in DMD patients. These observations strongly support use of the dystrophic chicken to advance knowledge of human neuromuscular diseases. (Supported by NIDDR Grant #G008300078.)

333.12 ANALYSIS OF HIGH MOLECULAR WEIGHT PROTEINS IN DUCHENNE AND BECKER MUSCULAR DYSTROPHY. R MEDORI*, R WATERSTON*, M BROOKE*, Washington University School of Medicine, St. Louis, MO.

We analysed muscles from biopsies of patients with Duchenne and Becker muscular dystrophy using polyacrylamide gel electrophoresis. Total muscle homogenates and myofibril preparations were processed for separation by polyacrylamide electrophoresis using 4% gels. Patients with neurogenic atrophy and different forms of myopathies other than dystrophy as well as normal fetal and mature muscles were used as controls. In preliminary work, muscle biopsies from patients with Duchenne and Becker muscular dystrophy show the absence of an approximately 500 KD polypeptide in the patients with muscular dystrophy. This high molecular weight protein may correspond to nebulin, which has been suggested to be the Duchenne dystrophy gene product. Efforts are underway to establish the relationship of the 500 KD protein to nebulin. The extra doublet of polypeptides in fetal and Duchenne muscle has been characterized as spectrin in Western blots. In the muscle affected with congenital dystrophy both the extra doublet of bands and the 500 KD polypeptide are present. A study of the Duchenne muscular dystrophy patients from this clinic is planned to determine if these preliminary findings can be generalized. Patients with an alteration in size of the putative nebulin band would be of particular interest. These findings open a new approach to the study of the pathogenetic mechanisms of Duchenne and Becker muscular dystrophy.

ANALYSIS OF HIGH MOLECULAR WEIGHT PROTEINS IN DUCHENNE AND BECKER MUSCULAR DYSTROPHY. R MEDORI*, R WATERSTON*, M BROOKE*,(SPON: R. Wilkinson) Dept. of Cell Biology & Physiology, Washington University Med. Ctr, St. Louis, MO 63110.

333.13 FIBER TYPE SPECIFIC ENZYMATIC TRANSFORMATION IN NEWLY FORMED HUMAN MOTOR UNITS. W.R. Turk* and L.A. Wargowski* (SPON: N.J. Lenn). Dept. of Neurology, Univ. of Virginia, Charlottesville, VA 22908

In mammalian skeletal muscle the motoneuron has a significant influence on the metabolic characteristics of the fibers it innervates. We have studied whether human skeletal muscle fibers within newly formed motor units acquire biochemical similarity following innervation by a common motoneuron.

Serial sections were obtained from the gastrocnemius muscle of a patient with motoneuron disease. Histochemical staining with myosin ATPase (pre-incubation pH 4.6) demonstrated a loss of the normal fiber type mosaic pattern and areas of fiber type grouping, which identify fibers that have been denervated and subsequentially reinnervated by a common motoneuron (Karpati G. & Engel W., Neurol. 18:447, 1968). Individual fibers within four regions of fiber type grouping were isolated and quantitatively assayed to determine their activity for lactate dehydrogenase (LDH) and adenylokinase (AK).

	Fiber Type	n	LDH*		AK*	
I	Motor Unit A	12	20.79 ± 3.30	15%	44.94 ± 5.62	13%
	Motor Unit B	10	7.30 ± 1.88	26%	24.99 ± 3.38	14%
	Random Fibers	18	9.30 ± 3.57	38%	29.77 ± 7.49	25%
	Total	40	12.30 ± 6.53	53%	33.11 ± 10.05	30%
IIA	Motor Unit C	13	39.80 ± 4.32	11%	82.13 ± 6.79	8%
	Random Fibers	20	37.14 ± 5.96	16%	85.87 ± 13.79	16%
	Total	33	39.19 ± 5.46	14%	84.70 ± 11.77	14%
IIB	Motor Unit D	7	42.29 ± 2.99	7%	103.37 ± 9.64	9%
	Random Fibers	20	44.66 ± 9.27	21%	113.45 ± 16.36	14%
	Total	27	44.10 ± 8.12	18%	110.84 ± 15.40	14%

*Enzyme activities are moles/kg dry wt/hr from duplicate samples expressed as mean ± S.D. and coefficient of variation. n=number of fibers.

The results demonstrate that fibers within newly formed motor units are enzymatically homogeneous when compared to randomly selected fibers of each fiber type. The variability within motor units composed of type IIA or IIB fibers approached the experimental error of the method (determined by multiple measures on individual fibers to be 5.9%). Within type I motor units there was greater variability than within type IIA and IIB motor units. This is in contrast to our previous examination of newly formed motor units in the rat using a nerve crush model where the extent of variability within newly formed motor units was identical regardless of fiber type (Nemeth P. & Turk W., J. Physiol. 355:547, 1984). These findings suggest a divergence with respect to the direction and extent of motoneuron directed fiber type transformation. Type II to type I transformation appears enzymatically less complete than type I to II transformation in human skeletal muscle. (Supported by NIH grant NS00957)

333.14 INCLUSION BODY MYOSITIS: A LIGHT MICROSCOPIC AND ULTRASTRUCTURAL ANALYSIS. M.J. Winger* and R.I. Roelofs* (SPON: D. Knopman). Dept. of Neurology, University of Minnesota, Minneapolis, MN 55455.

Inclusion body myositis (IBM) is thought to be a distinctive but rare myopathic disease of skeletal muscle. It is characterized by intramuscular rimmed vacuoles that stain basophilic with H&E, red on modified Gomori's trichrome and are acid phosphatase negative on cryostat sections. Final diagnosis depends on EM demonstration of intranuclear and/or sarcoplasmic fibrillary inclusions, 15-18 nm in diameter. Typically these patients develop painless extremity paresis in their sixth and seventh decades that is slowly progressive and involves proximal more than distal muscles. Serum creatine kinase may be normal or modestly elevated. The disease tends to be refractory to immunosuppressive therapy compared to polymyositis and dermatomyositis. Fewer than 60 cases have been reported in the literature. We report analysis of 686 consecutive muscle biopsies; 138 (20%) had features of an inflammatory myopathy and 38 of these had the characteristic light microscopic features of IBM. This latter group represented 5.5% of the total and 28% of the idiopathic inflammatory myopathies. To qualify for the diagnosis of probable IBM, at least 1% of muscle fibers had to contain the typical vacuoles on cryostat sections. None of the cases were felt to have any of the other rare conditions associated with a myopathy having rimmed inclusions. Seventeen of the 38 cases had ultrastructural evaluation; 9 demonstrated the pathognomic sarcoplasmic fibrillary inclusions, while 8 had sarcomembranous inclusions commonly seen in IBM but not considered diagnostic of the condition. All of these 8 cases had a low percentage (1-3%) of fibers containing rimmed vacuoles on light microscopy. Sampling error could account for the negative EM results. There was no light microscopic difference between the 9 proven cases of IBM, the 8 cases with only sarcomembranous inclusions and the remaining cases not studied with EM. It is likely that most of the 38 cases do in fact represent IBM, at least pathologically. As a tertiary referral center, our patient population is biased with cases that pose diagnostic or therapeutic dilemmas. However our high proportion and absolute numbers of cases with a pathologic diagnosis of IBM seem excessive. IBM may be a more common clinical pathologic disorder than previously recognized or conversely the histologic and ultrastructural features of IBM are less specific than generally appreciated.

SPECIAL LECTURE

334 SPECIAL LECTURE. NEUROETHOLOGY OF ELECTRIC FISH: THE ROLES OF ANALOGIES VS MODELS IN COMPREHENDING NEURAL SYSTEMS. Theodore H. Bullock, University of California, San Diego.

Loose, graphic analogies and formal models have distinct, mainly heuristic values as well as serious inadequacies. Nothing substitutes for specific anatomy and physiology of actual neural systems including the comparison of similar but contrasting systems. Nature often uses different solutions to common problems. Seven general neurobiological issues will be illustrated, mainly with examples from gymnotiform and mormyriform electric fish.

(1) The parliamentary analogy for control of graded behavior grades into oligarchy for switching of either-or behavior. The orchestra analogy of spatiotemporal pattern coding grades into a corporation or university analogy for decision and control.

(2) Corollary discharge is used in some taxa but not others, and at its best shows rapid learning of the reafference to be expected. (3) Special mechanisms act in some species to regularize pacemaker units, greatly reducing the variance of intervals, and others act to deregularize; some modulate pacemaker firing for social signaling. (4) Receptors and central neurons are tuned to respond best to a certain band of input signals, a frequency band in the cases here considered; the tuning may be more or less plastic if the internal milieu is conducive. (5) Multiple maps present several kinds of problems. One is the distinction between projection maps that preserve neighbor relationships and computed maps that extract parameters from converging afferents. Another is the distinction between maps that represent two equivalent dimensions of the input (e.g. somatotopic) and those represent only one or else 2 or 3 nonequivalent dimensions intersecting at some angle.

(6) The cerebellum presents an opportunity to compare functional organization in a conservative organ, highly developed in some fishes, with that in mammals and other classes. Apparent differences are a challenge to interpretation.

(7) Processing in afferent pathways, serial and parallel, from medullary input through midbrain, diencephalon and telencephalon, presents opportunity and challenge in other ways. Among the aspects to be discussed will be the meaning of the contrast between mammalian and fish telencephalon, the former showing responses readily and at high repetition rates, the latter only within a fractional mm of the hot spot and at extremely long intervals. The relation between unit and population responses, spikes and evoked potentials, exogenous and relatively endogenous event related potentials may give clues to the nature of evolution of neuronal cooperativity.

335 SYMPOSIUM. STIMULANT-INDUCED SENSITIZATION; BEHAVIOR AND NEUROPHARMACOLOGY. W.H. Riffee, University of Texas at Austin (Chairperson); B.A. McMillen, East Carolina University; G.V. Rebec, Indiana University; T.E. Robinson, University of Michigan.

Striatal dopamine (DA) exists in a large storage pool and a readily releasable pool, but the other monoamines do not. This distinction is important for both the acute effects of amphetamines and non-amphetamines and for the long term effects of stimulant or antipsychotic drugs on the dopaminergic system compared to the other monoaminergic systems. DA appears to be very much involved in the phenomenon of stimulant-induced sensitization. Many of the processes involved in the availability of DA within the dopaminergic neuron (transmitter synthesis, autoreceptor sensitivity, receptor-mediated control of synthesis and release as well as frequency of cell firing) have been studied and characterized as to their potential role in sensitization while other processes are yet to be explored in sufficient detail. Now, there are indications that certain drugs may interfere with the ability of central nervous system stimulants to induce sensitization.

One of the most important aspects of stimulant-induced sensitization is the effects that these drugs have on processes within the neuron which are not restricted to the nerve ending itself but extend to the cell body and its associated structures. Single-unit recording techniques have been used to investigate pre- and postsynaptic changes in monoaminergic systems during long-term amphetamine treatment. Such treatment increases impulse flow in dopaminergic and serotonergic neurones, and this effect is mediated in part by subsensitive autoreceptors. In sites downstream from monoaminergic terminals, including the neostriatum and substantia nigra pars reticulata, multiple amphetamine injections also accelerate neuronal activity. In these sites, however, the actions of amphetamine appear to reflect a complex interaction between monoaminergic mechanisms and other neurochemical systems.

Finally, the symposium will look at an overview of various stimulant drugs as well as stress and their enduring effects on brain dopamine systems and behavior. Additional information will be presented which discusses factors that are known to contribute to individual variation in sensitization and sucseptibility to the phenomenon.

336 SYMPOSIUM. A HEART-BRAIN PEPTIDE: THE ATRIAL NATRIURETIC FACTOR. J.M. Saavedra, NIMH (Chairperson); A. de Bold* Heart Institute, Ottawa; T. Inagami, Vanderbilt Univ.; C. Glembotski, San Diego St. Univ.; C. Saper, Univ. of Chicago; R. Quirion, Douglas Hosp., Quebec; A. Negro-Vilar, NIEHS, NC.

The atrial natriuretic factor (ANF) is a potent diuretic and natriuretic hormone released by the heart atria to the circulation. Peripherally, it inhibits renin and aldosterone secretion. In addition, circulating ANF has central actions, and the brain contains an endogenous ANF system and ANF receptors.

Dr. de Bold will define ANF biochemically and will analyze its production, storage and processing in the brain and peripheral tissues.

Dr. Inagami will describe the ANF forms in hypothalamus and other brain areas, its mechanism of secretion from neuronal tissues and its central antagonism of angiotensin II-induced hypertension.

Dr. Glembotski will compare the atrial and brain ANF molecular forms, and the molecular characteristics and the brain and peripheral ANF receptors using chemical crosslinking techniques, which demonstrate tissue-specific receptor heterogeneity.

Dr. Saper will describe the immunohistochemical evidence for the ANF system of neurons in the brain and will demonstrate that centrally administered ANF can inhibit baroresponsive vasopressin neurons.

Dr. Quirion will describe the characterization and distribution of brain ANF binding sites, including in human brain, their differences and similarities with peripheral sites, their alterations in disease states and their coupling to second messengers and other neurotransmitter systems.

Dr. Negro-Vilar will present evidence for a physiological antagonism between ANF and angiotensin in the central nervous system, with special emphasis on their interactions in relation to water intake.

The localization of brain ANF and its receptors, their brain-specific molecular forms, the central actions of ANF to decrease drinking, to lower blood pressure in hypertensive animals and to inhibit vasopressin secretion and saline preference, together with the very low numbers of brain ANF receptors in genetically hypertensive rats, and the presence of ANF receptors in the choroid plexus indicate that this peptide has important actions in central fluid and cardiovascular regulation and in the production of cerebrospinal fluid.

PROCESS OUTGROWTH IV

337.1 TIME-LAPSE STUDIES OF ENCOUNTERS BETWEEN SENSORY NEURON GROWTH CONES AND MOTONEURONS. M. G. Honig. Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109

In the chick hindlimb, sensory neurons grow along the appropriate pathways from the outset during normal development, but sensory neuron pathway selection is aberrant in the absence of motoneurons (Landmesser and Honig, Dev. Biol. 118:511). This result suggests that motoneuron (MN) axons guide outgrowing sensory neuron (SN) axons. To further address this possibility, I have begun to examine how the growth cones of SNs respond when they encounter the processes of MNs in dissociated cell cultures.

MNs and SNs were distinguished from one another and from other spinal cord neurons by labeling with highly fluorescent carbocyanine dyes (Honig and Hume, J. Cell Biol. 103:171). Prior to culturing, MNs from St. 25-27 embryos were retrogradely labeled by injection of the red dye, diI, into the lumbosacral spinal nerves. SNs were labeled green by dissociating lumbosacral dorsal root ganglia from St. 28-31 embryos in the presence of a second dye, diO. The cells were plated onto glass which had been coated first with polyornithine and then with laminin. The processes of the neurons were brightly labeled for the first few days in culture, during which time their interactions (in regions not complicated by the presence of other cells and processes) were assessed by videotaping. In most cases, the MN neurite under examination appeared to be an axon in that it ran a fairly long distance before branching.

A variety of behaviors have been seen. In some cases, the SN growth cone crossed the MN process. In other cases, the growth cone reoriented after its filopodia contacted the neurite, and instead of crossing, the growth cone grew roughly parallel to the neurite, typically separated by a distance of 5 - 20 μm, making repeated filopodial contact as it progressed. SN growth cones fasciculated only very rarely and for only short distances before diverging when they encountered MNs. This is likely to reflect, at least in part, the highly adhesive nature of the substrate, since fasciculation was also rare and brief when SNs encountered other SNs.

Finally, a common response was that the SN growth cone retracted from the MN. Sometimes this was followed by one of the behaviors described above or by growth in a direction away from the MN; in other cases the growth cone failed to progress any further for up to several hours. This last result is similar to the finding of Raper and Kapfhammer (Neurosci. Abst. 12:1335) that the growth cones of neurons originating from the peripheral nervous system (e.g. sympathetic neurons) tend to retract after they contact neurites of CNS origin (e.g. retinal neurons). However, the interactions between SN growth cones and MN processes (which normally encounter one another in the developing embryo) appear to be more varied and complex than those reported to occur between the more disparate sets of neurons. (Supported by NS21043 to R. Hume)

337.2 **STEREOTYPED PATHFINDING BY IDENTIFIED GROWTH CONES IN THE EMBRYONIC SPINAL CORD.** J.Y.Kuwada, A.Chitnis, & L.A.Lindamer. Dept. Biology & Institute of Gerontology, University of Michigan, Ann Arbor, MI 48109.

We are investigating how growth cones get to their targets in the simple spinal cords of zebrafish embryos by injecting dyes into individual neurons, backfilling neurons with axons in a single spinal tract with dyes, and by applying a monoclonal antibody (MAb ON1) we have generated that appears to recognize a subset of neurons in the embryonic CNS. MAb ON1 was generated by injecting membranes from optic nerves of adult carp into mice. It recognizes some of the axons in the early (up to 30 hr) embryonic CNS including the reticulo-spinal interneurons, neurons in the nucleus of the median longitudinal fasciculus (MLF), the C1 commissural neurons, and the Rohon-Beard neurons, but not the primary motor neurons nor any of the retinal neurons. By 52 hr MAb ON1 recognizes the segmental motor nerves, and in young adults many axonal tracts and nerves, including the optic nerve, are recognized.

Recent studies of axonal outgrowth in the embryonic fish (Eisen, et al., Nature, 1986; Kuwada, Science, 1986) demonstrated that the growth cones of identified neurons in the fish cord grow along precise routes to reach their targets. Likewise, we report that other embryonic growth cones extend in the embryonic CNS in a stereotyped fashion. The MLF contains the axons of a number of identified neurons in the fish CNS (Kimmel, et al., J.Comp. Neurol. 1982). Many of these neurons grow directly into the MLF. One of the most conspicuous hindbrain neurons, the M cell, projects its growth cone following approximately 18 hours of development at 29° C. In the next few hours it extends posteriorly and medially to cross the midline before proceeding posteriorly into the ventral portion of the spinal cord. Other large identified hindbrain neurons (RoL2, MiD1c, & MiD2c) grow into the MLF several hours after the M cell. These cells are followed by the neurons of the nucleus of the MLF found in the midbrain. Likewise, embryonic cord neurons project growth cones in a stereotyped fashion into their correct tracts. There are a set of large commissural interneurons that appear to be homologous to the C1 interneurons found in the embryonic cord of the Japanese medaka fish. These neurons appear not to be distributed in a strict segmental fashion: one is found every 1 or 2 hemi-segments and one does not always have a bilateral homologue in the same segment. By 22 hours of development the C1 neurons in anterior segments have projected growth cones that have extended ventrally and circumferentially, crossed the ventral midline and begun to grow anteriorly and dorsally on the other side. In the next several hours these growth cones extend into the mid-dorsal tract just ventral to the dorso-lateral fasciculus, the dorsal most spinal tract.

We are presently using the single cell techniques and generating more MAbs to learn more about how growth cones reach their targets in the vertebrate CNS.

Supported by research grants from NIH and the U of M.

337.3 INTRINSIC CELL POLARITY AND ORIENTATION OF AFFERENT PIONEER AXON OUTGROWTH IN THE GRASSHOPPER EMBRYO. Frances Lefcort and David Bentley. Neurobiology Group and Department of Zoology, University of California, Berkeley, CA, 94720.

In vitro, the site of growth cone emergence from neuroblastoma cells has been shown to be associated with microtubule organizing centers (Spiegelman et al, Cell 16, 253; 1979). What role, if any, might cellular control of the site of growth cone emergence play in the orientation of axon outgrowth in vivo? The pair of Ti1 pioneers are the first afferent neurons to initiate axonogenesis in grasshopper limb buds (Bate, Nature 260,54;1976). They are the progeny of an ectodermal mother cell situated at the distal tip of the limb, and extending from the apical to the basal surface of the epithelium (Keshishian, Dev.Bio. 80, 388; 1980). The mitotic spindle of this cell is aligned with the limb axis when it divides to produce the two pioneers, which thus have mirror image symmetry. In over 99% of limbs, the growth cone of the proximal pioneer emerges from the cell pole and is aligned with the former axis of the mitotic spindle (as indicated by the alignment of the pioneer nuclei). Therefore, while various extrinsic cues appear to orient these growth cones, it is possible that the initial orientation of axon outgrowth is due to intrinsic information (Bentley and Caudy, CSH Symp. Quant. Biol. 48, 573; 1983).

In most limbs, the distal pioneer extends its growth cone in close apposition to that of the proximal pioneer. In the minority of cases where this does not occur, the growth cone of the distal cell emerges from its distal pole and is also aligned with the former axis of the mitotic spindle, so that the two pioneers have mirror image configuration. This also suggests that growth cone emergence is initially determined by internal features of the cell.

How important might this internal information be in directing initial axon outgrowth from the pioneers toward their proximally located target, the CNS? To test this, we exposed grasshopper embryos to a pulse of 0.1 ug/ml cytochalasin D for 0.5 to 3 hours after the pioneer mother cell had divided, but before the initiation of pioneer axonogenesis, and then cultured the embryos for an additional twenty-four hours. Following cytochalasin treatment, some pioneer neurons initiated axonogenesis in non-proximal directions. In most cases, these growth cones reoriented proximally within 50 micrometers. Therefore, if internal information does control the direction of axon emergence (at least from the proximal pioneer), this factor is not essential for guidance of the axon in a proximal direction. On the other hand, it seems likely that internal information does control the site and direction of initial axon outgrowth, and that this underlies the very high incidence of proximally oriented axonogenesis.

337.4 THREE GENES ACT TOGETHER TO GUIDE SOME MIGRATING CELLS AND AXONS IN *CAENORHABDITIS ELEGANS*. B.D. Stern*, N. Ishii*, E.M. Hedgecock*, J. Culotti, and D.H. Hall. Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, NJ 07110, Mount Sinai Hospital Research Institute, Toronto, Ontario, and Albert Einstein College of Medicine, Bronx, NY.

Over fifty mutations in the genes *unc-5, unc-6, and unc-40,* disrupt the dorsalward or ventralward migrations of neuron growth cones, and other cells, along the epidermis in the nematode *Caenorhabditis elegans*. Mutations in the *unc-5* gene selectively disrupt dorsalward migrations, which include migrations of the axons of ventral nerve motorneurons, and of the excretory canals, the head mesodermal cell, the hermaphrodite distal tip cells, and the male linker cell. Mutations in the *unc-6* gene selectively disrupt these dorsalward migrations as well as ventralward migrations, which include migrations of the axons of lateral sensory neurons, and of the hermaphrodite anchor cell, and the male linker cell. In *unc-5* and *unc-6* mutant animals the axons of ventral nerve motorneurons which normally grow along the epidermis to the dorsal nerve are instead found in lateral positions. Mutations in the *unc-40* gene disrupt primarily the abovementioned ventralward migrations. In *unc-6* and and *unc-40* mutants the axons of the lateral sensory cells which normally grow ventrally into the ventral nerve are instead found to wander about and only sometimes reach the ventral nerve. Thus, in large part, the defects found in *unc-5* and *unc-40* mutants are each a subset of the defects found in *unc-6* mutants. Interestingly, certain *unc-6* hypomorphic mutations preferentially disrupt either the dorsalward or ventralward migrations mentioned above.

These mutants suggest that neuron growth cones and migrating mesodermal cells share some guidance cues provided by the epidermis. Nerve growth cones migrate between the epidermal cell membrane and the epidermal basal lamina, while the migrating mesodermal cells have direct contact with only the basal lamina. Thus, it seems likely that some of the guidance molecules coded for by these genes are located within the epidermal basal lamina.

One model to explain the functioning of these guidance genes is that the wild type*unc-6* gene product is a core molecule produced by all the epidermal cells. This molecule is modified by graded expression of the wild type *unc-5* (dorsal > lateral > ventral) and *unc-40* (ventral > lateral > dorsal) gene products. An alternative model is that the wild type *unc-5, unc-6,* and *unc-40* gene products are selectively expressed in the dorsal, lateral, and ventral epidermal cells, respectively. Using the technique of mosaic analysis we are attempting to determine the sites of expression of these genes.

We are currently attempting to molecularly clone these genes by the method of transposon-tagging. We have identified transposon-containing bands in *unc-5* and *unc-6* mutants, by Southern blot, which are presumed to be within these genes. A molecular understanding of these gene products should add to our current knowledge of the mutant phenotypes and help to understand how these genes function in cell guidance.

337.5 PEPTIDE MAPPING DEMONSTRATES THE EXISTENCE OF A FAMILY OF SENSORY NEURON AND GLIAL CELLS SPECIFIC 130 KD LEECH GLYCOPROTEINS. M.L. BAJT AND B. ZIPSER. DEPT. PHYSIOLOGY, MICHIGAN STATE UNIVERSITY, EAST LANSING, MI 48824.

Cell surface glycoproteins play important roles in the formation of brain patterns. The modulation hypothesis, advanced by Edelman, postulates that a small number of very ubiquitous adhesion molecules, among them N-CAM, can mediate pattern formation provided that their binding activities are epigenetically modulated. More restrictively distributed surface glycoproteins of 130 kD molecular weight were isolated from the leech (Haemopis, Hirudo) nervous system through monoclonal antibodies. In the adult leech, they a) define the system of sensory afferents (Lan3-2) and b) divide the system into three progressively smaller subsets (Laz2-369, Laz6-212, Laz7-79). Evidence consistent with the hypothesis that these surface glycoproteins play a role in specific axon tract formation comes from regeneration studies. A fifth glycoprotein of similar molecular weight, expressed by glial cells enveloping axons (Laz6-297), may provide a general permissive substrate for axonal growth.

We have begun a structural analysis of the group of 130 kD proteins asking whether they constitute a protein family in which individual members share a common protein core but differ in their oligosaccharides. Here, we are characterizing the protein cores in peptide mapping studies, using as probes our 5 mabs which appear to be directed against carbohydrate epitopes. Peptide mapping studies in SDS-PAGE gels using limited proteolysis with Staphylococcus aureus V8 Protease (0.2 to 2.0 ug/ml) demonstrate that the 130 kD band can be digested into several distinct smaller molecular weight fragments as analyzed by Western blotting. Proteolytic digestion of the 130 kD glycoprotein expressed by the full set of sensory afferents (Lan3-2), resulted in fragments centered around 92, 80, 45, and 40 kD. Proteolytic digestion of the 130 kD glycoprotein expressed by largest subset (Laz2-369) revealed similar molecular weight fragments: 90, 79, and 45 kD. The glial cell protein has a slightly lower molecular weight, about 125 kD, and proportionately its proteolytic fragments also have a slightly lower molecular weight which may arise from less glycosylation. Proteolysis of the 125 kD glial cell glycoprotein include 76, 36, and 33 kD fragments. Thus, the different neuronal and glial 130 kD glycoproteins are cleaved into similar peptide fragments when exposed to limited proteolysis. These results provide evidence in favor of a 130 kD protein family with a common, differentially glycosylated, core protein.

Our studies suggest that recognition molecules may come as epigenetically modified protein family which reduces the number of gene products necessary for creating specific brain patterns.

337.6 DEVELOPMENTALLY REGULATED APPEARANCE AND MODIFICATION OF SURFACE GLYCOPROTEINS LOCALIZED TO SPECIFIC CELLS IN THE LEECH EMBRYO. E.K. McGlade-McCulloh* and B. Zipser (Spon:K.J. Muller) Dept. Physiol. & Biophys., Univ. of Miami, Miami, FL 33101 and Dept. Physiol., Michigan State Univ., East Lansing, MI 48824

The development and regeneration of neurons relies upon cell-cell interactions which may be mediated by surface glycoproteins functioning as recognition/adhesion molecules. We have characterized the spatial and temporal expression of cell-type specific glycoproteins recognized by the monoclonal antibody (Mab) Lan3-2 during neuronal development in the leech, Hirudo medicinalis.

Fusiform cells located along the midline between primordial hemiganglia are the first cells during development of the CNS which are recognized by Mab Lan3-2. The midline cells are first found in anterior segments by day 7 and gradually appear more posteriorly forming a rostral-caudal chain over the course of 2 to 4 days. These midline cells occupy a landmark position toward which primordial ganglion cells move from their early bilateral positions. Shortly after the bilateral hemiganglia join, the midline cells cease to express antigen and may disappear. The midline cell's location and the timing of its transient existence suggest that it may have a role in the movement and fusion of primordial ganglia. Mab Lan3-2 later recognizes growing sensory afferent axons which continue to be recognized by the Mab into adulthood. Thus, within the nervous system of the embryonic leech the glycoproteins are expressed by two different cell types: 1) early, presumably transient cells and 2) sensory afferents which differentiate at a slightly later stage.

On immunoblots, embryonic (day 8) glycoproteins recognized by Mab Lan3-2 were of a higher molecular weight than those from adults. Adult neuronal glycoproteins occur as a major broad band centered at 130 kD and minor bands at 103 and 95 kD. In contrast, the embryonic forms of these glycoproteins occur as a major broad band centered at 160 kD and two minor bands at 140 and 130 kD. Additionally, there is a 240 kD glycoprotein species expressed by embryonic cells. Other surface glycoproteins (e.g. NCAM) undergo a molecular weight conversion during development which is postulated to play a role in brain pattern formation. In other systems, surface glycoproteins play a role in cell-cell or cell-matrix interactions and may serve as receptors for recognition molecules such as nerve growth factor, fibronectin and/or laminin. Perhaps transiently expressed glycoproteins of the fusiform midline cells in the leech play a role in gangliogenesis. (Supported by NIH grants RO1-NS20607 & 5T32-NS07044-11.)

337.7 Labelling of Subsets of Early Forming Axon Tracts in the Rat by a Monoclonal Antibody to the Limbic System-Associated Membrane Protein (LAMP). H. L. Horton* and P. Levitt, Dept. of Anatomy, Medical College of Pennsylvania, Philadelphia, Pa. 19129.

Unique cell surface molecules may play a role in the formation of specific neuronal connections by serving as recognition molecules in the processes of pathfinding and synaptogenesis. Such a molecule would be distributed on functionally and anatomically related neurons, as is the Limbic System Associated Membrane Protein (LAMP) (P. Levitt, Science 223:299, 1984). In the present study, we found an early and selective expression of LAMP on neurons and developing axon pathways. Dot-blot assays demonstrate the presence of LAMP-immunoreactivity by E13, followed by a substantial increase during fetal development. Immunocytochemical staining of fetal brains reveals that LAMP is expressed in both cellular areas and on certain fiber tracts during development, including the fimbria/fornix, stria terminalis, internal capsule and corpus callosum. Neurons which will express LAMP in adulthood appear to express the antigen shortly after they are born. For example, in the thalamus the earliest staining occurs by embryonic day 15, about 24 hours after the earliest birthdates (J. Altman and S. A. Bayer, JCN 188:473, 1979). At this time, subsets of fibers of the internal capsule are also immunoreactive. These appear to originate from the newly generated LAMP-immunoreactive thalamic neurons. The selective expression of LAMP on subsets of the internal capsule fiber bundles can readily be seen by comparing cresyl violet and LAMP-stained sections. This is being confirmed by double labeling of frozen sections with antibodies to LAMP and neurofilament protein and by electron microscopic analysis. The LAMP-immunoreactive fibers can be traced during fetal development to specific cortical areas that also express the cell surface protein. Similar patterns of staining, on subsets of LAMP-immunoreactive fibers originating from LAMP-immunoreactive cells, also occur within the corpus callosum and fimbria/fornix. The presence of a common epitope on related neurons and fibers suggests that LAMP may be a model target and pathway recognition molecule in the developing vertebrate brain. Supported by NSF BNS-8645272 and March of Dimes Basic Research Grant 1-919.

337.8 **CHARACTERIZATION AND CLONING OF FASCICLIN II IN GRASSHOPPER** A. Harrelson, K. Zinn*, P.M. Snow*, M. Bastiani, J. Schilling*,[1], and C.S. Goodman. Dept. Biol. Sci., Stanford University, Stanford CA 94305, and [1]California Biotechnology.

One important form of neuronal recognition during development is the selective affinity that growth cones display for specific axon pathways. Previous cellular studies using insect embryos gave rise to the labeled pathways hypothesis which predicts that axon fascicles in the embryonic neuropil are differentially labeled by surface recognition molecules. To identify candidates for such molecules, we generated monoclonal antibodies (MAbs) which recognize surface antigens expressed on subsets of axon fascicles in the grasshopper and Drosophila embryos. We used these MAbs to identify and purify three different surface glycoproteins which meet these criteria: two in grasshopper (fasciclin I and II; Cell 48:745, 1987) and one in Drosophila (fasciclin III; Cell 48:975, 1987). We are using molecular genetic approaches to determine the structure of these proteins and to study their function.

The fasciclin II (FII) protein is localized primarily on longitudinal axon bundles in the developing grasshopper CNS. At an early stage of development it is expressed on the growth cones and transiently on the cell bodies of only a few neurons. The protein first appears on the most medial of the three major longitudinal tracts, soon thereafter on the middle tract, and finally on the most lateral tract. After 50% of development, the protein is localized on all of the longitudinal tracts but on none of the commissural pathways. Thus FII is regionally expressed on only certain portions of the neurons. For example, axons express FII where they run in longitudinal tracts, but do not express it where they run in commissural bundles.

FII was purified and the amino acid sequences of portions of two proteolytic fragments were determined. This information was used to design oligonucleotide probes which were used to isolate clones encoding FII from grasshopper genomic and cDNA libraries. We also isolated a FII cDNA clone by screening a cDNA expression library with an antiserum against FII; this clone also hybridizes to the oligonucleotide probe. We are presently determining the structure of FII by cDNA sequence analysis, and searching for the homologous gene in Drosophila.

337.9 **MOLECULAR GENETIC ANALYSIS OF FASCICLIN I FROM GRASSHOPPER AND DROSOPHILA: SEQUENCES OF cDNA CLONES ENCODING MULTIPLE FORMS OF THE PROTEIN** L. McAllister*, K. Zinn*, P.M. Snow*, T. Elkins, J. Schilling*,[1], G. Makk*,[2], and C.S. Goodman (SPON: M.J. Bastiani). Dept. Biol. Sci. and [2]Dept. Psychiatry, Stanford University, Stanford CA 94305, and [1]California Biotechnology.

We previously reported the purification and characterization of fasciclin I (FI), a 70 kD cell-surface glycoprotein which is expressed on a subset of axon pathways in the grasshopper embryo (Cell 48:745, 1987). We have since identified a second, 37 kD form of FI. Both forms of FI are membrane-associated, but neither is an integral membrane protein, as both can be stripped from membranes by high pH treatment. We have determined the amino acid sequence of the N-terminus and of portions of two fragments from the 70 kD FI. This information was used to design oligonucleotide probes which were used to isolate clones encoding FI from grasshopper genomic and cDNA libraries.

Northern blot analysis indicates that there are at least three FI mRNAs (~3.6, 3.2, and 1.8 kb in length). We have isolated full-length cDNA clones corresponding to each of these size classes, and have determined the sequences of a 3.2 and 1.8 kb clone. These two cDNAs encode proteins of 70 kD and 37 kD, respectively. The 70 kD protein consists of a signal sequence, three imperfect copies of a 37 amino acid sequence spaced at 150 a.a. intervals (suggesting three structural domains), and no transmembrane region. The third copy of the repeat is truncated and is immediately followed by a very basic C-terminal sequence. The 37 kD protein corresponds to the N-terminal half of the larger protein (containing only the first of the three domains), followed by a short unique C-terminal peptide. We are generating antibodies against the unique C-terminal portions of the two forms of FI in order to study their localization in the grasshopper embryo.

We have used the grasshopper cDNA to isolate homologous cDNAs from Drosophila. These also exist in multiple forms. Limited sequence analysis indicates that the Drosophila sequence is 60-70% homologous to grasshopper FI. The Drosophila FI gene maps to position 49F on the polytene chromosomes. We are currently generating antibodies to Drosophila FI for localization studies, and making deletion mutatations to determine its function during neuronal development.

337.10 **GENETIC ANALYSIS OF FASCICLIN III IN DROSOPHILA: DELETION OF THE GENE LEADS TO ABNORMAL AXON FASCICULATION** J. R. Jacobs, N. H. Patel*, T. Elkins and C. S. Goodman. Dept. Biol. Sci., Stanford University, Stanford CA 94305.

We previously reported the characterization and cloning of fasciclin III (FIII), a group of highly related surface glycoproteins which are expressed on a subset of neurons and axon pathways in the Drosophila embryo (Cell 48:975, 1987). Here we report on genetic and ultrastructural studies aimed at determining the function of FIII.

In each segment of the CNS at hr 11, FIII is expressed on the surface of 52 of the 153 axons in the two commissures; these axons are fasciculated in 5 bundles (3 in the anterior and 2 in the posterior commissure). In addition, FIII is expressed on the continuation of one of these fascicles as it turns posteriorly and laterally toward the intersegmental nerve, and on the medial nerve. FIII is also expressed on parts of glial cells which contact these labeled axons. FIII is regionally expressed on only certain portions of the neurons. For example, axons express FIII where they run in a labeled commissural fascicle, but then cease to express it where they turn longitudinally.

The FIII gene maps to position 36E1 on chromosome 2L. The gene is deleted in embryos which are trans-heterozygous for the deficiencies VA18 and H20. These embryos show a clear but subtle abnormal phenotype in the developing CNS. In contrast, other deficiencies which delete flanking genes but not the FIII gene, do not show this phenotype.

At the ultrastructural level, the mutant phenotype fits the predictions based on normal FIII expression. The anterior commissure in particular is broader in the mutants due to abnormal trajectories of many commissural axons. The RP1/RP2 neurons (which normally express FIII) fail to complete their normal migration towards the midline. The trajectory of the RP1 axon is often abnormal and variable from segment to segment. Other axons which normally express FIII are also abnormal in their trajectory. These results suggest that fasciclin III is a neuronal recognition molecule involved with selective fasciculation and other specific cell interactions. We are presently screening for lethal point mutations in the FIII gene, and reconstructing the CNS in mutant vs. wild type embryos.

337.11 **MOLECULAR ANALYSIS OF FASCICLIN III IN DROSOPHILA: SEQUENCES OF cDNA CLONES ENCODING MULTIPLE FORMS OF THE PROTEIN.** P.M. Snow*, N.H. Patel*, and C.S. Goodman. Dept. Biol. Sci., Stanford University, Stanford CA 94305.

In the previous abstract we reported on genetic and ultrastructural studies aimed at determining the function of fasciclin III (FIII), a group of highly related surface glycoproteins which are expressed on a subset of neurons and axon pathways in the Drosophila embryo (Cell 48:975, 1987). Here we report on a molecular genetic analysis of FIII cDNA clones aimed at determining the sequence and structural domains of the different forms of the protein.

Immunoprecipitation experiments indicate that FIII consists of at least four distinct but related proteins (80, 66, 59 and 46 kD). Two dimensional peptide maps indicate that the proteins are highly related at the level of their primary structure. The proteins were purified by immunoaffinity chromatography and antisera generated against each of the four forms. The antisera were used for cDNA expression cloning to identify a single gene. That this gene encodes FII was confirmed by in situ hybridization and genetic deficiency analysis. Northern blot analysis identified at least four mRNAs, suggesting that different mRNAs might encode the different forms of the protein.

In order to identify full length cDNA clones encoding the different forms of the protein, several other cDNA libraries were screened with one of the initial cDNA inserts. These screens resulted in the isolation of 50 cDNA clones which fell into at least two classes based on Eco R1 restriction sites. The longest representatives of each class were subcloned and sequenced. The two cDNAs encode proteins which are identical at the N-terminus (apparent extracellular domain), but which diverge at the C-terminus. At least one of the two possesses a transmembrane region characteristic of integral membrane proteins. Antisera against peptides specific for each of the two forms are being generated in order to study their tissue distribution in greater detail. In addition, the cDNA clones are being used to screen for other genes encoding potentially related proteins.

337.12 THE 5B4 ANTIGEN EXPRESSED ON SPROUTING NEURONS IS A MEMBER OF THE NCAM FAMILY. Leland Ellis[1], Purita Ramos[1,*], Roohangiz Safaei[2,*] and Celik Kayalar[2,*]. Howard Hughes Medical Institute and Department of Biochemistry, University of Texas Health Science Center, Dallas, Tx. 75235-9050[1]; Department of Chemistry, University of California, Berkeley, Ca. 94720[2].

Monoclonal antibody (mAb) 5B4 recognizes a developmentally regulated membrane glycoprotein whose expression on neurons is coincident with neuronal sprouting (Ellis et al., J. Cell Biol. 101 1977-1989, 1985; Wallis et al., J. Cell Biol. 101 1990-1998, 1985). In fetal rat brain, mAb 5B4 identifies a diffuse antigen of ~ 185-255 kDa, while the antigens recognized in adult rat brain are of ~ 180 and 140 kDa. Furthermore, the 5B4 antigen expressed in fetal brain carries the unusual carbohydrate moiety α-2,8-linked polysialic acid (Rosenberg et al., Dev. Brain Res. 30 262-267, 1986), which is a characteristic of neural cell adhesion molecules (NCAMS; Rutishauser, Nature 310549-554, 1984; Edelman, Ann. Rev. Biochem. 54135-169, 1985).

To further explore the relationship of the 5B4 antigen to the NCAM family, we utilized mAb 5B4 to screen a λgt11 library prepared from mRNA of postnatal week two rat brain. λ5B4.1 contains a cDNA insert of ~ 500 bp, and encodes a β-galactosidase fusion protein of ~ 130 kDa which reacts on a Western blot with mAb 5B4. The DNA sequence of this insert reveals an open reading frame of 95 amino acids, whose sequence is ~ 70% homologous with the carboxy terminus of chicken NCAM (Hemperly et al., PNAS 83 3037-3041, 1986). The localization of the 5B4 epitope to the cytoplasmic domain of the molecule is consistant with previous results of indirect immunofluorescence studies of cultured neurons with mAb 5B4, where visualization of staining required prior permeabilization of the cultures with the nonionic detergent saponin (see above refs.). Rescreening of the library with the λ5B4.1 insert yielded an additional clone with an insert of ~ 1700 bp. Characterization of this insert by restriction enzyme mapping demonstrates that λ5B4.1 is contained within the 3' end of λ5B4.2. DNA sequencing reveals that the more 5' sequences of λ5B4.2 continues the open reading frame amino proximal. This deduced amino acid sequence shares significant homology with chicken NCAM extending from the end of the extracellular domain through the transmembrane and cytoplasmic (the so-called long form; see above reviews) domains.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: VESTIBULAR SYSTEM II

338.1 SENSORY CODING BY THE VESTIBULAR LABYRINTH OF THE LARVAL FROG. S.L. Cochran and J.T. Hackett. Dept. Physiology, Univ. of Virginia Med. School, Charlottesville, VA 22908.

Tadpoles (Rana catesbeiana, premetamorphic) exhibit a prominent ocular counter-rolling in response to tilt of the animal. The gain of this static reflex is approximately one. The head of these tadpoles can be maintained in vitro with the brain exposed (forebrain removed) and shows spontaneous and light-aroused eye movements and swimming-like contractions of the neck muscles. The compensatory response of the eyes to head tilt persists in the in vitro head preparation for several days following isolation, facilitating elucidation of the neuronal networks mediating these reflexes. Bilateral section of the optic nerves does not affect the static gravitationo-ocular reflexes, while lesions of the VIIIth nerves abolish them. Examination of the labyrinth reveals that the inner ear end organs are structurally and functionally similar to those of the adult frog. Hair cells and VIIIth nerve afferents are well-developed in the amphibian papilla, lagena, sacculus, utriculus, and the three canals. Synaptic profiles between the hair cells and the afferents show the same specializations at the electronmicroscopic level (i.e.--chemical, Type I) as are found in the adult. Efferent terminals also contact the hair cells. In the isolated labyrinth, intracellular recordings from individual afferents (at their exit from the end organs) reveal the presence of spontaneously-occurring EPSP's that often provoke action potential generation. Kynurenic acid (bath-applied), a glutamate receptor antagonist, reversibly reduces the amplitude of these EPSP's, indicating that the pharmacology of this synapse is glutamatergic, as is that of the adult. Intracranial recordings from these afferents in the isolated head suggest that the tadpole brain receives similar sensory information as its adult counterpart. Some cells respond selectively to rotation about specific axes (yaw, pitch, roll) in a phasic manner with no apparent response to static head position per se. These dynamic units most likely arise from the canals. Other axons respond to vibration and probably are saccular in origin. Within the anterior portion of the VIIIth nerve, there are units (apparently small), whose tonic discharge rate is related to head angle with respect to the horizontal plane implying these fibers communicate gravity sensing information and most likely arise from the utriculus. Most of these axons respond phasically to tilt as well. These preliminary studies of the vestibulo-ocular reflex in vitro suggest that the vestibular labyrinth in the tadpole conveys similar specialized sensory information as it does in the adult. The decrease in the gain of this reflex as the tadpole develops into a frog is thus more likely due to central than to peripheral reorganization of the neuronal networks involved in this reflex.

Supported by NSF grants BNS 84-06259, BNS 86-16738 and by NASA.

338.2 THREE-NEURON VESTIBULO-OCULAR REFLEX ARC IN THE ADULT FLATFISH: EXCLUSIVE ROLE OF SECOND-ORDER VESTIBULAR NEURONS FOR THE ADAPTATION OF COMPENSATORY EYE MOVEMENTS. W. Graf and R. Baker. The Rockefeller University, New York, NY 10021, and Dept. Physiol. & Biophysics, New York University Med. Ctr., New York, NY 10016.

Flatfish offer a natural paradigm for studying adaptive changes in the vestibulo-ocular reflex (VOR) because of the 90 deg relative displacement of vestibular versus extraocular muscle coordinates after metamorphosis. Structurally, this arrangement requires a neuronal pathway from the horizontal semicircular canals to muscles that move the eye vertically (J. Neurophysiol. 54: 887-889, 900-916, 1985). The present study in the winter flounder, Pseudopleuronectes americanus, extends the previous work to include the entire three-neuron reflex arc of the VOR. We have focused on the bilateral symmetry of the vestibular system, especially the projections of primary vestibular afferents, and on the trajectories of axons ascending in the MLF. Single cell morphology of second-order vestibular neurons linked to the right or left horizontal canal, visualized with the intracellular HRP injection method, revealed a qualitatively bilaterally symmetric distribution of neurons with terminals in either both superior rectus and inferior oblique motoneuron pools or with the antagonists to these muscles, the inferior recti and superior obliques (trochlear nuclei). Surprisingly, all second-order neurons had contralaterally ascending main axons, and even some cell somata of stained neurons were found contralateral to the stimulated horizontal canal. The latter organization, in particular, has never been observed in any other vertebrate VOR system. Vertical canal related second-order neurons were not different from that described in other vertebrates. Visualized by extra- and intracellular HRP methods, primary afferents from the semicircular canals terminated in the five subnuclei of the vestibular complex (anterior, magnocellular, descending, tangential, posterior), and also in the eminentia granularis and the medial reticular formation, as described in other teleost fishes. The intracellular HRP data of primary vestibular afferents showed that no axon collaterals crossed the midline, and thus offered little explanation for the peculiar contralateral location of vestibular nucleus neurons.

In summary, our data indicate a thoroughly bilateral symmetric distribution of post-metamorphic second-order VOR neurons for both excitation and inhibition linking the horizontal canals to vertical eye muscles. In light of normal primary vestibular afferent, second-order vertical vestibular neuron, and oculomotor organization, the species-specific horizontal vestibular neurons are both necessary and sufficient for adaptation of the VOR in the adult flatfish. - Supported by NIH grant NS20358.

338.3 **GABA-IMMUNOCYTOCHEMISTRY OF THE VESTIBULAR COMPLEX IN WEAVER AND PCD MUTANT MICE.** U. Grüsser-Cornehls* and B.G. Grover* (SPON: European Neuroscience Association) Dept. of Physiol., Freie Universität Berlin, Arnimallee 22, 1000 Berlin 33 (Germany).

In the mutant mouse Weaver (B6CBA) almost all cerebellar granular cells die in the first postnatal weeks, while in the PCD mutant (C57BL6J) almost all Purkinje cells die in the first postnatal month. Cerebellar processing is disrupted in both mutants but they differ in the way in which the malfunction is able to affect downstream structures such as the vestibular complex, as indeed electrophysiological experiments have demonstrated (Grüsser-Cornehls, U., *Soc. Neurosci.*, 8: 524, 1983; Helmchen, Ch. et al., *Pflüg. Arch. Suppl.*, 405: R56, 1985). The present investigation was undertaken to determine whether changes in GABA-immunocyto-chemistry occur in the vestibular nuclei of these mutants and whether they can be related to plasticity mechanisms which might underly normal cerebellar function in relation to motor learning.

In material immuno-reacted for GABA using the PAP method, experiments started with K.-P. Hoffmann and A. Horn in Ulm, stained boutons may be identified in apposition to somata and dendrites. In normal mice GABA positive boutons in the superior and lateral vestibular nuclei range in size from 0.3 to 2 µ in diameter and may be roughly categorized as small, medium and large. The large boutons are comparable in size to those identified in the lateral vestibular nucleus of cats as Purkinje-cell terminals using EM-degeneration methods (Mugnani, E. and Walberg, F., *Exp. Brain Res.*, 4: 212-236, 1967). The fact that in the PCD mutants only small- and medium-sized terminals are found in the lateral and superior nuclei also suggests that the large boutons are of Purkinje-cell origin. In Weaver mutants, stained boutons in these nuclei are almost all of the large size and there is no obvious increase in the number of terminals per unit area. It is not yet clear whether the increase in large GABA terminals in Weavers represents an increase in the number of Purkinje cell terminals at the expense of interneuron terminals or an increase in size of existing terminals.

In contrast, cytoplasmic staining of GABA positive cells, in the vestibular nuclei only, is consistently weaker in Weaver mutants than in normals or PCD mutants, suggesting either a depletion of intracellular GABA stores due to higher release rate or a turning off of GABA production in vestibular nuclei GABA-ergic neurons in Weavers.

The immunocytochemical results suggest that the derangement of cerebellar function in Weaver (in which the anatomical integrity of the output channels is preserved) results in a cascade of compensatory mechanisms located extrinsicly to the cerebellum (profusion of large terminals and changes in cytoplasmic GABA levels in the vestibular nuclei). Such mechanisms, on a smaller scale, could partially underlie the 'cerebellar' contribution to motor learning.

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338.4 CONVERGENCE OF NECK AND VESTIBULAR INFLUENCES ON NEURONS IN THE VESTIBULAR NUCLEI OF THE DECEREBRATE CAT. J. Kasper*, R.H. Schor, and V.J. Wilson. The Rockefeller University, New York, NY 10021.

When the head moves on the body, the tonic neck and vestibular reflexes interact to produce a coordinated postural response in the limbs. Part of this convergence takes place in the vestibular nuclei (Boyle and Pompeiano, J. Neurophysiol. 45, 1981). We examined the spatial and temporal properties of this interaction by recording the responses of neurons in the lateral and inferior vestibular nuclei. Stimuli consisted of combinations of sinusoidal rotations in vertical planes (roll, pitch, intermediate planes) delivered to the whole animal (vestibular stimulus), body with stationary head (neck stimulus), and head with stationary body (combined).

We found 44 cells which responded to both neck and vestibular stimuli, 15 of them identified as vestibulospinal neurons. The vestibular input to these cells originated in vertical semicircular canals and utricle. All but five neurons responded better to roll than to pitch, with the majority excited by ipsilateral side down tilt. From their response orientations and dynamics, 7 cells appeared to receive input from a single vertical canal, 10 from the utricle, 6 from both vertical canals, and 12 from canal and utricle.

The dynamics of the response to neck stimulation shows, on average, a gain increase (about 3-fold per decade) and phase lead of 30 to 60 degrees over a frequency range of 0.02 to 1 Hz. This response is similar to that seen in neck spindle afferents excited by neck rotation (Chan et al., J. Neurophysiol. 57, 1987).

We determined the orientations of the neck and vestibular response vector (the stimulus direction producing the maximal response) in 33 of these cells. In 21 cells, the vector orientations pointed in opposite directions (within 30 degrees). Only a single cell with vectors in the same direction was observed.

In response to head rotation on a stationary body, neck and vestibular contributions often opposed each other, resulting in no net neural modulation. Such cancellation was observed in 12 neurons, all with opposite neck and vestibular vector orientations and similar response gain and phase to these two stimuli. In 7 neurons, the interaction of these inputs produced a response during head rotation which had fairly flat gain and phase near position at all frequencies, despite the fact that the neck or vestibular signal often showed a considerable dynamic response. Such neurons appear to be encoding head position in space.

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338.5 OPTOKINETIC RESPONSES OF SECOND-ORDER VESTIBULAR NEURONS IN THE RABBIT. J.F. McGurk and W. Graf. The Rockefeller University, New York, NY 10021.

Direction selective retinal ganglion cells provide an important sensory input for the performance of compensatory eye movements. While there have been a number of studies on visual-vestibular interaction in the horizontal system, no information is available regarding the spatial organization of the optokinetic input to vestibular nucleus neurons in the vertical systems.

We recorded intracellularly from second-order vestibular neurons linked to the vertical canals projecting to the oculomotor/trochlear nuclei in the rabbit. Axons of contralaterally (excitatory) and ipsilaterally (inhibitory) ascending neurons were penetrated with micropipettes filled with 2 M KCl in the MLF caudal to the trochlear nucleus. Neurons were identified by their monosynaptic potentials following electrical stimulation via labyrinth implants. Neurons were first characterized with hand-held visual patterns to establish their receptive field properties, and later by a planetarium projector to determine the axis of visual field rotation producing the best response. The planetarium projector could be oriented in any direction in space via a gimble system. Typically, data were taken at nine orientations for binocular and monocular stimulations each.

All recorded vestibular neurons received binocular visual input. Excitatory anterior canal (AC) neurons were excited by upward and backward movement of the hand-held patterns in the anterior three quadrants of the ipsilateral hemifield, and by downward and backward movement in the posterior three quandrants of the contralateral hemifield. This response was consistent with rotation of the entire visual world about an axis passing from approximately 135 deg ipsilateral to 45 deg contralateral (0 deg represents an axis pointing rostrally). Excitatory posterior canal (PC) neurons were excited by upward and forward movement in the ipsilateral posterior three quadrants, and by downward and forward movement in the contralateral anterior three quadrants of the visual field. This response was consistent with rotation about an axis passing from 45 deg ipsilateral to 135 deg contralateral. Ipsilaterally projecting neurons could be driven from any portion of the visual field consistent with rotation about their respective canal axis. Quantitative analysis of fully characterized neurons showed that on average, AC neurons (n=4) responded best to rotation about an ipsilateral axis of 136 deg (AC axis = 135 deg; vertical recti axis = 151 deg), and PC neurons (n=3) responded best at an ipsilateral axis of 43 deg (PC axis = 32 deg; obliques axis = 43 deg). These results demonstrate that the optokinetic input to second-order vestibular neurons is coded in coordinates comparable to the vestibular and/or extraocular muscle sensory and motor axes.

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338.6 **SINGLE UNIT RESPONSES IN THE VESTIBULAR CORTEX OF SQUIRREL MONKEYS.** W.O. Guldin* and O.-J. Grüsser (SPON: European Neuroscience Organization). Dept. of Physiol., Freie Universität Berlin, Arnimallee 22, 1 Berlin 33, Germany.

A new vestibular cortical area is described in Squirrel monkeys (Saimiri sciureus). This area is situated in the fundus of the Fissura lateralis in area ritp (Pandya and Sanides, *Z.Anat.Entwickl.-Gesch.*,139:127,1973) or Ri according to Jones and Burton (*J.Comp.Neurol.*,168:197,1976). A comparable vestibular area was described by Grüsser, Pause and Schreiter (Roucoux and Crommelinck [eds.], *Physiological and Pathological Aspects of Eye Movements*, 251,1982) in the Macaque monkey as the parieto-insular vestibular cortex (PIVC).

About fifty percent of the PIVC neurons recorded in Squirrel monkeys were responsive to vestibular stimulation (sinusoidal pitch, roll or jaw) as well as to large-field visual stimulation. Most units also showed activation during stimulation of neck receptors. A few units also reacted to stimulation of other parts of the body such as limbs or back.

Nearly all recorded vestibularly activated cells responded to rotation in more than one of the semicircular canal planes. Each unit appeared to have a "preferential plane" of rotation in space for which the response is maximal. The data collected suggest that all possible preferential planes are represented in the PIVC. As a rule, PIVC units respond to angular acceleration and not to steady tilt in darkness.

There is some indication in the present data that units with similar preferential planes were located close to one another suggesting the existence of a barrel- or cluster-like organization of the PIVC.

The parieto-insular vestibular cortex seems to be a high-order center for vestibular somatosensory and visual integration that processes information about the localization of the body and head in space.

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338.7 BIOMECHANICAL CONSTRAINTS LIMIT THE NUMBER OF DEGREES OF FREEDOM OF THE VERTEBRATE HEAD-NECK ENSEMBLE: ITS CONSEQUENCES FOR THE POSTURAL SYNDROME FOLLOWING HEMILABYRINTHECTOMY. C. de Waele*, W. Graf, and P.P. Vidal (Sponsor: E.E. Brink). Lab. Physiologie Neurosensorielle du C.N.R.S., 75270 Paris Cedex 06, France, and The Rockefeller University, New York, NY 10021, USA.

In a previous study we demonstrated a stereotyped resting posture of the head-neck arrangement in a number of vertebrates: the cervical vertebral column is oriented vertically, and the horizontal semicircular canals are tilted upward from earth horizontal (Exp. Brain Res. 61: 549-559, 1986).

The present investigation first quantified the range of motion of the different articulations of the head-neck ensemble in monkey, cat, rabbit and guinea-pig by x-ray photography and dissection. Our data indicate that biomechanical constraints limit the number of possible solutions how an animal can perform a given orienting movement. For example, in the sagittal plane, the upper cervical vertebrae allow only flexion, whereas the lower cervical and upper thoracic vertebrae only allow extension of the vertebral column. Although there is about a 90-100 deg range of motion in the atlanto-occipital joint, the head cannot be flexed beyond a certain limit. By comparing these constraints to the head-neck posture of alert unrestrained animals, we conclude that, when at rest, assuming a vertically oriented cervical vertebral column, animals hold their heads at the extreme point of flexion in the atlanto-occipital articulation. Similarly, the cervical and upper thoracic vertebrae are locked into the limits of their range of extension. At this position, mechanical constraints almost exclusively provide the biomechanical means of maintaining resting position with minimum energy expenditure (Soc. Neurosci. Abst. 11, 25.25, 1985). Furthermore, at this position the horizontal semicircular canals will appear tilted upward from earth horizontal. From that resting position, lowering of the head is only possible by moving the C6-Th3 vertebrae out of their extreme extension into the flexion direction. Secondly, a radiological study was performed on the postural syndrome following hemilabyrinthectomy in guinea pigs. X-ray photographs from lateral and from above were taken every 30 minutes during the first 72 hours following surgery. Head position in space resulted from a) lateral head deviation by rotation mainly at C1/C2, b) longitudinal twist of the vertebral column via rotation about C6-Th3, c) crossed extension of the legs. Compensation for each of these deficits followed different time courses.

In conclusion, our results suggest that during normal and pathological motor activity, biomechanical constraints limit the degrees of freedom of the skeleton. These constraints are thought to simplify neuronal operations subserving motor control and plasticity.

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338.8 HEAD AND EYE MOVEMENTS DURING CIRCULAR LOCOMOTION. D. Solomon* and B. Cohen, Dept. of Neurology, Mt. Sinai Sch. of Med. NY, NY 10029

The gain of visual, vestibular and somatosensory inputs on gaze velocity (eye velocity + head velocity) was determined during circular locomotion for two monkeys. Animals wore a harness tethered to a .3 meter radial arm fixed to a freely rotating center post and walked or ran around the perimeter of a circular platform. The platform and post were mounted on a motorized rate table, so that the running animal's velocity in space could be reduced or nulled. Horizontal and vertical eye position in the head was measured with electro-oculography (EOG), and differentiated to obtain eye velocity. An angular velocity sensor (Watson Industries) measured head velocity in the yaw plane. A similar device mounted on the center post measured the animal's net angular velocity re earth. Head on body velocity was determined by subtracting post velocity from yaw head velocity. The animal's gait velocity re the platform was obtained by subtracting platform angular velocity from the animal's angular velocity re earth. One monkey spontaneously circled to the left in his cage, and had asymmetrical responses to passive rotation and optokinetic stimulation. It ran equally well in either direction on the platform, however, with velocities in excess of 300 deg/sec. A second animal had no directional bias. While running in light at a constant velocity, compensatory slow phase gaze velocities were maintained in excess of 200 deg/sec at around unity gain. Phase plots of head vs. eye velocity indicated that both eye and head movement contributed to the slow phase velocity seen during any beat of nystagmus. Eye velocity developed earlier in the slow phase but declined as head velocity increased. Synchronous fast head and eye movements constituted the fast phases. Continuous gaze velocity during running in darkness occurred in directions in which there was substantial velocity storage on passive rotation or during OKN and OKAN. The gain of the steady state slow phase gaze movements during running in darkness was 0.6. In contrast, during passive rotation with the head fixed, slow phase velocities decayed to zero over the dominant time constant of the VOR. When the animal stopped running, attenuated post rotatory nystagmus occurred only in the eyes. The platform was counter-rotated during running to decrease or eliminate angular velocity re earth and linear acceleration. Eye and head nystagmus still occured without vestibular stimulation during stationary running in darkness, and after-nystagmus was observed in the same direction after stopping, as in stepping around in humans, described by Bles (1981). These studies show that combined head and eye velocity is affected by vision, vestibular sensation and somatosensory information. The maintenance of gaze velocity during stationary running in darkness indicates a role for proprioceptive and motor signals in the generation of velocity storage.

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338.9 EFFECTS OF GRAVITY ON THE PRINCIPAL AXES OF VELOCITY STORAGE IN THREE DIMENSIONS. Theodore Raphan and Bernard Cohen. Dept. of Computer and Information Science, Brooklyn College of CUNY, Brooklyn, New York, and Depts. of Neurology and Neurophysiology, Mount Sinai School of Medicine, New York, New York.

Rotation of the visual surround that induces horizontal optokinetic nystagmus (OKN) excites the velocity storage integrator and induces horizontal optokinetic after-nystagmus (OKAN). If horizontal OKN is induced while monkeys are tilted with regard to gravity, then the OKAN which follows has a vertical component; if monkeys are supine or prone then the OKAN has a roll component (Raphan & Cohen, 1987). The cross coupled components of the nystagmus do not appear immediately but build up and decay slowly with dynamics consistent with those associated with velocity storage. This indicates that the principal axes of the velocity storage integrator in three dimensions i.e those axes along which velocity storage is maximal are dependent on the orientation of the head with regard to gravity. The purpose of this study was to determine the orientation of these axes as a function of angle of head tilt in response to optokinetic stimulation. To this end, oblique optokinetic stimuli were given with monkeys upright as well as on their sides. If monkeys were upright when they received an oblique stimulus at any angle, the direction of the OKN was oblique and followed the stimulus but only horizontal OKAN was induced and the vertical component was essentially absent. This is consistent with the fact that that pure vertical OKN with subjects upright is associated with little vertical OKAN (Matsuo & Cohen, 1974). It indicates that the animal's vertical is the principal axes for storage in the upright position. When animals are on their sides, vertical OKAN tends to be stronger and is asymmetrical, being weaker for downward than for upward nystagmus (Matsuo & Cohen, 1974). If subjects were given an oblique optokinetic stimulus such that the cross coupling would tend to produce a vertical component in the same direction as that of the stimulus, the vertical component of both upward and downward OKN and OKAN were stronger. If the oblique stimulus was such that the cross coupling would tend to produce a vertical component opposite to the direction of that component induced by the stimulus, then the vertical component of the OKAN was cancelled. The vertical cross coupling was stronger with greater angles of tilt. Consequently, the angle of the stimulus relative to the animal that augmented or cancelled the vertical component increased becoming more oblique. This tended to maintain the principal oblique axes for velocity storage constant in space. The data suggest that gravity orients the principal axes of the velocity storage integrator towards the space vertical and attempts to maintain them spatially invariant regardless of head orientation.

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338.10 **EFFECTS OF ROTATION ECCENTRICITY & TARGET DISTANCE ON VISUAL SUPPRESSION OF VESTIBULO-OCULAR REFLEX.** J. Goldberg & C. Cox*. Department of Otorhinolaryngology and Clayton Neurotology Laboratory, Baylor College of Medicine, Houston, TX 77030

Body rotation about a vertical axis positioned behind the eyes imparts a linear as well as angular acceleration to the eyes relative to a stationary visual target. We have previously shown that vestibulo-ocular reflex (VOR) gain, measured as the ratio of eye to head angular velocity, exceeds 1.0 in human subjects during such eccentric rotations. Visual suppression of the high-gain VOR can be expected to increase in difficulty with increasing rotation eccentricity and target distance, since VOR gain increases as a function of these kinematic parameters. Alternately suppression could be independent of the kinematics.

Eight normal subjects were rotated sinusoidally at 0.2 Hz, 120°/s amplitude in a servo-driven chair while fixating a small target light attached to the chair. They were seated so that the rotation axis passed near the midpoint of the inter-ocular distance ('concentric rotation') or about 20 cm behind it ('eccentric rotation'). Target position was varied along the midline in a randomized sequence of viewing distances from about 65 cm to the near point of accomodation/convergence of each subject. Every subject could fixate the target at least as close as 20 cm without difficulty. Smooth eye velocity was computed from digitized left eye position measured by electro-oculography. Statistical analysis was carried out on data obtained from each subject for 50 and 25 cm distances during concentric and eccentric rotations.

Suppression of VOR during concentric rotation resulted in mean eye velocity amplitudes of 5.7°/s (range: 0.7-21.9) for the 50 cm distance and 6.5°/s (1.0-26.6) for the 25 cm distance. During eccentric rotation eye velocity increased to 7.2°/s (0.9-27.4) for the 50 cm distance and 8.9°/s (1.9-29.6) for the 25 cm distance. While suppression performance was quite variable between subjects, the effects of rotation eccentricity and target distance on eye velocity were consistent and statistically significant within subjects (two-way ANOVA on repeated measures, $p<0.05$).

Our results show that slow-phase eye velocity increases with rotation eccentricity and visual target proximity during visual suppression of VOR as it does during VOR. The single kinematic factor that differs between the two situations - target rotation in space - does not appear to change this relationship.

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338.11 MODIFICATION OF THE VESTIBULOOCULAR REFLEX AND OPTOKINETIC RESPONSE. H.Cohen, B.Cohen, T.Raphan, Depts. of Neurology, Mount Sinai School of Medicine, N.Y., N.Y. 10029, and Computer and Information Sciences, Brooklyn College, Brooklyn, N.Y. 11210

Habituation and adaptation of the vestibuloocular reflex (VOR) optokinetic nystagmus (OKN), and optokinetic after-nystagmus (OKAN) were studied in rhesus monkeys. Habituation is a relatively permanent decrement in the time constant after repeated testing; adaptation is a temporary incremental or decremental modification of the gain, in response to a change in the stimulus. The goal was to determine the time course of these processes and establish whether either or both are controlled by the cerebellar nodulus and uvula. Animals were tested with steps of velocity about the vertical axis, at velocities varying from 30 to 150 deg/sec while eye movements were recorded with a scleral search coil system. First, the animal was tested repeatedly to habituate the time constant of the VOR to a stable level. Then, the animal wore magnifying lenses (x 2.0) and reducing lenses (x .5) for varying time periods to manipulate the gain. The animal also wore control lenses (x1.0) for 24 hours. The lenses were bolted to the head and the animal was free to move about her cage. The gain of the VOR and OKAN, tested in darkness, adapted approximately 30% after wearing magnifying or reducing lenses for up to 120 hours although substantial adaptation occurred within 4 hours, and most adaptation took place within 24 hours. OKN and OKAN gains were more variable than VOR gains. Time constants changed inversely with changes in gain, decreasing as initial eye velocity increased, and vice versa. This is similar to findings in normal monkeys. Therefore adaptation of the VOR gain does not affect the VOR time constant directly. Upon removal of the lenses the gain returned to preadapted levels. Continued testing, including adaptation trials, reduced the time constants further. The asymptote for habituation of the OKAN time constant was 2-3 sec, and of the VOR time constant was near the cupular time constant. Thus, velocity storage was essentially eliminated in the fully habituated animal.

After adaptation training the nodulus and uvula were surgically ablated in one monkey. Post-operatively, VOR and OKAN gains were affected little. The VOR and OKAN time constants rose immediately to the pre-habituation level and were unaffected by repeated testing. The ability to adapt the VOR gain, however, remained intact. These data suggest that the VOR gain and time constant are controlled separately. Habituation is due to decreased ability to store activity related to slow phase velocity. The nodulus and uvula are involved in control of the VOR time constant, but are not primarily involved in control of its gain.

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338.12 CONDITIONAL ADAPTATION OF VESTIBULOOCULAR REFLEX (VOR) DIRECTION S.I. Perlmutter*, S.A. Rude*, B.W. Peterson, F.R. Robinson, and J.F. Baker. Dept. Physiology, Northwestern University Medical Ctr., Chicago, IL 60611; and Dept. Physiology and Biophysics, University of Washington, Seattle, WA, 98195.

Although adaptation of the VOR has been well studied, it is not clear whether such plasticity generalizes or is specific to the exact conditions of adaptation. We are examining the specificity of adaptation of VOR direction.

The VOR rapidly adapts the direction of its compensatory eye movements when whole-body rotations are paired with orthogonal optokinetic motion. After adaptation, VOR eye movements are seen in the optokinetic direction (cross-axis gain ~=0.3 at 120 min.) when the animal is rotated in darkness.

We tested for specificity of VOR direction adaptation by exposing cats to combined vestibular and optokinetic stimulation in two body postures. The direction of optokinetic motion was reversed when body orientation was changed so that VOR adaptation in opposite directions was required for the two body orientations. Each animal was trained while lying on both its left and right sides to make horizontal eye movements (earth-vertical) in response to pitch (earth-horizontal) whole-body rotations. For example, during training on the left side, when the head was moved in the ventral direction (clockwise as seen from above), the visual world moved to the cat's right (upward relative to earth); during training on the right side, when the head was moved ventrally the visual world moved to the cat's left. Ten minute adaptation sessions on the left and right sides were interleaved for two hours of training on each side. Horizontal and vertical electro-oculographic recordings during 0.25 Hz rotations on the left and right in the dark were made before, during, and after the four hour adaptation period. Saccades were removed from the records, and the adaptation was measured as the vectorial difference between pre- and post- adaptation gain and phase of cross-axis VOR.

In all five experiments on three cats, we observed adaptation specific to training posture. Cross-axis eye movements during rotation in the dark while the animal was lying on one side attained gains (slow phase eye velocity / rotation velocity) of 0.1-0.35 after 120 minutes of training on that side. The adaptive movements on the two sides were in opposite directions relative to the same whole-body rotation; that is, they were in phase with the optokinetic training stimulus experienced on each side.

These results suggest that VOR directional adaptation occurs through plastic changes in highly specific neural pathways which are dependent on animal orientation. It seems likely that static signals from the otoliths relay information about body orientation and provide conditioning cues that differentiate these pathways.

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BIOCHEMICAL AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT III

339.1 FUNCTIONAL GABA AND GLYCINE RECEPTORS PRECEDE GLUTAMATE RECEPTORS DURING THE DEVELOPMENT OF THE RAT SPINAL CORD. R.N.Mandler, A.E.Schaffner, E.A.Novotny*, G.D.Lange and J.L.Barker. Lab. of Neurophysiology, NINCDS, NIH, Bethesda, MD 20892.

We have utilized voltage-sensitive dyes in conjunction with flow cytometry to study the functional effects of putative aminoacid transmitters on embryonic rat spinal cord cells. This strategy allows sampling of relative membrane potential for large cell populations with single cell resolution. Rat embryos at embryonic (E) days 12 to 20 were used. Spinal cords were dissected and treated with papain for 45 min. Cellular suspensions were stained with acridine orange and ethidium bromide, to assess viability or with anionic voltage-sensitive dyes, to assess changes in membrane potential. Stained suspensions were studied in a flow cytometer for their fluorescence and light scatter properties under control and experimental conditions. Over 90% of cells obtained throughout the entire embryonic period were viable. GABA, muscimol or glycine did not change the membrane potential distribution at resting conditions (physiological medium). However, incubation with GABA and glycine in low-Cl^- saline revealed depolarizing responses that were detected early (E12). Furthermore, incubation with GABA and glycine in high K^+ or in batrachotoxin revealed hyperpolarizing responses that were also detected early. Bicuculline and strychnine blocked GABA and glycine responses, respectively. Depolarizing responses to glutamate, aspartate, kainate and quisqualate in physiological saline, and to NMDA in low Mg^{++} were detected at E14-15. Dose-response treatments starting at nanomolar concentrations up to high micromolar were done for inhibitory and excitatory aminoacids at all ages; detectable effects were seen with submicromolar concentrations for inhibitory and excitatory agonists, indicating that this strategy is a sensitive assay of receptor coupled changes in membrane excitability. The results suggest that GABA and glycine activate Cl^- conductance mechanisms relatively early in the development of the rat spinal cord, and that physiological responses to excitatory aminoacids appear later in embryogenesis.

339.2 FLOW CYTOMETRIC ANALYSIS OF A VENTRAL-DORSAL GRADIENT IN THE DEVELOPMENT OF BATRACHOTOXIN AND KAINATE SENSITIVITIES IN EMBRYONIC RAT SPINAL CORD. A.E. Schaffner, R.N. Mandler, E.A. Novotny*, G.D. Lange and J.L. Barker. LNP, NINCDS, NIH, Bethesda, MD 20892.

We have used flow cytometry in conjunction with voltage-sensitive dyes to examine the development of Na^+ channels and glutamate receptors in embryonic spinal cord cells. There is evidence for a rostral-caudal and ventral-dorsal gradient in the production and maturation of spinal cord cells. We were interested in determining if the development of pharmacological responses would follow a similar pattern. Pieces of spinal cords corresponding to cervical, thoracic and lumbo-sacral segments were removed from 15-day rat embryos and hemisected into dorsal (DH) and ventral halves (VH). Cells were dissociated after 45 min in papain and stained with either acridine orange (AO) to assess cell viability or the oxonol dye $DiBaC_4(3)$ to assess changes in resting membrane fluorescence properties after various pharmacological manipulations. AO staining revealed that 95% of the cells in the preparation were viable. Over the length of the cord, cells from the VH scattered more light than cells from the DH, presumably due to the presence of large motoneurons in the VH. Cells were incubated with micromolar batrachotoxin (BTX), a sodium channel agent. Both DH and VH contained cells which were relatively insensitive to BTX and cells which were depolarized in the presence of BTX. The BTX responses were blocked by TTX. The extent of the depolarization was greater in cells from the VH. Within each segment, cells from the VH but not the DH were depolarized in the presence of 50 μM kainic acid, a glutamate receptor agonist. Although we could not demonstrate marked rostral-caudal gradients in the development of these sensitivities, gradients may be more apparent in either younger or older embryos. In contrast to the rostral-caudal axis, there are clear differences in the ventral-dorsal direction at all spinal levels with regard to kainate and BTX sensitivity. This is in agreement with previous studies that indicate that cells in the VH, specifically motoneurons, differentiate before cells in the DH. The results suggest that functional forms of electrical and chemical excitability are not expressed uniformly throughout the rat spinal cord at this time in development. The application of indicator dyes and flow cytometry to anatomically discrete areas in spinal cord and brain throughout development should allow a systematic study of the developmental appearance of specific aspects of electrophysiological function.

339.3 SYMPATHETIC PREGANGLIONIC NEURONS IN CULTURE DISPLAY RECEPTORS TO MULTIPLE NEUROTRANSMITTERS. B. Clendening * and R.I. Hume. (SPON: S.S. Easter). Dept. Biology, Univ. of Michigan, Ann Arbor, MI 48109

Most mature CNS neurons express receptors for several different neurotransmitters. We wondered whether, during development, the different receptors on a single cell might be regulated independently, or in a co-ordinated manner. We have approached this issue by characterizing the transmitter sensitivity of an identified class of CNS neurons in cell culture.

We used methods developed by Honig and Hume (J. Cell Biol. 103:171) for labelling sympathetic preganglionic neurons (SPN) in embryonic chick spinal cords, and then maintaining them in dissociated cell culture. Whole cell recordings were used to characterize the responses of SPN to a battery of neurotransmitters which are generally believed to be released from terminals presynaptic to SPN in mature animals in vivo. We studied responses to serotonin, norepinephrine, gaba, glycine and glutamate. All were applied by pressure ejection from puffer pipettes, and each transmitter was tested at 10 μM.

Serotonin and norepinephrine both produced relatively small hyperpolarizations from resting potential and under voltage clamp they produced small outward currents (< 100 pA) at rest. Glutamate produced relatively large depolarizations from rest, often eliciting long trains of action potentials. Under voltage clamp glutamate produced relatively large inward currents (>100 pA) at rest. As has been noted by others these inward currents got larger as the cells were depolarized over the range of -45 to -20 mV. Under our recording conditions, with relatively high intracellular chloride (E_{Cl} = -5 mV), gaba and glycine produced depolarizations from rest, and under voltage clamp they produced currents that reversed at E_{Cl}. Only two neurotransmitters were tested on any one cell, however since almost all SPN responded to each transmitter, we conclude that SPN in culture are sensitive to at least 5 different transmitters.

We have made two interesting observations concerning the regulation of transmitter sensitivity. First, application of a transmitter is sometimes much more effective if applied to a region other than the cell body. Furthermore, the site of maximal sensitivity is not necessarily at the same location for different transmitters. Second, in some cultures the gaba sensitivity was 10 times higher than usual, whereas in these same cultures the sensitivities to glycine, serotonin and norepinephine were not atypically high. Thus it seems likely that receptors for different neurotransmitters on the same cell can be regulated by relatively independent mechanisms.

339.4 TRANSFERRIN IMMUNOCYTOCHEMISTRY REVEALS CHANGING PATTERNS DURING RETINAL DEVELOPMENT. G.D.Zeevalk & A.G.Hyndman. Rutgers Univ. Dept. Biol.Sci., Piscataway N.J. 08855

The serum glycoprotein transferrin is the major transporter of iron to cells. In recent years, evidence has accumulated to suggest that transferrin might be functioning in unique roles in the nervous system, possibly involving neurite outgrowth, synaptogenesis, target cell differentiation and behavior modulation. Transferrin or its receptors have been found in central neurons in chick, sheep and mouse and peripheral neurons in chick. None of the work on the brain distribution of transferrin or its receptor has included the retina. In this study we have looked for the presence of transferrin in the developing chick retina, as well as, its pattern of distribution and any changes in distribution that may occur with development. Sites of iron stores in the retina also were examined to determine if these correlated with regions of transferrin distribution. Immunocytochemical examination of retinas from embryonic day 6 to 3 weeks post hatching revealed that transferrin was differentially distributed in retinal layers. Furthermore, the pattern of transferrin distribution changed with developmental age. At day E6, transferrin was found in 2 distinct bands which were located in the area of the Muller cell endfeet. By day E9, additional regions of transferrin immunoreactivity could be found in the inner and outer plexiform layers (IPL,OPL) and the nerve fiber layer (NFL). These latter three bands became more prominent from E9 until E17 as the synaptic layers and nerve fiber layer increased in size, density and maturation. Perikarya in the nuclear layers were negative. At day E17 and later, the newly forming outer segments of photoreceptor cells were strongly reactive for transferrin while somas of the photoreceptor cells, in the ONL, were negative. Retinas from chicks 1 day to 3 weeks post hatching retained strong immunoreactivity for transferrin in the photoreceptor cell outer segments and OPL, lessened immunoreactivity in the IPL and loss of immunoreactivity in the NFL. Iron distribution at all ages showed only 2 bands that locally corresponded to the Muller cell endfeet. Iron stores were not found in the synaptic layers or photoreceptor cell outer segments. These studies suggest an iron storage function for retinal glia and a role for transferrin in neuronal development and differentiation.

339.5 TRANSFERRIN CAN ALTER ELECTROPHYSIOLOGICAL AND NEUROTRANSMITTER PROPERTIES OF RETINAL NEURONS A.G. Hyndman, P.E. Hockberger, G.D.Zeevalk & J.A. Conner, Dept of Biol. Sci., Rutgers Univ., Piscataway N.J. 08855 & Dept of Molec. Biophysics, Bell Labs, Murray Hill, NJ 07974

Transferrin, an iron transport protein, may be important in the regulation of neural differentiation. During synaptogenesis in the chick retina, transferrin has been localized within the plexiform & optic nerve layers (Zeevalk & Hyndman, this issue). These unique locations suggest a possible role for transferrin in synaptogenesis and led us to examine the effects of transferrin on the electrophysiological properties of retinal neurons and on the development of glutamate binding, uptake and stimulated release in vitro. Retinal neurons from 8 day chick embryos were grown in basal medium supplemented with catalase (5.6U/ml) and insulin (0.83uM) plus or minus transferrin (62.5nM). Cultures used in whole cell patch recordings were supplemented with 10% fetal bovine serum. Using whole cell patch recording, we examined the influence of transferrin on the electrophysiological properties of neurons with a bipolar morphology after 6 days in vitro. Pressure application of transferrin evoked an inward current in most cells recorded from under voltage clamp conditions. In normal saline, the amplitude of the evoked current was larger at a holding potential of -40mV compared to -80mV. This response was identical to the response evoked by glutamate applied iontophoretically. Both responses showed less voltage sensitivity in Mg++ free PBS and on many occasions were found on the same cell. Coupled with these observations, we found that K+ stimulated release of preloaded 3H-glutamate in cultures maintained for 6 days in vitro occurred only when cultures were maintained in transferrin or when transferrin was present during the assay. Glutamate binding & uptake in retinal cultures increased between 2 and 5 days in vitro. This change was mainly due to an increase in the number of neurons which developed specific glutamate binding or high affinity uptake in vitro. The presence of transferrin during the culturing period did not alter these properties. These studies indicate that transferrin may have neurotransmitter like actions of its own, and may function as a neuromodulator in the developing retina. This work was funded by AFOSR, Grant # F49620-85-C-0009 to JC & PHS RR 07058-21 to AH.

339.6 IDENTIFICATION AND PURIFICATION OF AN AXONALLY SECRETED PROTEIN THAT IS DEPOSITED ON THE SURFACE OF NEURONAL SOMAS AND PROCESSES. P. Sonderegger, E.T. Stöckli*, M.A. Rüegg*, P.F. Lemkin*§, P. Streit**, T.B. Kuhn*, and M. Heller*, Institute of Biochemistry and *Brain Research Institute, University of Zurich, CH-8057 Zurich, Switzerland, and §Image Processing Section, DCBD, NIH, Frederick, MD 21701.

Proteins secreted from axons have been suggested to contribute to axon growth and synapse formation and might be involved in the transmission of a variety of axon-derived regulatory signals during neurogenesis. In order to identify axonally secreted proteins, dorsal root ganglia neurons from chicken embryos were cultured in a compartmented cell culture system that allows separate access to neuronal cell somas and axons. The proteins synthesized by the neurons were metabolically labeled by addition of [^{35}S] methionine to the compartment containing the cell somas; the proteins released from the axons were harvested from the culture medium of the axonal compartment. Two-dimensional SDS- PAGE/fluorography, followed by computerized gel-image analysis, revealed two axonally secreted proteins (ASPs) with apparent molecular weights of 54-60 kD and 132-140 kD; they were termed ASP54-60 and ASP132-140, respectively. Both ASPs were found to be secreted from a variety of neuronal cell cultures, but not from any of the non- neuronal cultures investigated, and hence, might be neuron-specific. Virtual absence of these proteins from the axonal protein pattern suggests constitutive secretion. Using coordinates and spot morphology in two-dimensional SDS-PAGE as a means for identification, one of these proteins, ASP132-140, was subsequently purified from chick embryonic vitreous fluid by a four step chromatographic procedure. Polyclonal antibodies were raised. The identity of the purified protein and the in vitro identified axonally secreted protein was confirmed by immunoprecipitation. Immunoblotting experiments revealed that ASP132-140 is exclusively present in vitreous fluid of the embryo but not of the adolescent or adult chicken. The tissue localization of this protein was investigated by light and electron microscopy. Light microscopic localization by indirect immunofluorescence or indirect immunoperoxidase on embryonic spinal cord cultures revealed circumscribed, extracellular depositions of ASP132-140 on neuronal somas and processes, but not on nonneuronal cells. In immuno-electron microscopy these extracellular depositions of ASP132-140 were prevalently between the membranes of adjacent neuronal elements; most frequently they were encountered in the neuropil.

The distinct localization and the occurrence during development suggest a role for ASP132-140 in the organisation of intercellular contacts between neurons during neurogenesis.

339.7 MONOCLONAL ANTIBODY CAT-301 RECOGNIZES A PROTEOGLYCAN SPECIFIC TO THE SURFACE OF SUBSETS OF MAMMALIAN CNS NEURONS. S. Zaremba* and S. Hockfield (SPON: M. DeSantis). Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

A proteoglycan unique to subsets of mammalian CNS neurons is shown here to react specifically with monoclonal antibody Cat-301. Previous immunohistological studies with Cat-301 have shown that the antigen (1) is associated with subsets of neurons, (2) is localized on the surfaces of neuron cell bodies, (3) is expressed relatively late in nervous system development, and (4) requires normal neuronal activity early in development in order to be expressed. The present work shows that antigens reacting specifically with Cat-301 can be purified from urea/CHAPS (3-[(3-cholamidopropyl)dimethylammonio]1-propanesulfonate) extracts of membrane preparations from either hamster or guinea pig CNS. Two pools of immunoreactive material are isolated by DEAE (diethylaminoethyl) ion-exchange column chromatography from extracts of either species; within each species, the two pools are apparently distinguishable only by the ionic strength at which they elute from the column. On Western blots, specific Cat-301 immunoreactivity appears as a broadly migrating species in the stacking gel. Protease treatment totally abolishes immunoreactivity. In contrast, Cat-301 staining on Western blots is altered by incubation with chondroitinase ABC or hyaluronidase, enzymes that degrade glycosaminoglycan chains. The characteristic, broadly migrating species disappears and a sharply defined immunoreactive band is generated that runs just inside the resolving gel and is larger than the 400,000 dalton subunit of laminin. Further incubation of the chondroitinase-treated pools with endoglycosidase F has no effect on Cat-301 immunoreactivity. These biochemical data indicate that the Cat-301 antigens are proteoglycans and suggest, but do not prove, that the Cat-301 epitope is a polypeptide. This raises the possibility that discrete subsets of mammalian CNS neurons may express characteristic cell surface proteoglycans through a mechanism of selective gene expression.

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339.8 MOLECULAR EVIDENCE FOR EXPERIENCE-DEPENDENT DEVELOPMENT OF MOTORNEURONS. R. Kalb* and S. Hockfield (SPON: S. Gobel). Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT.

Monoclonal antibody Cat-301 recognizes a surface antigen on subsets of neurons in many areas of the mammalian CNS, including Y-cells in the cat dorsal lateral geniculate nucleus (LGN) and spinal motorneurons in several species. We have shown previously that the Cat-301 antigen is expressed relatively late in postnatal development with a time course that matches that of late developmental features of both LGN and spinal cord neurons. In the visual system of cats and primates the anatomy and physiology of neurons can be profoundly altered by depriving an animal of normal visual experience early in life (Hubel and Weisel, J. Physiol. 206: 419). The developmental time course of the expression of the Cat-301 antigen in the LGN correlates with the period during which visual deprivation can alter LGN Y-cells. Neonatal visual deprivation (by monocular lid suture or dark rearing) suppresses Cat-301 expression on Y-cells while deprivation in adult animals has no effect on antigen expression. These results suggested that the onset of Cat-301 immunoreactivity provides a positive molecular marker for the end of a critical period in cat visual system development.

To determine if Cat-301 might be a general marker for experience-dependent development in other areas of the CNS we have examined antigen expression on hamster spinal cord motorneurons. Here we report that Cat-301 antigen expression on motorneurons is tied to early neuromuscular experience. Cat-301 immunoreactivity develops on hamster motorneurons between postnatal days 7 and 14. Altering neuromuscular activity by sciatic nerve crush or thoracic hemicordotomy inhibits Cat-301 expression on motorneurons if performed at postnatal day 5, before the normal onset of Cat-301 immunoreactivity. The loss of Cat-301 is relatively selective as other motorneuron antigens are unaffected. In adult animals nerve crush or cordotomy has no effect on Cat-301 immunoreactivity, demonstrating that Cat-301 expression is not simply dependent on ongoing neural activity. These observations suggest that motorneurons, like LGN neurons, require some pattern of neuronal activity during a critical period in development and, further, that the phenotypic changes in neurons consequent to early experience are reflected by the expression of specific molecules. The identification and characterization of such molecules may yield a description of the molecular mechanisms of experience-dependent development. (Supported by BNS-8544681 and the Klingenstein Foundation).

339.9 BRAIN OPIOID RECEPTOR REGULATION IN THE DEVELOPING ANIMAL. J. Habas[1], A. Tempel[2] and G.A. Barr[3] (SPON: A.B. Johnson[2]). [2]Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, USA, [1]Dept. of Medicine, SUNY/Downstate Medical Center, Brooklyn, NY 11203, USA, [3]Dept. of Psychiatry, Albert Einstein College of Medicine, Bronx, NY 10461, USA.

Chronic treatment with opiate agonists and antagonists have been shown to induce changes in brain opioid receptor density in adult rats. The development of these receptors and their regulation by exogenously administered opioids in perinatal pups has been less well characterized. We examined opioid receptor regulation in brains of neonatal rats after chronic agonist and antagonist treatment. Two regimens of drug treatment were used: naltrexone or morphine was administered to 1) dams or 2) pups of non-treated mothers starting on day of birth. Pups (ages 0-28 days) from each treatment group were sacrificed and brains assayed for specific opioid receptor types. The highly specific ligands used were [^{3}H]D-Ala^2,N-Me-Phe^4,Gly-ol^5-enkephalin for u receptor assays and [^{3}H]D-Pen^2-D-Pen^5-enkephalin for delta assays. Scatchard analysis revealed a 115% increase in brain u opioid receptors on the day of birth in offspring of naltrexone-treated mothers. Daily naltrexone administration to offspring of non-treated mothers induced a 78% increase in brain u opioid receptors. In a separate series of experiments, dams were treated chronically with morphine sulfate for 1 week prior to parturition. Chronic agonist treatment produced a 35% decrease in brain u opioid receptors of pups at postnatal day 0. Daily morphine administration (8 days) to offspring of non-treated mothers also induced a 30% decrease in brain u opioid receptor density. Subsequently, the number of receptors increased to control levels. The observation of a pronounced up- and downregulation of u opioid receptors following chronic pre- and/or postnatal treatment of opioid antagonist and agonist raised the question as to the behavioral consequences of these changes. In order to address this issue, morphine-induced forepaw analgesia was measured in pups treated either prenatally or postnatally with either morphine or naltrexone. Chronic morphine treatment of pregnant rats produced a statistically significant decrease in the morphine-induced analgesic response or tolerance in offspring. Conversely, chronic naltrexone treatment of pregnant rats resulted in an enhancement of the morphine-induced forepaw analgesic response. These data show a behavioral correlate for the observed changes in u opioid receptor numbers following postnatal treatment with morphine or naltrexone as the time course for the induced changes are well-correlated with the behavioral data. (This work is supported by NIH grant No. NS21973).

339.10 CHARACTERIZATION OF A TYPE II CALCIUM/CALMODULIN-DEPENDENT KINASE FROM NEUROBLASTOMA/GLIOMA CELLS. M.L. Vallano and C.M. Beaman-Hall* Dept. of Pharmacology, SUNY/Health Science Center, Syracuse, NY 13210.

A type II calcium/calmodulin-dependent protein kinase (CaM Kinase II) is a proposed regulator of a variety of cellular events, including cytoskeletal protein function. In order to elucidate the role of this kinase in cytoskeletal function, a neuroblastoma/glioma hybrid cell (NG-108CC15) that elaborates cytoskeletal-filled neurites under specific conditions of cell culture (treatment with dibutyryl cAMP) was selected as a model. Initial studies were directed towards the partial purification and biochemical characterization of CaM Kinase II in these cells. A combination of differential centrifugation, ion-exchange chromatography, and calmodulin-affinity chromatography were employed to partially purify CaM Kinase II activity from whole cell homogenates. The putative kinase in NG-108CC15 cells was compared with authentic CaM Kinase II purified from rat brain cytosol. Based upon subunit molecular weight, calmodulin-binding properties, calmodulin-dependent autophosphorylation, time course, substrate specificity, requirement for divalent cations, ATP and calmodulin, CaM Kinase II was identified in these cells. In addition to the autophosphorylated subunits of CaM Kinase II, several other endogenous proteins whose identity remains to be determined served as substrates for the kinase.

In a second series of experiments, cell differentiation was induced by addition of 1 mM dibutyryl cAMP to the culture medium. A combination of morphological, biochemical and electrophysiological criteria were applied to establish that differentiation had occurred. After several days in culture, CaM Kinase II activity was measured in control cells versus treated cells and a significant increase in activity was observed in differentiated cells. Using a combination of gel electrophoresis and scanning densitometry, a significant increase in the staining of several proteins was observed in the treated cells compared to control cells. We suggest that there is an increase in the amount of CaM Kinase II associated with the cytoskeleton in treated cells and that this enzyme may play an important role in regulating cytoskeletal function in these cells.

339.11 NEUROTENSIN IS PRESENT IN LARGE AMOUNTS IN THE PRINCIPAL INFERIOR MEDULLARY OLIVARY COMPLEX OF THE HUMAN YOUNG INFANT. J.-J. Vanderhaeghen and P. Mailleux* laboratories of Neuropathology and Neuropeptide Research and of Pathology and Electron Microscopy, Brugmann and Erasme Hospitals, Université Libre de Bruxelles,808 route de Lennik,B-1070,Brussels,Belgium.

Human brains were obtained at autopsy less than ten hours post mortem from adults, fetuses and young infants (therapeutic abortus, sudden death syndrome, non neurological diseases). Medulla was processed for immunohistochemistry (10 to 50 micrometers thick, 4 % paraformaldehyde fixed-sucrose, cryostats sections), radioimmunoassays (supernatants of 10 % freshly dissected inferior medullary olivary complex homogenized in 0.06 M potassium phosphate buffer, 0.01 M EDTA, Ph 7.2 and boiled for 10 minutes) or receptors autoradiographies (25 micrometers thick, unfixed, cryostat sections). Neurotensin 1-13 (NT) radioimmunoassay was performed with iodinated I^{125} Neurotensin (Amersham). Antiserum was raised in rabbit by injection of NT coupled to thyroglobulin (Sigma) or to albumin (kindly from L. Jennes). Immunohistochemistry was performed using the PAP technique of Sternberger.

In infants from birth to one year, an intense labelling of nerve terminals was present in all parts of the principal medullary olivary complex (principal oliva) but not in the accessory oliva. The labelling disappeared around 8 years of age and was completely absent in the adult. In the 6 months infant or in 75 year old adult, respectively 582 +/- 106 and 5 +/- 0.1 picomoles of NT equivalent (+/- SEM) per g wet weight (n=3) of medullary olivary complex was present. This material eluted as one main peak together with NT on a sephadex G25 column equilibrated with ammonium acetate buffer. Using autoradiography after binding of iodinated NT I^{125}, high levels of displaceable binding sites were present in the principal oliva of a six months old infant but not in an adult .

The presence of NT in nerve terminals of the infant principal oliva but not in the accesory paroliva suggests that they belong to a descending pathway. The accessory paroliva receive they afferences from the spinal cord but the principal oliva receive them from the cerebral cortex, the basal ganglia and the mesencephalic tegmentum. Several NT containing nerve cell bodies have been found in colchicinised rat in the mesencephalic tegmentum where we observed numerous NT nerve fibers but no NT cell bodies during the first year of age. NT neurons, in the human hippocampus (Brain research, 375, 351, 1986), have been shown to be present in the young child but to disappear in adult age.

Data supporting the implication of trophic factors including neuropeptides (Nature, 325, 617, 1987) in the development of the nervous system are rapidly accumulating. NT nerve terminals in principal oliva especially during the first year of age, may play a role in the development of that nucleus projecting to the cerebellum which also completes its development during that year. Supported by Belgian FRSM (3.4523.86-89), Queen Elisabeth Foundation (Neurobiology, 1986-87) and National Lotery (1986).

339.12 TEMPERATURE AND LIGHT INDUCED CHANGES IN NEONATAL RAT PINEAL GLAND N-ACETYLTRANSFERASE ACTIVITY. G. Torres*, K.A. Haak*, and L.D. Lytle. Department of Psychology, University of California, Santa Barbara, CA 93106.

The activity of N-acetyltransferase (NAT), a pineal gland enzyme important for the synthesis of the methoxyindoleamine endocrine hormone, melatonin, varies diurnally. Interestingly, the high nocturnal activity of the enzyme can be rapidly suppressed by exposing animals to brief presentations of light (Klein and Weller, 1972). Nocturnal light inhibits adult rat pineal gland NAT activity via a circuit that involves portions of the retina, brain, spinal cord, and sympathetic nervous system. Changes in other environmental stimuli [e.g., long-term exposure to high ambient temperatures (Nir and Hirschmann, 1978)] also affect adult pineal gland NAT activity, but the mechanisms involved in these changes are unknown.

We initially determined whether changes in environmental temperature might differentially affect NAT activity in the pineal glands of newborn animals whose sense organs and nervous systems were incompletely developed. Neonatal male or female albino rats were exposed to different temperatures (22 °C or 35 °C) for a 4 hr period during the middle of the light phase of a 12:12 hr day:night cycle (17 ft candle fluorescent lights on at 0700 hr). Unlike animals older than 12 days of age, 4 day old rats showed large increases in NAT activity following exposure to the 22 °C vs. 35 °C ambient temperature. Interestingly, animals pretreated with 6-hydroxydopamine (to destroy the sympathetic nervous system) failed to show cold-induced elevations in pineal gland enzyme activity.

We also studied possible maturational changes in light-induced inhibition of nocturnal pineal gland NAT activity. Four day-old animals maintained at 35 °C were exposed briefly (1 min) or for longer (4 hr) periods of time to nocturnal light (17 ft candles) 2 hr after the onset of darkness. The long (but not short) light exposure significantly reduced pineal gland NAT activity, indicating that photic stimuli may affect the enzyme at an earlier age than thought previously. Whether this light induced inhibition of enzyme activity in 4 day old animals depends on mechanisms different from those involved in the retina-pineal gland circuit remains to be determined.

GABA AND BENZODIAZEPINE: RECEPTORS

340.1 ISOLATION AND PURIFICATION OF ENDOGENOUS LIGAND FOR BENZODIAZEPINE RECEPTOR FROM PIG BRAIN. H.S. Lin*, C.T. Lin and J.-Y. Wu. Dept. of Physiol., Penn State Univ. Col. of Med., Hershey, PA 17033.

The purification of endogenous ligand for benzodiazepine (BZ) receptor was performed by homogenizing pig brain gray matter in distilled water to make a 10% homogenate, followed by ultracentrifugation at 100,000 X g for 1 hr. The supernatant liquid obtained was filtrated through Amicon membrane which has an exclusion limit of 10,000-dalton. The filtrate was concentrated by heating to about one seven hundredth of the original volume. The concentrated sample was extracted by a mixture of chloroform/methanol (2:1) for overnight. The supernatant solution obtained after brief centrifugation was completely air dried. The dried sample which was resuspended in minimal amount of distilled water was reextracted with petroleum ether in a water bath at 37°C for 3 hrs. The organic phase and aqueous phase were separated after brief centrifugation. The ligand activity which was determined by binding assay was found to be mainly in the aqueous phase. The active fraction was further purified by HPLC using C-18 column which was eluted with a linear gradient of buffer A (25 mM ammonium acetate) and buffer B (40%, 50 mM ammonium acetate and 60% acetonitrite). The ligand activity was found to be eluted at 70% buffer B. The HPLC purified ligand preparation was eluted as a single, symmetrical peak at 220 nm on a second HPLC column. The purified preparation had an absorption maximum at 215 nm (between 200-340 nm) which is different from any BZ endogenous ligands that had been reported. The purified ligand preparation inhibited the binding of flunitrazepam (FNZP) and RO5-1788 in a competitive manner; whereas, no effect was observed on the binding of RO5-4864 to the peripheral BZ receptor in both pig and rat preparations. The competitive nature between the isolated ligand and FNZP was further demonstrated in autoradiographic experiments in which the binding of [^{3}H]FNZP to BZ receptor in the hippocampus and cerebral cortex was greatly reduced in the presence of either unlabeled FNZP or purified endogenous ligand. However, the nature of the endogenous ligand whether it is an agonist, antagonist or inversed agonist has not been determined yet. (Supported by NIH grants #NS20978, #NS20922 and #EY05385.)

340.2 THE BRAIN GABA RECEPTOR: CLONING AND FUNCTIONAL EXPRESSION OF THE cDNAs ENCODING ITS SUBUNITS AND LOCALIZATION BY IN SITU HYBRIDIZATION. P.R. Schofield*, B.D. Shivers, H. Rodriguez*, L. Rhee*, J. Ramachandran*, M. Darlison*, N. Fujita*, D. Burt*, A. Stephenson*, T. Glencorse*, V. Reale*, E. Barnard* and P.H. Seeburg* (SPON: C. Cusick) ZMBH, University of Heidelberg, Im Neuenheimer Feld 282, D-6900 Heidelberg, FRG; Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080, USA and MRC Molecular Neurobiology Unit, Hills Road, Cambridge, CB2 2QB, UK.

Gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the vertebrate brain, produces neuronal hyperpolarization by activating a chloride channel that forms an integral component of the GABA receptor. The channel activity of this receptor is modulated allosterically by a variety of clinically significant drugs because of anxiolytic (e.g. benzodiazepines, barbiturates) anxiogenic (e.g. ß-carbolines) or convulsant (e.g. picrotoxin) activities.

We have used affinity-purified bovine GABA receptor to generate peptide sequences and have thus cloned bovine cDNAs encoding both subunits, and ß, of the GABA receptor. The GABA receptor subunits are homologous in both primary sequence (35% identity between and ß subunits) and transmembrane topology. Homology is also seen with subunits of the nicotinic acetylcholine receptor suggesting the existence of a superfamily of chemically-gated ion channels.

Co-expression of both and ß RNA's in Xenopus oocytes produces a functional receptor and chloride ion channel which is potentiated by benzodiazepines and barbiturates and blocked by picrotoxin and bicuculline.

We have used these bovine sequences to isolate the corresponding rat and human cDNA's. The former have been used to map the tissue and cellular distribution of GABA receptor in the rat brain. The latter have been used for directed mutagenesis and electrophysiological analysis of these mutants.

340.3 MOLECULAR CLONING OF THE LIGAND-BINDING SUBUNIT OF THE GLYCINE RECEPTOR. H. Betz, G. Grenningloh*, A. Rientiz*, B. Schmitt*, E.D. Gundelfinger*, C. Methfessel*, M. Zensen* and K. Beyreuther*. ZMBH, Universität Heidelberg, FRG, Max-Planck-Institut für Biophysikalische Chemie, D-3400 Göttingen, FRG, and Institut für Genetik, D-5000 Köln, FRG.

Glycine is a major inhibitory neurotransmitter in the CNS of vertebrates and invertebrates. The glycine receptor was purified by affinity chromatography and shown to be a heterooligomeric glycoprotein containing three polypeptides of 48, 58 and 93 K. The 48 K polypeptide contains the ligand-binding site of the receptor. The 93 K polypeptide is a cytoplasmically localized peripheral membrane protein of the postsynaptic glycine receptor complex. Using microsequencing and oligonucleotide screening approaches, cDNA clones encoding the strychnine-binding 48 K-polypeptide were isolated and analysed. The deduced protein exhibits the same structural organization and significant sequence homology to neuronal and muscle acetylcholine receptor polypeptide. In particular, two invariant cysteines of the extracellular region and the putative transmembrane regions M1 to M4 are all found at equal positions in these receptors. Our data suggest that the glycine and the nicotinic acetylcholine receptors, and possibly other receptors as well, are structurally related products of a family of genes encoding neurotransmitter-gated ionic channels.

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340.4 PREFERENTIAL AFFINITY OF ^{3}H-2-OXO-QUAZEPAM FOR TYPE I BENZODIAZEPINE RECOGNITION SITES IN THE HUMAN BRAIN

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Quazepam and its active metabolite 2-oxo-quazepam (2-oxo-quaz.) are two benzodiazepine (BZ) hypnotics containing a trifluoroethyl mojety on the ring nitrogen at position 1, characterized by their preferential affinity for Type I BZ recognition sites. In the present study we characterized the binding of ^{3}H-2-oxo-quaz. in discrete areas of the human brain. Saturation analysis demonstrated specific and saturable binding of ^{3}H-2-oxo-quaz. to membrane preparations from human cerebellum. Scatchard plots of saturation data indicated a single population of binding sites in this brain area. Hill plot analysis of displacement curves of ^{3}H-Flunitrazepam (^{3}H-FNT) binding by 2-oxo-quaz. yelded Hill coefficients significantly less than unity in the cerebral cortex, hippocampus, caudate nucleus, thalamus and pons suggesting the presence of more than one binding site for 2-oxo-quaz. in these brain areas. Self and cross displacement curves for ^{3}H-FNT and ^{3}H-2-oxo-quaz. bindings in these brain areas indicated that 2-oxo-quaz. binds with different affinities to two population of binding sites. High affinity binding sites were more abundant in the cerebellum (96% of total sites), cerebral cortex and thalamus, whereas low affinity sites were predominant in the caudate nucleus and pons. Competition studies of ^{3}H-2-oxo-quaz. (2nM) and ^{3}H-FNT (0.5 nM) using unlabelled ligands indicated that compounds which preferentially bind to Type I sites, such as ethyl-ß-carboline-3-carboxylate, quazepam, 2-oxo-quaz. and halazepam were more potent at displacing ^{3}H-2-oxo-quaz. than ^{3}H-FNT from cerebral cortex membrane preparations. On the other hand, flurazepam, temazepam and nitrazepam, compounds which have similar affinity for Type I and Type II sites displaced ^{3}H-2-oxo-quaz. and ^{3}H-FNT with similar Ki values. The results suggest that ^{3}H-2-oxo-quaz. may be used for selectively studying Type I BZ recognition sites in the human brain. We also studied the modulation by ɣ-aminobutyric acid (GABA) of ^{3}H-2-oxo-quaz. binding to well-washed cerebral cortex membrane preparations. The "in vitro" addition of GABA concentration-dependently (10^{-8} - 10^{-4} M) enhanced the specific binding of ^{3}H-2-oxo-quaz. This effect was potentiated by the presence of chloride ions in the incubation buffer. Scatchard analysis of saturation curves indicated that GABA decreased the apparent dissociation constant (K_D) while did not modify the maximum number of binding sites (Bmax).

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340.5 ASTROCYTIC BENZODIAZEPINE RECEPTOR - PHARMACOLOGICAL EVIDENCE THAT IT MAY BE INVOLVED IN CONVULSANT AND ANXIETY STATES. L. Hertz* and A.S. Bender* (SPON: J.S.Richardson), Dept. of Pharmacol., Univ. of Saskatchewan, Saskatoon, Sask. S7N 0W0 Canada

It is now well established that astrocytes possess receptors for not only the "peripheral-type" benzodiazepine ligand PO 5-4864 but also such clinically relevant drugs as diazepam and flunitrazepam and that the pharmacological profile for the astrocytic and the neuronal benzodiazepine receptor are profoundly different (Bender and Hertz, J. Neurochem., 43, 1319, 1984; J. Neurosci. Res. in press). The astrocytic benzodiazepine receptor may be correlated with a voltage-dependent calcium channel (Bender and Hertz, Europ. J. Pharmacol. 110, 287, 1985), and atrial natriuretic peptide, 8-bromo 3',5'cyclic GMP and phorbol-ester, compounds which may be involved in modulation of calcium channels, all displace astrocytic diazepam binding potently.

The anti-anxiety, anticonvulsant and/or sedative effects by benzodiazepines might equally well be mediated by the astrocytic and the neuronal benzodiazepine receptor. We have therefore compared potencies of several convulsant and anticonvulsant drugs as well as of anxiogenic and anxiolytic compounds in displacing [^{3}H]diazepam binding in primary cultures of either astrocytes or neurons. The two convulsants picrotoxinin and pentylenetetrazole inhibited diazepam binding in astrocytes with IC_{50} values in the low nanomolar range but had much less effect on neuronal binding. The excitatory barbiturates CHEB and DMBB were also more potent on astrocytic than on neuronal binding. The anticonvulsant drugs carbamazepine and phenobarbital were ten times more potent in displacing astrocytic benzodiazepine binding than in displacing neuronal binding, whereas phenytoin was equipotent. Valproic acid, trimethadione and ethosuximide were also more active at the astrocytic than at the neuronal binding site. For all of the anticonvulsant drugs the IC_{50} values in the astrocytes were comparable to the therapeutic plasma levels. The endogenous anxiogenic compound octadecaneuropeptide (ODN; anxiety peptide) was much more potent in inhibiting astrocytic (IC_{50} 140 nM) than neuronal (IC_{50} value 7 µM) binding (Bender and Hertz, Europ. J. Pharmacol. 132, 335, 1986). Classical anxiolytic drugs like diazepam had relatively similar IC_{50} values (approximately 10 nM) in astrocytes and in neurons. A novel group of drugs, the thienotriazolodiazepines WE 973 and WE 1008, which have pronounced effects on conflict behavior and are potent anticonvulsants, were, however, less potent in astrocytes (IC_{50} values > 100 nM) than in neurons. Brotizolam which, in addition, has sedative effects displaced both astrocytic and neuronal binding potently whereas the convulsant KW 1937 was much more potent in astrocytes (IC_{50} value 5 nM) than in neurons.

340.6 INTERACTIONS OF PYRETHROID INSECTICIDES WITH THE PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR. L. Devaud* and T.F. Murray (Spon: J.E. Morris), College of Pharmacy, Oregon State University, Corvallis, OR 97331.

The neurotoxic effects of pyrethroids have been well characterized. Investigating the results of low dose administration of pyrethroids on seizure susceptibility we have previously reported that pyrethroids exert potent proconvulsant effects when tested in the pentylenetetrazol (PTZ) seizure threshold model. (Devaud et al, Europ. J. of Pharmacol., 120: 269 (1986)). This effect is stereoselective with 1RcisαS cypermethrin and 1Rcis permethrin (insecticidally active) each causing a dose dependent reduction in seizure thresholds while their insecticidally inactive isomers were devoid of proconvulsant activity. 1RcisαScypermethrin was the most potent pyrethroid tested, producing a maximal 33% reduction in mg/kg PTZ required to elicit the first myoclonic twitch. The peripheral-type benzodiazepine receptor (PTBR) has been implicated as a site of action for pyrethroids. Pretreatment with PK11195, an antagonist of the PTBR, blocked the proconvulsant effects of deltamethrin, permethrin and cismethrin. Having established the proconvulsant activity of both Type I and Type II pyrethroids, the interactions of pyrethroids with the PTBR were investigated using radioligand binding techniques. We now report that the results of competition binding studies were in agreement with published reports in that Ro5-4864 and PK11195 were potent displacers of [^{3}H]Ro5-4864 from a rat olfactory bulb membrane preparation. The IC50 for this effect was 2.9 ± 0.33 nM for Ro5-4864 and 2.8 ± 0.28 nM for PK11195. Deltamethrin effectively displaced [^{3}H]Ro5-4864 binding with an IC50 of 40.3 ± 8.7 nM and a maximal displacement of 85-90% of specific binding. 1RcisαS cypermethrin was equipotent with deltamethrin as an inhibitor of [^{3}H]Ro5-4864 with an IC50 of 43.1 ± 6.1 nM. The 1ScisαR isomer of cypermethrin, which lacked proconvulsant activity was 350 fold less potent than the 1RcisαS isomer as an inhibitor of [^{3}H]Ro5-4864 binding. In addition, the maximal inhibition of [^{3}H]Ro5-4864 produced by the 1ScisαR isomer of cypermethrin was 47% whereas the maximal displacement produced by the 1RcisαS isomer was 88-95% of specific binding. Type I pyrethroids also interact with the PTBR. The insecticidally active 1Rcis permethrin (but not the insecticidally inactive 1Scis isomer) displaced [^{3}H]Ro5-4864 with an IC50 of 1.50 ± 0.01µM. Other Type I pyrethroids, such as allethrin and cismethrin, also inhibited [^{3}H]Ro5-4864 binding, with an IC50 values of 2.3 ± 0.3 µM and 7.2 ± 3.3 µM, respectively. These indicate a general correlation between proconvulsant activity of pyrethroids and in vitro affinity as inhibitors of binding to the PTBR. Moreover, these results provide additional evidence for this site as a locus of a neurotoxic action of pyrethroid insecticides. (Supported in part by a grant from the Oregon State University Environmental Health Sciences Center.)

340.7 MICROSCOPIC ANALYSIS AND ALLOSTERIC MODULATION OF [^{3}H]-SR 95531 BINDING TO GABA$_A$ RECEPTORS IN THE RAT BRAIN. R.T. McCabe, James K. Wamsley, J.P. Yezuita* and R.W. Olsen[1]. Depts. of Pharmacology and Toxicology and Psychiatry, Univ. of Utah, SLC, UT 84132, and [1]Dept. of Pharmacology and the BRI, UCLA, LA, CA 90024.

One of the two GABA receptor subtypes, the GABA$_A$ receptor, is coupled to a chloride ionophore and exists in both high- and low-affinity conformations. A pyridazinyl-GABA derivative, SR 95531 [2-(3'-carbethoxy-2'-propyl)-3-amino-6-paramethoxy-phenyl-pyridazinium bromide], has recently been reported to have selective GABA$_A$ antagonist properties. In the present study, we investigated the binding of [^{3}H]-SR 95531 using autoradiographic techniques. Furthermore, based on experiments showing allosteric interactions at the GABA receptor, modulation of [^{3}H]-SR 95531 binding by various benzodiazepine (BZ) and barbiturate receptor ligands was examined in several brain areas.

For autoradiography, tissue sections from rat brain were prepared for assay. Dissociation, association, saturation and displacement studies were performed to determine the optimal binding conditions. In all subsequent experiments, tissue sections were incubated for 15 min in ice-cold 10 mM K_2HPO_4/KH_2PO_4 buffer (pH 7.5) containing 100 mM KCl and [^{3}H]-SR 95531, and then rinsed (3x5 min) in buffer alone before drying. Nonspecific binding was defined by 10^{-4} M GABA or 10^{-4} M SR 95531. For homogenate studies, membranes were prepared from whole brain or regions representing cortex, striatum, hippocampus, midbrain-thalamus, medulla-pons and cerebellum. Tissues were immersed for 30 min in ice-cold buffer containing [^{3}H]-SR 95531 and separation of bound from free ligand was performed by centrifugation or filtration. Nonspecific binding was identified with 10^{-4} M GABA. Microscopic analysis indicates that intermediate to high densities of [^{3}H]-SR 95531 binding were found in areas which also contain intermediate to high densities of low-affinity GABA$_A$ sites. A K_d of 42.4 nM and B_{max} of 105.8 fmol/mg tissue was obtained from Scatchard analysis of saturation data. The binding of [^{3}H]-SR 95531 to membranes occurred at low-affinity GABA$_A$ receptor sites. Results from displacement studies, to determine pharmacological specificity, demonstrate that [^{3}H]-SR 95531 is displaceable by GABA$_A$ ligands and preferentially inhibited by GABA$_A$ antagonists. Allosteric modulation data indicate that [^{3}H]-SR 95531 binding is inhibited by barbiturates and several BZ receptor ligands that varied with brain region. Numerous barbiturates exhibited a stereoselective inhibition of [^{3}H]-SR 95531 binding in whole brain.

The binding of [^{3}H]-SR 95531 selectively identifies GABA$_A$ receptors which correspond to the low-affinity sites previously reported in homogenate assays. Our results show that this agent is a new probe for obtaining specific labeling of the low-affinity GABA$_A$ receptor.

340.8 THE LOCALIZATION OF GABA$_A$ RECEPTOR-LIKE IMMUNOREACTIVITY IN RAT CEREBELLUM: A LIGHT AND ELECTRON MICROSCOPIC STUDY. D.L. Meinecke, J. Tallman, and P. Rakic. Section of Neuroanatomy, Yale U. School of Medicine. 333 Cedar Street New Haven, CT 06510-8001.

The inhibitory action of GABA appears to be mediated mainly through a GABA$_A$ receptor complex which gates chloride channels. Therefore, knowledge of the precise location of these receptors in normal and disease states may provide insight into inhibitory synaptic mechanisms in the CNS. Sensitive and high resolution localization of GABA$_A$ receptors can be achieved with immunocytochemical techniques using monoclonal antibodies against proteins of the GABA$_A$ receptor complex. The present study examines the distribution and ultrastructural localization of GABA$_A$ receptor-like immunoreactivity in rat cerebellum with a monoclonal antibody (E9) to a 50KD subunit of the GABA$_A$/benzodiazepine receptor complex (Sweetnam et al., 1986 Psychopharm. Bull. 22).

Cerebellar hemispheres were excised from rats after cardiac perfusion with 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1M PO_4 buffer. Sections of cerebellum were cut at 20um with a Vibratome and incubated in buffered dilutions of E9 and control sera overnight. Sections were next immunoreacted with the ABC-peroxidase system and 0.08% DAB, post-fixed in osmium ferricyanide, and flat embedded in epoxy resin.

Light and electron microscopy showed that GABA$_A$ receptor-like immunoreactivity was located in all three layers and the five neuronal types of the cerebellar cortex and in neurons of the deep nuclei. Glial cells and white matter were not immunoreactive. Immunoreactivity was most intense in the neuronal cell bodies and dendrites where reaction product was localized mostly over free ribosomes and rough endoplasmic reticulum, but not in the nucleus, while axonal shafts and synaptic terminals were not reactive. In the molecular layer stellate and basket cells, small diameter dendrites belonging to these cells, and large Purkinje cell dendrites were immunoreactive. However, reaction product was not present in Purkinje dendritic spines, nor axon terminals forming synapses with these spines. Purkinje cell bodies were strongly immunoreactive but synaptic terminals on their somas were unreactive. In the granule layer reaction product was located in the perikaryal cytoplasm and dendrites of granule and Golgi II cells, as well as in mossy fiber terminals. In contrast, GABAergic axon terminals of Golgi II cells forming synapses with mossy fiber terminals were free of reaction product. Consequently, E9 antibody was differentially distributed over cerebellar cells and their subcellular compartments. These data indicate that GABA$_A$ receptors may be located in the post-synaptic elements of cell bodies and their processes known to form synapses with GABA-containing axon terminals.

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340.9 IN VITRO AND IN VIVO BINDING CHARACTERISTICS OF BENZODIAZEPINE RECEPTOR LIGANDS IN THE CNS: QUANTITATIVE AUTORADIOGRAPHY AND IMAGE ANALYSIS. J.G. Richards, J.M. Séquier*, R. Glinz* and H. Möhler*, Pharma Res. Dept., F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basle, Switzerland.

The therapeutic effects of benzodiazepine minor tranquillizers are triggered by their selective interaction with allosteric binding sites on GABA$_A$ receptors which regulate the gating function of chloride channels in the CNS. Although the cellular and subcellular distribution of benzodiazepine receptors can be resolved immunohistochemically using monoclonal antibodies (Richards et al., J. Neurosci., in press), a quantitative analysis of receptor numbers, their affinity and regulation in microscopic regions of the CNS is only possible using radiohistochemical techniques.

We determined the regional affinity and GABA-shift of receptor ligands (agonists and partial agonists) in ^{3}H-meclonazepam and ^{3}H-flumazenil binding respectively (to rat brain sections) in vitro. Moreover, in order to further characterize drug-receptor interactions in discrete brain regions under more physiological conditions, the regional affinity and time-course of binding inhibition by agonists and partial agonists were determined in ^{3}H-flumazenil binding (to mouse brain) in vivo. For the measurement of binding data by quantitative autoradiography, we used tritium brain paste calibration standards and an image analyser (ASBA^R, Wild-Leitz, Zürich). The influence of the variable lipid content of brain regions on tritium absorbance (quench effect) was investigated by comparing the in vitro binding of a benzodiazepine receptor ligand (Ro 16-0154) radiolabelled with either ^{3}H or ^{125}I.

The findings reveal, 1) the need for re-calibration of commercially available standards, 2) the absence of a quench effect with tritium, 3) the absence of a correlation between the pharmacological profile (agonist, partial agonist) of a drug and its regional affinity for benzodiazepine binding sites in vitro or in vivo, 4) a correlation between the pharmacological characteristics of a drug and its GABA-shift in vitro as well as its duration of binding inhibition in vivo.

340.10 **DISTRIBUTION AND LATERAL MOBILITY OF BENZODIAZEPINE RECEPTORS ON SPINAL CORD AND CEREBRAL NEURONS**
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GABA is the most abundant inhibitory neurotransmitter in the central nervous system and appears to exert its main effects via a GABA receptor that gates a Cl^- channel in the subsynaptic membrane. These receptors contain a modulatory unit, the benzodiazepine receptor, which potentiates GABA-gated channel openings.

To determine the topography and mechanisms governing the distribution of these receptors on neurons, microfluorimetry and fluorescence recovery after photobleaching (FRAP) were used to measure the density and lateral mobility of fluorescently labeled benzodiazepine receptors on neuronal cells in culture. We have synthesized several fluorescent benzodiazepine derivatives. One of these, 7-nitroben-2-oxa-1,3-diazole-1012S (NBD-1012S) was synthesized, purified to homogeneity, and tested in equilibrium binding. Competitive binding of NBD-1012S with [^{3}H]-flunitrazepam on adult chick brain membranes, membranes prepared from chick neuronal cultures, or on neuronal cultures yields K_is of 30 pM, 29 pM, and 46 pM respectively. By microfluorimetry neurons could be specifically labeled by NBD-1012S; the cell soma was predominately labeled and 85 % of the labeling could be displaced by 300 nM unlabeled 1012S or by 300 nM clonazepam. In some neurons, hotspots were observed on the cell body. FRAP experiments on chick neurons indicate that 30 % of the labeled receptors on the same are immobile ($D \leq 10^{-12}$ cm^2/s). The mobile fraction had an average lateral diffusion coefficient of 2×10^{-10} cm^2/s, while those receptors on processes were completely immobilized. On rat spinal cord motoneurons, a significantly greater fraction of receptors were immobilized on the soma (63 %). In contrast, voltage-dependent Na^+ channels and tetramethyrhodamine-phosphatidylethanolamine were very mobile on the cell soma ($D = 10^{-9}$ cm^2/s). General glycoproteins identified by tetramethylrhodamine-succinylConcanavalin A were diffusely distributed with 60% of these sites having only moderate mobility (5×10^{-10} cm^2/s). The results indicate that benzodiazepines are largely restricted to the neuronal soma and are immobilized there. Hence, the forces which restrict benzodiazepine receptor diffusion and allow Na^+ channels to move freely must have some measure of molecular specificity (Supported by NIH grants DK 17436, NS 11535, and NS 24606).

340.11 BICUCULLINE-INSENSITIVE [^{3}H]GABA BINDING TO HOMOGENATES OF CATFISH CENTRAL NERVOUS SYSTEM. G. Tunnicliff*and J.M. Myers* (SPON: H.C. Stanton). Laboratory of Neurochemistry, Indiana University School of Medicine, Evansville, IN 47712.

At least 2 subtypes of GABA receptors have been described in the mammalian central nervous system. $GABA_A$ receptors are linked to Cl^- flux whereas evidence exists that $GABA_B$ receptors are associated with both Ca^{2+} and K^+ currents. In catfish brain it has previously been shown that [^{3}H]GABA binding occurs (Mathis, C.A. and Tunnicliff, G., Comp. Biochem. Physiol. 78C:479,1984.) that has properties similar to $GABA_A$ binding in rat brain. The present study has revealed that a second, and possibly a third, type of GABA binding site exists in catfish brain, both of which are sodium ion independent. One of these sites resembles the $GABA_B$ binding site in mammalian brain since it requires Ca^{2+} and can be inhibited by baclofen. However, this inhibition was not very potent and even at high baclofen concentrations maximum inhibition was only 43%. Moreover, the dissociation constant (Kd) for GABA binding was 4 µM, a value that is about 40 times higher than that measured in rodent brain. At high concentrations isoguvacine was able to substantially increase the inhibition of [^{3}H]GABA brought about by baclofen. This suggests a third GABA binding site exists in catfish brain that is sensitive to high isoguvacine. Neither of the binding sites described is able to be inhibited by bicuculline.

340.12 MODULATION OF FLUNITRAZEPAM BINDING IN INTACT PRIMARY CULTURED SPINAL CORD NEURONS BY GABAergic DRUGS. A.K. Mehta* and M.K. Ticku. Division of Molecular Pharmacology, Department of Pharmacology, Univ. of TX Hlth. Sci. Ctr., San Antonio, TX 78284-7764.

[^{3}H]Flunitrazepam binding and its modulation by $GABA_A$ergic drugs was studied in intact primary cultured spinal cord neurons. The specific benzodiazepine binding to intact cells was rapid, exhibiting high affinity and saturability, with an apparent K_D of 6 nM and B_{max} of 822 fmol/mg protein. The association as well as dissociation of [^{3}H]flunitrazepam binding exhibited monoexponential kinetics. Benzodiazepines such as flunitrazepam, clonazepam, diazepam and RO 15-1788, as well as β-carbolines such as methyl-6, 7-dimethoxy-4-ethyl-β-carboline-3'-carboxylate (DMCM) displaced specifically-bound [^{3}H]flunitrazepam in a concentration-dependent manner. In intact cells, RO15-1788 (K_i = 1.7 nM) was the most potent displacing agent, followed by DMCM (K_i = 4.7 nM) and RO5-4864 (K_i > 5,000 nM) was the least potent. Conversely, specific [^{3}H]flunitrazepam-binding to intact cells was enhanced in a concentration-dependent manner by GABA agonists like muscimol (EC_{50} = 2.6 µM) and GABA (EC_{50} = 14 µM). The EC_{50} values of muscimol and GABA are similar to their K_m values for increased ^{36}Cl-influx in these neurons (Lehoullier and Ticku, Neurosci. Abst. 12:186(#533.3), 1986). The enhancing effect of GABA agonists was susceptible to reversal by bicuculline and picrotoxinin. Specific [^{3}H]flunitrazepam binding was also enhanced by drugs which facilitate GABAergic transmission, like etazolate (EC_{50} = 2.4 µM), (+)etomidate (EC_{50} = 11 µM) and pentobarbital (EC_{50} = 200 µM). These results suggest that the intact cultured spinal cord neurons exhibit the properties of benzodiazepine-GABA receptor-ionophore complex thereby probably providing an ideal in vitro assay preparation to study GABA synaptic pharmacology.

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NEURAL PLASTICITY IN ADULT ANIMALS II

341.1 DL-AMINOPHOSPHONOVALERATE (APV) BLOCKADE OF HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) PREVENTS AN LTP-ASSOCIATED INCREASE IN PROTEIN F1 PHOSPHORYLATION. D.J. Linden*, F-S. Sheu, and A. Routtenberg. (SPON: Z. Iqbal). Cresap Neurosci. Lab., Northwestern Univ., Evanston, IL, 60201.

Previous work from this laboratory has shown a selective increase in the phosphorylation of protein F1 (47K, 4.5pI) measured in vitro following induction of hippocampal LTP. Protein F1 phosphorylation is also positively correlated with the persistence of the potentiated response measured 1 hour or 3 days after LTP induction [Br. Res.(1985) 343:137,(1986) 399:205]. Application of APV, an antagonist of the NMDA class of glutamate receptor reversibly blocks initiation of hippocampal LTP, while leaving low-frequency synaptic transmission unimpaired. However, APV blocks only the initiation of LTP, as APV has no effect on a response once potentiated [J. Physiol.(1982)334:33]. If increased phosphorylation of protein F1 were critical for the persistence of LTP and were a consequence of LTP initiation, then blockade of LTP initiation by APV should prevent an LTP-induced increase in protein F1 phosphorylation.

Rats were implanted with a stimulating electrode in the perforant path and a multi-barrelled micropipette to record field potentials and deliver drugs in the dentate hilus. APV (192 pmol) and vehicle control solutions were applied by micropressure ejection over a 2 min period; high-frequency stimulation (HFS) to induce LTP was provided by 8 stimulus trains (400 Hz, 8 pulses, .4 msec pulse width); 60 min following HFS, the hippocampus was rapidly frozen for study of protein phosphorylation.

Three groups (n=8/group) received either APV immediately before HFS (potentiated response completely blocked, 101% of baseline), APV immediately after HFS, or vehicle before HFS (responses 147% and 149% of baseline respectively). Significant and selective increases in protein F1 phosphorylation of the APV-post HFS (10.21% of total) and vehicle-pre HFS (10.01%) groups relative to the APV-pre HFS group (7.53%; overall difference p<.005 by ANOVA) were observed. Protein F1 phosphorylation was positively correlated with the magnitude of potentiation observed immediately (+.859) and 60 min (+.903) following HFS.

The result herein, that blockade of LTP prevents the associated increase in protein F1 phosphorylation, indicates that protein F1 phosphorylation may be critical for the persistence of the LTP response, and furthermore that phosphorylation of protein F1 is a consequence of the activation of APV-sensitive initiation mechanisms (supported by MH25281-13 and AFOSR87-0042 to A.R.).

341.2 INDUCTION, ENHANCEMENT AND BLOCKADE OF HIPPOCAMPAL SYNAPTIC POTENTIATION: DOSE-DEPENDENT EFFECTS OF PHORBOL 12,13-DIBUTYRATE (PDBu) P. Colley* and A. Routtenberg (SPON: R. Sekuler). Cresap Neuroscience Laboratory, Northwestern Univ., Evanston, Ill 60201

Protein kinase C (PKC), in synergism with a calcium signal, plays a central role in signal transduction and transmembrane signalling in nervous tissue [Science (1984) 225 1365]. Such a mechanism may underlie synaptic potentiation in brain. Phorbol ester, a PKC activator by translocating the kinase to the membrane, enhanced dentate gyrus synaptic responses only in combination with 2 trains of 400 Hz afferent stimulation (2TP) in the intact hippocampus [Br. Res. (1986) 374 378]. In contrast, induction of synaptic potentiation has been observed by others [J. Neurosci. (1986) 8 475]. To clarify this issue, we explored a range of PDBu dosages on PKC regulation of synaptic potentiation.

PDBu, diluted in a 10% ethanol/TRIS vehicle (Veh), was applied iontophoretically in dosages of 6, 12 or 20 pmol, as determined by ejection of 3H-PDBu into test tube. A low dose of PDBu (6 pmol) given alone without 2TP had no effect on synaptic activation, yet in synergism with 2TP induced a growth of the initial potentiation (126% at T=120 min) similar to that seen in the dentate hilus using 32 pmol of phorbol (TPA). If the synergistic enhancement of low dosages of PDBu and 2TP were mediated by PKC activation as proposed, then one would expect an increase in membrane PKC activity. Using lysine-rich histone as substrate to measure PKC activity [Science (1986) 231 587], animals who showed growth of the response over two hours regardless of the magnitude of the initial potentiation after 2TP had 32% more membrane PKC activity compared to animals receiving 12 pmol of PDBu or Veh which showed near baseline responses.

12 and 20 pmol of PDBu delivered alone enhanced the baseline population EPSP slope (twoway ANOVA at T=120 min: df=3,108 p<.001) in a way similar to that seen in the CA1 region of hippocampal slice. Surprisingly, doses which produced this enhancement blocked initial potentiation by 70% when applied just prior to 2TP in a second experiment (oneway ANOVA at T=0 min: F=16.41 df=3,12 p<.001). Whether this block of initiation is mediated by direct activation of PKC, a modulatory role of PKC, or by a non-PKC mediated mechanism can not yet be determined. PKC does appear to have an essential role, however, in the maintenance of synaptic potentiation induced with high frequency stimulation. (Supported by MH25281-13, and AFOSR87-0042 to A.R.)

341.3 PROTEIN KINASE C (PKC) INHIBITORS PREVENT MAINTENANCE OF HIPPOCAMPAL LONG-TERM POTENTIATION (LTP) D. Lovinger*, K. Wong*, K. Murakami*, and A. Routtenberg. (SPON: J.P. Rosenfeld). Cresap Neurosci. Lab., Northwestern Univ., Evanston, IL 60201.

If PKC activation were crucial for maintenance of potentiation, as we have proposed [Prog. Br. Res., 69, 1987, p. 411], then PKC inhibitors should decrease the endurance of potentiation. The failure to observe such a result would be strong evidence against our proposal. We applied PKC inhibitors [mellitin (MEL), polymyxin B (PMB) or H-7] by micropressure ejection to the perforant path synaptic zone in the dentate gyrus 10 min after LTP. Each of the 3 inhibitors induced decay of potentiation in contrast to the persistence observed in Tris-ejected or no ejection controls ($F=21.44$, $df=2,117$, $p<.001$ using MEL, $F=156.61$, $df=1,150$, $p<.001$ using PMB). Synaptic responses returned to baseline within 50 min of MEL or PMB application, and potentiation induced at this time did not differ from that seen in controls. Given the differing effects of the 3 inhibitors on protein kinases other than PKC, their elimination of potentiation likely resulted from actions on PKC. To assay PKC subcellular distribution we measured phosphorylation of exogenous histone H-1 using hippocampal cytosol and membrane from animals given PMB or Tris 10 min after LTP. PMB-treated animals exhibited a decreased percentage of membrane-associated PKC activity relative to animals given Tris (PMB = 68.75±4.29%, Tris=85 ± 4.95%). Thus inhibitors may act by reducing membrane association of PKC following LTP.

MEL treatment 1 hr after LTP produced decay of potentiation, but not to pre-LTP baseline levels ($F=45.53$, $df=2,225$, $p<.001$). MEL treatment 4 hr after LTP was without effect. Thus the importance of PKC activity for LTP maintenance might peak within the first hour after potentiation and decrease thereafter. Alternatively, PKC translocated to the membrane following the onset of potentiation may become resistant to inhibitor action.

MEL or PMB given 15 min before LTP did not affect initial potentiation, but decreased its persistence ($F=19.81$, $df=2, 171$, $p<.001$). The effect of PKC inhibitors on potentiation contrasts with that of the NMDA receptor antagonist APV which does not affect maintenance when given after LTP, but blocks initiation [Trends Pharm. Sci., 6, 407, 1985, p. 407] and PKC substrate phosphorylation when given before LTP [see Linden et al., this meeting]. These data suggest that initiation and maintenance of potentiation constitute separable molecular processes. (Supported by MH25281-13, and AFOSR87-0042 to A.R.)

341.4 TWO GROWTH CONE-ENRICHED C-KINASE SUBSTRATES AND TWO VESICLE-ASSOCIATED PHOSPHOPROTEINS ARE DIRECTLY CORRELATED WITH PERSISTENCE OF LONG-TERM POTENTIATION: A QUANTITATIVE ANALYSIS OF TWO-DIMENSIONAL GELS. R.B. Nelson*, D.J. Linden*, A. Routtenberg (SPON: T. Bleck). Cresap Neuroscience Laboratory, Northwestern University, Evanston, IL 60201.

Translocation of protein kinase C (PKC) from cytosol to membrane and increased phosphorylation of protein F1 (47 kDa, pI 4.5) are correlated with long-term potentiation (LTP; Akers et al., Science, 231:587, 1986; Lovinger et al., Brain Res., 399:205, 1986). Although protein F1 alone was related to LTP persistence in previous studies, not all phosphoproteins can be analyzed by one-dimensional SDS-PAGE, in particular co-migrating phosphoproteins and minor phosphoproteins competing against background label. We therefore used quantitative analysis of two-dimensional gels to relate individual phosphoproteins to measures of LTP, after inducing LTP in the intact dorsal hippocampal formation. Of 15 protein spots analyzed, the phosphorylation of 4, including protein F1, were directly correlated to persistence of LTP over 10 min (measured as growth or decay of the potentiated population spike amplitude), but not to the spike amplitudes themselves. Two of these phosphoproteins, protein F1 and an acidic 80 kDa protein (Albert et al., PNAS, 83:2822, 1986) are PKC substrates and the major phosphoproteins seen in a purified fraction of neural growth cones (Nelson et al., Soc. Neurosci., 11:927, 1985; Katz et al., J. Neurosci., 5:1402, 1985). The other two phosphoproteins are relatively minor species stimulated by cAMP, and to a lesser extent, by phorbol esters and calmodulin. The molecular weight, isoelectric point, charge heterogeneity, and phosphorylation dependencies of these latter two proteins suggest they are the immunologically-related synaptic vesicle proteins IIIa and IIIb (Browning and Greengard, Soc. Neurosci., 10:196, 1984). The synaptic vesicle proteins Ia and Ib were not correlated to persistence of LTP. The present findings suggest that multiple PKC substrates and a specific subset of synaptic vesicle phosphoproteins may be important in the mechanism underlying persistence of LTP. All four of these proteins appear to be heavily or exclusively represented in the pre-synaptic terminal. The enrichment of both protein F1 and 80k in growth cones is consistent with our proposal that a common molecular mechanism involving PKC substrate phosphorylation may underlie both normal neurite growth in developing brain and neural plasticity at adult synapses (Routtenberg, Prog. Brain Res., Vol. 69, 1986). Supported by MH25281-13 and AFSOR87-0042 to A.R.

341.5 **LEUPEPTIN, A THIOL-PROTEINASE INHIBITOR, BLOCKS HIPPOCAMPAL LONG-TERM POTENTIATION IN CHRONICALLY IMPLANTED RATS.** U. Staubli, M. Baudry & G. Lynch, Center for Neurobiology of Learning and Memory, U. of Calif. Irvine, CA 92717.

We recently reported that stimulation applied in a pattern that mimics the naturally occurring theta rhythm induces a potentiation (LTP) of hippocampal field potentials that is stable for one to several weeks (Staubli & Lynch, in press). It has been proposed that partial degradation of submembraneous cytoskeletal proteins, in particular brain spectrin, by a calcium-activated protease is a crucial step in the translation of intense physiological activity into the morphological changes that accompany LTP (Lynch & Baudry, 1984). If so, then drugs that inhibit calpain would be expected to interfere with the induction of LTP. Leupeptin is a reasonably potent antagonist of purified calpain but does not freely enter intact cells. However, intraventricular perfusion of the drug for several days does produce a selective impairment of some forms of learning and causes at least a partial inhibition of calpain activity (Staubli *et al.*, 1985). Accordingly, we have used the chronic perfusion technique to test if leupeptin also affects LTP.

Animals were implanted bilaterally with stimulating electrodes in the Schaffer-commissural system and with one recording electrode in the apical dendrites of field CA1. Osmotic pumps containing either leupeptin (20 mg/ml) or saline and that infused 0.5 *u*l/hr into the lateral ventricle were used. In some rats the pumps were inserted at the same time as the electrodes and in others only after several days of physiological recording. Rats that did not exhibit stable physiological responses for 3 days were dropped from the study. Baseline testing was begun after at least 3 days of infusion and continued for 3 days. "Theta bursting" stimulation (Larson & Lynch, 1986) was used to induce LTP. The control group (n=11) exhibited an immediate and robust LTP effect that remained in place 24 and 48-72 hrs later. Only three of the thirteen leupeptin treated rats exhibited evidence of LTP and in only one of these was a sizeable effect recorded over several days. The difference in amount of LTP between the 2 groups was highly significant. The leupeptin filled pumps were disconnected in seven rats and testing was resumed after a delay of 5-8 days. Potentiation was observed in all of these animals immediately after bursting stimulation and in five for several days thereafter. Thus, elimination of leupeptin removed the block on LTP induction.

Two lines of evidence indicate that leupeptin did not substantially affect baseline physiology: 1) the evoked responses were unchanged from the drug-free condition in the rats in which this analysis was conducted; 2) disconnecting the pumps did not affect the size of the response from that recorded during infusion. Accordingly, it is not likely that the striking blockade of LTP induction caused by leupeptin is due to a generalized disturbance in the physiological properties of the hippocampus. (Supported by AFOSR grant #86-0099.)

341.6 MULTIPLICATIVE INTERACTION BETWEEN LONG-TERM POTENTIATION AND SHORT-TERM FACILITATION IN CRAYFISH NEUROMUSCULAR SYNAPSES. C.L. Keenan, D.A. Baxter, & T.H. Brown, Division of Neurosciences, Beckman Research Institute, City of Hope, Duarte, CA 91010.

We investigated the interaction between long-term potentiation (LTP) and short-term facilitation (STF) in crayfish opener-excitor (OE) neuromuscular synapses. At the same time, we also began to explore the optimal patterns of stimulations for LTP induction.

In each experiment, an attempt was made to induce LTP by stimulating the OE axon 300 times within 30 s. A constant stimulation rate appeared to be more effective in inducing LTP than a sequence of brief but high-frequency stimulations. In 27 of 34 preparations, stimulating the OE at a constant frequency of 10 Hz (300 stimulations in 30 s) induced LTP (72% mean increase above the control level 30 min after the stimulation). In contrast, in 10 of 10 preparations, a series of short bursts of tetanic stimulations (also a total of 300 stimulations in 30 s) failed to induce LTP (9% mean increase). Subsequent stimulation at a constant rate of 10 Hz (for 30 s) induced LTP in all 10 preparations (63% mean increase).

Several characteristics of STF, including the magnitude (F_e) and accumulation (F_n) of STF during short trains of stimuli and tonic stimulation (Bittner and Sewell, J. Comp. Physiol. 109:287, 1976), were monitored before and at least 30 min after LTP induction. The results indicated that the LTP-induced increase in transmitter release (Baxter, Bittner and Brown, Proc. Natl. Acad. Sci. 82: 5978, 1985) had a multiplicative (not additive) effect on STF. The parameters F_e and F_n were not altered by LTP.

The expression of STF and LTP may therefore be mediated through independent processes. McNaughton (J. Physiol.324:249, 1982) observed a similar multiplicative interaction between LTP and STF in the hippocampal formation. He noted that a simple multiplicative interaction could arise if LTP increased the mean quantal size $\bar{q}$ and STF increased the mean quantal content m. However, in the OE synapses LTP, is accompanied by an increase in m but not $\bar{q}$ (Baxter, et al., 1985). Alternatively, it is possible that STF and LTP selectively and differentially increase the binomial release parameters (Brown, Perkel and Feldman, Proc. Natl. Acad. Sci. 73: 2913, 1976) $\bar{n}$ and $\bar{p}$ (whose product is m), a possibility that appears testable in this system (Keenan and Brown, Soc. Neurosci. Abstr. 12: 605a, 1986).

We are now studying the biophysical mechanisms underlying the multiplicative interaction between STF and LTP in OE synapses. We are also exploring the possible behavioral role of LTP in fine tuning the motor system that controls the claw, the operation of which depends on the development of STF. (Supported by NIH grant NS21561, AFOSR contract F49620 and a McKnight Foundation Award.

341.7 TRANSITION FROM LONG-TERM POTENTIATION TO KINDLING IN THE PYRIFORM CORTEX. J. S. Stripling and D. K. Patneau. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Electrical stimulation of the olfactory bulb (OB) elicits a potential in the pyriform cortex (PC) consisting of an initial wave (period 1) encompassing both a mono- and di-synaptic population EPSP, followed by period 2, which is temporally associated with inhibition of PC pyramidal cells. Repeated high-frequency trains of OB stimulation too brief (10 pulses each) to trigger a seizure produce a selective long-term potentiation of period 2 in the PC (Soc. Neurosci. Abstr. 10:76, 1984). In contrast, a single long (100 pulse) high-frequency train at the same current intensity triggers a focal electrographic seizure and produces a distinctly different pattern of effects. An increase in period 2 is seen, as with stimulation below seizure threshold, but in addition a long-term increase in period 1 occurs (Brain Res. 361:61-69, 1985). This pattern of effects suggests that long-term potentiation in the PC involves enhancement of inhibitory mechanisms, and that kindling superimposes upon this effect an increase in excitatory input to PC pyramidal cells. This contrasts sharply with the hippocampus, where potentiation of the population EPSP is a prominent effect of high-frequency stimulation below seizure threshold. The present experiment examined whether it is possible to produce an increase in period 1 in the PC without triggering a seizure.

Male Long-Evans rats were chronically implanted with electrodes in the OB and PC. Animals received high-frequency stimulation of the OB (100 pulses at 100 Hz) at increasing current intensities until a focal electrographic seizure was triggered. Test potentials were evoked in the PC by OB stimulation before and after each stimulation train. Potentiation of period 1 was reliably seen not only following a seizure, but also following stimulation trains 1-2 current steps below seizure threshold.

These results indicate that the occurrence of a seizure is not essential for the potentiation of period 1, but that stimulation close to seizure threshold is needed. Whether this potentiation is due to an increased output from the OB or to increased transmission at the synapses between lateral olfactory tract fibers and PC pyramidal cells is currently under investigation. Potentiation of period 2 can be produced by much briefer trains which are far below seizure threshold and cause no long-term alterations in period 1, even with extensive repetition. Thus the potentiation of period 2 in the PC appears to involve a separate mechanism which is more likely to be activated by naturally occurring patterns of neural activity than the mechanism responsible for potentiation of period 1.

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341.8 RECOVERY OF FUNCTION AFTER UNILATERAL SENSORIMOTOR CORTEX LESIONS: CONTRIBUTION OF THE CONTRALATERAL CORTEX. T.M. Barth, T.A. Jones*, and T. Schallert. Dept. of Psychology Univ. of Texas at Austin, TX 78712

Unilateral damage to the sensorimotor cortex in rats causes a somatosensory asymmetry ipsilateral to the lesion that is readily quantified using bilateral tactile extinction tests (T. Schallert et al., *Pharm. Biochem Behav.*, 19:753, 1983; T.M. Barth et al., *Neurosci. Abstr.*, 10:1146, 1984). Previous work has shown that recovery (a gradual reduction in the magnitude of sensory asymmetry) occurs even after hemidecortication (T.Schallert & I. Q. Whishaw, *Behav. Neurosci.*, 98:518, 1984). These data suggest that cortex ipsilateral to the lesion is not necessary for recovery to occur, and that the contralateral cortex and/or subcortical brain areas may be involved. In the present study we examined the effects of lesions in the homotopic contralateral cortex following recovery from unilateral brain damage.

Unilateral lesions were placed in three different areas of the rat cortex: 1) the anteromedial cortex; 2) the caudal forelimb sensory + motor area; 3) the rostral forelimb motor area. An ipsilateral sensory asymmetry and subsequent recovery were observed in each of the lesion groups. After recovery (i.e. two consecutive postoperative tests in which an animal showed no sensory asymmetry) half of the rats in each unilateral lesion group received a second lesion in the homotopic contralateral cortex (i.e. two-stage bilateral lesion) and the other half were given a sham surgery (i.e. lesion + sham). For comparison, additional groups of rats were given simultaneous bilateral lesions (i.e. one-stage bilateral lesion).

The results showed that rats receiving two-stage bilateral lesions in either the caudal or rostral forelimb area exhibited a sensory asymmetry ipsilateral to the ***second*** lesion that was comparable in magnitude to that observed after the initial unilateral lesion. No change in sensory asymmetry was observed in the respective lesion + sham or one-stage bilateral lesion groups. In contrast, rats with two-stage bilateral lesions in the anteromedial cortex did not exhibit a sensory asymmetry after the second lesion and were not different from the lesion + sham or one-stage bilateral groups.

These data suggest that subareas of the rat cortex may be distinguished by comparing the effects of two-stage bilateral lesions and that the role of the homotopic contralateral cortex in recovery may differ depending on the area damaged.

341.9 CHRONIC STABILITY OF RECEPTIVE FIELDS AT LOCI IN NORMAL, ADULT SOMATOSENSORY CORTEX. M.B. Calford, Neuroscience Laboratory, Department of Physiology and Pharmacology, University of Queensland, St Lucia, Queensland, 4067, Australia.

Somatosensory cortex of adult mammals is known to be capable of dramatic reorganization following peripheral nerve damage, or amputation (Wall, J.T. and Kaas, J.H., in *Synaptic Plasticity*, Cotman, C.E., ed., Guildford Press, 1985). An unresolved issue is the extent to which the ability of cortex to quickly adapt to a changed afferent input, maintaining responsiveness and topography, is reflected in plasticity of somatotopy in normal animals.

Chronic (2 to 6 weeks) multiunit recordings were obtained through implanted, floating electrodes (teflon-coated, stainless steel; 24 μm diameter) from somatosensory cortex of the little red flying fox. Previous studies of flying fox somatosensory cortex (Calford *et al.*, *Nature*, 313: 477-479, 1985) revealed a normal mammalian pattern with at least three somatotopic fields. No check on the physical stability of the floating electrodes was possible. However cutaneous receptive fields on the head, snout, back, chest, arm or pro-wing of 25 electrodes from 6 animals recorded over periods of 2 to 6 weeks were remarkably stable. This suggests that the electrodes were fixed in position and that somatosensory cortex does not show plasticity in normal animals. This result contrasts with plasticity demonstrated acutely in the same species following amputation of the thumb. Loci that once represented the digit are immediately responsive to adjacent regions. These new receptive fields (on the arm and wing) are larger than fields found in this region of normal animals and always extend to some part of the wound. The acute result demonstrates that connections exist to supply a locus in somatosensory cortex with input from an area far larger that its receptive field. The chronic result indicates that the subset of this input that forms the receptive field is stable over time in normal animals.

341.10 TOPOGRAPHICAL REORGANIZATION IN THE SECOND SOMATOSENSORY AREA (SII) AFTER REMOVAL OF SELECTED PORTIONS OF POSTCENTRAL CORTEX T.P. Pons, P.E. Garraghty, and M. Mishkin, Lab Neuropsychology, NIMH, Bethesda, MD 20892, and Dept of Brain Sciences, MIT, Cambridge, MA 02139.

We used electrophysiological recordings to assess the fate of the SII body representation following lesions of selected portions of the postcentral strip (areas 3a, 3b, 1, and 2). Six to eight weeks following the cortical lesions, single or multiunit activity elicited by peripheral somatic stimulation was recorded with tungsten microelectrodes in the SII region of 9 hemispheres in 5 adult macaques (4 Macaca mulatta and 1 M. fascicularis) anesthetized with a mixture of nitrous oxide and halothane. Our results yielded two major findings.

First, as previously reported, elimination of the representations of any body part in the postcentral strip eliminated that, and only that, representation in SII (5 hemispheres). Second, six weeks after removal of the postcentral hand representations, a procedure that functionally deafferented the SII hand representation, the cortical space in SII previously representing the hand was not silent. Instead, the normally adjacent representations of the body expanded into this vacated region. The expansion was most obvious for the representation of the foot, which occupied only 5-12% of SII in each of five unoperated hemispheres, but occupied 55-80% of SII in each of three hemispheres that received lesions of the postcentral hand representations. This massive reorganization extended over half of SII in the rostral caudal dimension, for a distance up to 7mm. In three additional cases we recorded from the SII region of adult macaques (M. mulatta) 24 hours after removal of the postcentral hand representations. In these cases there appeared to be no expansion of the foot or trunk representation. Instead there was a large "silent" zone in the expected location of the SII hand representation. Thus the results obtained at 6-8 weeks after the lesions are not completely attributable to a simple "unmasking" of preexisting inputs.

These findings provide evidence for a previously unrecognized degree of cortical plasticity in adult primates in that the reorganizational changes occur over 5 or more millimeters of cortex. The results require major revisions of current theories which tend to confer static properties on cortical maps.

342.1 SEROTONIN RECEPTOR BINDING AND AGONIST PROPERTIES OF 6-CARBOXAMIDO-4-DIPROPYLAMINOTETRAHYDROBENZINDOLE. N. R. Mason, R. W. Fuller and M. E. Flaugh*. Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285.

Several analogs of the ABC tricyclic partial ergoline, 4-dipropylaminotetrahydrobenzindole (DATB), were synthesized with a protophilic group at position 6 and were tested as serotonin agonists based on their structural similarity to serotonin. The 6-methoxy, 6-nitro, 6-cyano and 6-carboxamido derivatives all inhibited binding of tritiated serotonin (^{3}H-5HT) to 5HT1 sites in membranes from rat frontal cortex with IC-50 values of approximately 100 nM or less. The 6-bromo derivative and the parent compound with no substituent at the 6 position were slightly less potent. The methoxy, carboxamido and bromo derivatives had much lower affinity for 5HT2 receptors labeled in frontal cortex membranes with ^{3}H-spiperone (IC-50 values greater than 1000 nM) and for dopamine D2 receptors labeled in striatal membranes with ^{3}H-spiperone.

Competition curves for inhibition of ^{3}H-5HT binding by 6-carboxamido-DATB were biphasic, indicating differential affinity for different binding sites. Experiments in the presence and absence of 1 μM spiperone to block binding of ^{3}H-5HT to 5HT1A sites revealed that 6-carboxamido-DATB had high affinity for the 5HT1A site (IC50=1 nM) and low affinity for the 5HT1B site (IC50 >1000 nM). The (+) and (-) enantiomers of 6-carboxamido-DATB had similar affinities for 5HT1A sites as the racemate. The racemate and the enantiomers showed similarly high affinity for 5HT1A sites when ^{3}H-8-hydroxy-2-dipropyl-aminotetralin was used as the radioligand.

6-Carboxamido-DATB given to rats at doses of 0.03 mg/kg s.c. and higher decreased hypothalamic concentrations of 5-hydroxy-indoleacetic acid, indicating a decreased serotonin turnover presumably secondary to activation of synaptic receptors for serotonin. The two enantiomers were equipotent in producing this effect and in decreasing the accumulation of 5-hydroxy-tryptophan in hypothalamus following decarboxylase inhibition by NSD 1015. The racemate and enantiomers also increased serum corticosterone concentration in the same dose range, an effect produced by other 5HT1A agonists including 8-hydroxy-2-dipropyl-aminotetralin, buspirone and ipsapirone.

These data indicate that both enantiomers of 6-carboxamido-DATB are potent serotonin agonists with selectivity for the 5HT1A receptor subtype.

342.2 STRUCTURE-ACTIVITY RELATIONSHIPS OF NITRO AND AMINO PHENYLPIPERAZINES ON [^{3}H]SEROTONIN BINDING. S.G. Britt*, G.T. Redpath*, S.R. VandenBerg, Dept. of Path., Univ. of Virginia Sch. of Med., Charlottesville, VA 22908

The multiple specific binding sites for serotonin (5-HT) in the CNS may mediate the variety of its effects, but there is currently limited information on the structural correlates of receptor agonist/ antagonist activities and the functional significance of the various binding sites. Trifluoromethylphenyl piperazine (TFMPP), an agonist at the high affinity [^{3}H]5-HT binding site(s), and a number of other non-indoleamine ligands have been recently used to study populations of these binding sites (Glennon, R.A., J. Med. Chem. 30: 1-12, 1987). The majority of phenyl piperazines which have been used are halogenated compounds and comprise a relatively limited group with respect to polarity and electronic character. We have synthesized a series of mono- and disubstituted nitro and amino phenyl piperazines, which differ significantly in structure from the original halogenated compounds, to further elucidate the structure-activity relationships of this class of compounds.

The nitro phenyl piperazines (NO_2PP) were prepared by dehaloamination of a substituted fluoronitrobenzene with piperazine, or by a reaction involving ring closure of bis (chloroethyl) amine with a substituted aniline. The amino phenyl piperazines (NH_2PP) were prepared by catalytic hydrogenation of the nitro precursors. The pure compounds were characterized by TLC, NMR, mass spectrometry and elemental analysis. Radioligand displacement studies in a 50mM TrisHCl, 6mM $CaCl_2$, 10μM pargyline HCl buffer (pH 7.4) supplemented with antioxidants were done with 2nM [^{3}H]5-HT (New England Nuclear, 24-30 Ci/mmol) using 5μM d-LSD to define non-specific binding, in a lysed P2 membrane fraction prepared from bovine cerebral cortex.

The rank order of affinity for some of these compounds in comparison to previously characterized phenyl piperazine analogues was TFMPP > $2NO_2$PP > PP > $3NO_2$PP ≈ mianserin > $2NH_2$PP > $2NO_2$-$4NH_2$PP > $4NO_2$PP > $4NH_2$PP and affinity constants derived from computer analysis (McPherson adaptation of "LIGAND") ranged from 110 nM to 93,000 nM. Change in the position of nitro substitution was accompanied by a 3-18 fold reduction of the affinity while change from nitro to amino substitution at a given position reduced the affinity 8-10 fold. This rank order could also be demonstrated with human cortical membranes. These substituted phenyl piperazines will be useful in subsequent studies to more clearly establish the structural correlates of serotonin agonist interaction with receptor binding site(s).

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342.3 **ASCORBATE EFFECTS ON REVERSIBLE [^{3}H] SEROTONIN BINDING TO BOVINE FRONTAL CORTEX MEMBRANES.**
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There has been controversy over the use of ascorbate as an antioxidant in [^{3}H] serotonin binding assays. Both beneficial and deleterious effects have been reported. Ascorbate is also present in the brain in concentrations approaching those used in binding assays. It seems possible that ascorbate could have both antioxidant and physiologically meaningful effects in binding assays. To test for non-antioxidant effects of ascorbate, however, conditions must be established where [^{3}H] serotonin binding is reversible without exogenous antioxidants. We have utilized the endogenous antioxidant capacity of membranes (Kovachich and Mishra Neurosi.Letts. 52, 153-158, 1984: Todd and Ciaranello Brain Res., 400, 245-258, 1987) to study the effects of ascorbate on reversible binding.

Conditions for [^{3}H] serotonin binding to bovine frontal cortex membranes were established where binding was reversible and saturable in the absence of ascorbic acid. Membranes were added to assay tubes first and binding was initiated by the addition of [^{3}H] serotonin. Greater than 95 percent of binding was dissociable under our conditions. There were two components of binding with kds of 1.33 ±0.26 nM (5-HT_1) and 124.8 ±56.9 nM (5-HT_2) and Bmaxs of 0.16 ±0.02 fmoles/ug and 0.80 ± 0.24 fmoles/ug, respectively (mean ± standard deviation, n = 3). At pH 7.4, increasing ascorbate (0 to 5.7 mM) significantly enhanced binding affinities (0.90 ± 0.04 nM and 29.6 ±17.3 nM, mean ± standard deviation, n = 3, at 5.7 mM ascorbate) without changing Bmaxs. At lower pHs (pH 6.6 to pH 7.1) 5-HT_1 binding was reduced in the presence of ascorbate while 5-HT_2 binding was not affected.

These results confirm that ascorbate is not necessary for reversibility of [^{3}H] serotonin binding in vitro. In addition, conditions were found where combinations of ascorbate and hydrogen ion concentrations could enhance or decrease [^{3}H] serotonin to different binding site subtypes. The hydrogen ion and ascorbate concentrations used here are in the physiological range. It is possible that changes in ascorbate and pH could potentiate or attenuate serotonergic responses in either normal or extreme metabolic circumstances.

342.4 [^{3}H]-DIHYDROERGOTAMINE: VALIDATION AS A RADIOLIGAND FOR SEROTONERGIC 5-HT_{1B} BINDING SITES IN RAT BRAIN AND DEMONSTRATION OF 5-HT_{1B} GUANINE NUCLEOTIDE SENSITIVITY. Mark W. Hamblin, Kayvan Ariani*, Peter I. Adriaenssens*, and Roland D. Ciaranello. Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305.

The 5-HT_{1B} binding site is a putative 5-HT receptor subtype that may represent a cerebral cortical presynaptic autoreceptor in rats. The only 5-HT_{1B} radioligands available to date, [^{3}H]-5-HT and [^{125}I]-iodocyanopindolol, both suffer from lack of specificity and relatively high non-specific binding. We now describe the binding characteristics of [^{3}H]-dihydroergotamine ([^{3}H]-DE) to 5-HT_{1B} type binding sites. The high affinity and low non-specific binding seen with this compound may make it the preferred radioligand in many studies.

[^{3}H]-DE labels a population of sites in rat cerebral cortex and hippocampus membranes with high (K_d<0.1nM) affinity. This binding is both saturable and reversible. Kinetic and competition studies indicate heterogeneity of binding sites, with significant contributions to binding made by alpha adrenergic and 5-HT_{1A} serotonergic sites, as well as 5-HT_{1B} sites. When phentolamine (500nM) and spiroxatrine (500nM) are included to mask alpha and 5-HT_{1A} binding, all remaining specific binding shows affinities (K_i's) for other drugs characteristic of 5-HT_{1B} sites: 5-HT, 29nM; RU 24969, 2.4nM; ±21-009, 0.75nM; ±cyanopindolol, 1.6nM; DPAT, 4100nM; and mesulergine, 4500nM. Under these 5-HT_{1B} specific conditions, [^{3}H]-DE binding is typically about 90% specific and has a K_d of approximately 50pM. The B_{max} is 96 fmol/mg protein in hippocampus membranes and 77 fmol/mg protein in cortex membranes. [^{3}H]-DE dissociation from 5-HT_{1B} sites is slow, with a dissociation $t_{1/2}$ of 2.1 hours at 37° C, 17 hours at 25° C, and >48 hours at 4° C, a feature that may make [^{3}H]-DE useful in the study of solubilized 5-HT_{1B} site proteins.

[^{3}H]-DE 5-HT_{1B} specific binding is weakly inhibited by the addition of GTP or Gpp(NH)p. In contrast, addition of guanine nucleotides lowers the affinities of the 5-HT_{1B} agonists 5-HT and RU 24969 7-fold in [^{3}H]-DE competition studies. This suggests that the 5-HT_{1B} site has a G-protein effector-linkage and that [^{3}H]-DE is a weak partial agonist at this receptor.

342.5 STRUCTURE-ACTIVITY RELATIONSHIPS OF A SERIES OF TETRAHYDROPYRIDYL-INDOLES AT SEROTONIN$_{1A}$ AND SEROTONIN$_2$ RECEPTORS. D.L. Nelson, A.L. Killam*, G. Lambert*, S.S. Nikam*, B. Weck*, and A.R. Martin*. College of Pharmacy, Univ. of Arizona, Tucson, AZ 85721.

Based on findings that the tetrahydropyridylindole RU 24969 is an agonist at serotonergic receptors, a series of 22 analogs of this compound was synthesized to carry out a systematic study of the structural features that determine high potency and selectivity between subtypes of serotonin (5-hydroxytryptamine, 5-HT) receptors. The compounds were evaluated at 5-HT$_{1A}$ sites, measured by the binding of [^{3}H]8-OH-DPAT, and 5-HT$_2$ sites, measured by [^{3}H]ketanserin binding. In addition, certain compounds were also tested at functional 5-HT$_2$ receptors found in the vasculature. The starting compound for all structural modifications was 3-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-indole (MTHPI). MTHPI itself showed little discrimination between the two binding sites, while substitution at position 5 of the indole generally resulted in increased potency at 5-HT$_{1A}$ sites with either no change or decreased potency at 5-HT$_2$ sites (see examples below).

Substitution at C-5	Apparent K_i, nM 5-HT$_{1A}$	5-HT$_2$
H	146	67
OCH_3	21	897
OH	62	356
F	68	45
Cl	26	51
Br	15	48
CO_2CH_3	19	3,665

In contrast to substitution at position 5, addition of a benzyl group at position 1 resulted in almost complete loss of affinity for the 5-HT$_{1A}$ site (K_i> 10,000 nM) with an increased affinity for the 5-HT$_2$ site (K_i= 15 nM). Studies of selected compounds at peripheral vascular models of 5-HT2 receptors produced unexpected results. In the rabbit femoral artery the compounds tested acted as agonists, while in the rat aorta they acted as antagonists. As an example, RU 24969 produced contractions in the rabbit femoral artery ($EC_{50} \simeq$ 100 nM) that appeared to be antagonized by the 5-HT$_2$ antagonist ketanserin. In the rat aorta RU 24969 itself antagonized the actions of 5-HT. The data from these studies show that structural modifications of a simple, partially rigid analog of 5-HT can produce compounds that have relatively high potency and that can discriminate between 5-HT receptor subtypes. Such compounds should aid in defining the nature of the ligand recognition sites of these receptors. (Supported by NIH grants NS16605 and NS01009).

342.6 DIFFERENTIAL EFFECTS OF SULFHYDRYL REAGENTS ON BINDING TO BRAIN SEROTONIN (5-HT) RECEPTOR SUBTYPES. C.A. Stratford, G.L. Tan*, M.W. Hamblin, and R.D. Ciaranello. Dept. of Psychiatry and Behavioral Sciences, Stanford Univ. Stanford, CA 94305

The sulfhydryl reagents N-ethylmaleimide (NEM) and para-chloromercuribenzoate (PCMB) reduce binding of [^{3}H]8-hydroxy-2-(di-propylamino)tetralin (DPAT) to 5-HT$_{1A}$ and of [^{3}H]5-HT to 5-HT$_{1B}$ and 5-HT$_{1D}$ binding sites in rat cortex and bovine frontal cortex. IC_{50}'s for NEM and PCMB on binding to these subtypes range from 20 to 40 micromolar. Binding of [^{3}H]mesulergine to 5-HT$_{1C}$ binding sites and of [^{3}H]ketanserin to 5-HT$_2$ sites is reduced only at much higher concentrations of either NEM or PCMB (20-50,000 and 500 micromolar, respectively). Binding of [^{3}H]dihydroergotamine (DE) to 5-HT$_{1B}$ sites is not as sensitive to NEM as is binding of [^{3}H]5-HT to this site. The rank order of NEM sensitivity of the binding sites is as follows (compound listed in parentheses indicates tritiated ligand used to define the site):
5HT$_{1A}$(*DPAT) $\approx$ 5HT$_{1B}$(*5-HT) $\approx$ 5HT$_{1D}$(*5-HT) >> 5HT$_{1B}$(*DE) $\geq$ 5HT$_2$(*Ketanserin) $\approx$ 5HT$_{1C}$(*Mesulergine).
These data suggest that the determination of sulfhydryl sensitivity may not be intrinisic to the receptor subtype, but rather to the type of ligand (agonist or antagonist) used to label specific sites.

The effects of the sulfhydryl reagents on [^{3}H]5-HT binding are prevented by pre-incubation of membranes with dithiothreitol, indicating sulfhydryl specificity of the effect. Prior and concommitant incubation of membranes with serotonin or other serotonergic agents (DPAT, mesulergine, spiperone) does not appear to protect the sites from the effects of NEM, suggesting that the sensitive sulfhydryl groups are not at the ligand binding sites.

5-HT competes with [^{3}H]DE for binding to rat 5-HT$_{1B}$ binding sites with an IC_{50}-derived $K_{D(app)}$ of 16nM. Addition of either GppNHp or NEM to the assay shifts the 5-HT competition curve to the right. When incubated together, the effects of these agents are not additive. In other receptor systems, such as the bovine brain GABA-B receptor, NEM sensitivity of agonist binding has been shown to be attributable to sulfhydril groups present on receptor-associated G-proteins (Asano, T. and Ogasawara, N., Mol. Pharmacol., 29:244, 1986). In such systems, non-additivity between GppNHp or GTP and NEM was also observed. Work is in progress to determine the extent and nature of linkage of each of the 5-HT receptor subtypes to G-protein(s).

342.7 **CHARACTERIZATION OF 5-HT2 RECEPTORS IN A CELL LINE DERIVED FROM THE 7315a ANTERIOR PITUITARY TUMOR.** **P. McGonigle, K. Nicklaus, K. A. Neve, N. Cocero and P. B. Molinoff. (SPON: R.B. Murray). Dept. of Pharmacology, University of Pennsylvania, Phila., PA 19104.**

Dispersed cells from 7315a transplantable anterior pituitary tumors were plated on protamine-coated plates in Dulbecco's modified Eagle's medium (DMEM) enriched with horse serum, fetal bovine serum (FBS) and rat hypothalamic extract. After 3 weeks, hypothalamic extract was removed and the concentration of horse serum was reduced. Cells that attached to protamine-coated plates were subcultured repeatedly. Cells were cloned by limiting dilution after plating at a theoretical density of 0.1 cell/well and are presently being maintained on uncoated tissue culture plates in DMEM with FBS supplemented with penicillin G (100 μg/ml) and streptomycin (100 μg/ml). This cell line was selected for further study on the basis of a high density of specific binding sites for ^{125}I-NAPS and ^{3}H-spiroperidol. A preliminary saturation experiment with ^{3}H-spiroperidol yielded a linear Scatchard plot and a K_d of 1 nM. Nonspecific binding was defined with 2 μM (+)-butaclamol. The binding of ^{3}H-spiroperidol was inhibited by ketanserin and pipamperone, but was not inhibited by the D-2 antagonist sulpiride. These data are consistent with the presence of 5-HT2 receptors that are labelled with ^{3}H-spiroperidol.

These sites were further characterized in binding assays with ^{125}I-LSD. Nonspecific binding was defined in the presence of 100 nM ketanserin. At 37^{o}C, the binding of ^{125}I-LSD (0.25 nM) reached equilibrium in 45 min and remained stable for at least an additional 45 min. Scatchard analysis of the equilibrium binding of ^{125}I-LSD (.05-2.5 nM) resulted in linear plots and yielded a K_d of 0.53 $\pm$ .02 nM and a B_{max} of 90 $\pm$ 29 fmol/mg protein. Studies of the inhibition of the binding were performed with a number of competing ligands including ketanserin, pipamperone, domperidone and sulpiride. The inhibition curves for the competing ligands had Hill coefficients close to 1.0 suggesting that ^{125}I-LSD is labelling only a single class of receptors in this cell. The order of potency for these competing ligands, ketanserin > pipamperone > domperidone > sulpiride, was consistent with an interaction at the 5-HT2 receptor. Thus, this clonal line of cells appears to contain a relatively high density of 5-HT2 receptors. (Supported by NS 18591 and GM 34781)

342.8 ACTIVATION AND REGULATION OF THE SEROTONIN 5-HT$_{1C}$ RECEPTOR ON THE CHOROID PLEXUS BY CSF-BORNE SEROTONIN. James Giordano and Paul R. Hartig. Environmental Neurobiology Program, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD 21205.

The serotonin 5-HT$_{1C}$ receptor is found at high densities on epithelial cells of the choroid plexus. This tissue forms an interface between the CSF and the blood circulation, forming an important component of the blood-CSF barrier. Since the epithelial cell layer of the choroid plexus does not receive significant direct innervation by serotonergic fibers, it has been suggested that the 5-HT$_{1C}$ receptor is activated by serotonin contained in fluids which bathe this tissue. An unresolved question has been whether this receptor is located on the apical surface of this cell layer, where it may be activated by serotonin from the ventricular CSF, or whether it is located on the basal or lateral surfaces, where it may be activated by serotonin in blood serum. In the present study we investigated the source of activation of the 5-HT$_{1C}$ receptor by measuring in vivo labeling of the choroid plexus following intravenous (iv) and intracerebroventricular (icv) injections of ^{3}H-5-HT. Following iv injection, we observed a slow labeling of choroid plexus sites that were four-fold more abundant than the total 5-HT$_{1C}$ site density in this tissue. The amine transport inhibitor hexamethonium reduced choroid plexus labeling by 95% following iv injection of doses that do not block the serotonin 5-HT$_{1C}$ receptor. We conclude that blood-borne ^{3}H-5-HT does not label the choroid plexus 5-HT$_{1C}$ site but may label a transport site in this tissue. In contrast, icv injection of ^{3}H-5-HT labels a saturable site in the choroid plexus that was relatively unaffected by co-administration of hexamethonium. In vivo saturation labeling studies of this site revealed a site density (apparent B_{max} = 3060 fmol/mg protein), binding affinity (apparent K_d = 72 nM), and antagonist binding properties in good agreement with previous in vitro homogenate binding studies on the 5-HT$_{1C}$ receptor. We conclude that the 5-HT$_{1C}$ receptor can be labeled by CSF-borne, but not by blood-borne, serotonin. These data suggest that the 5-HT$_{1C}$ receptor is located on the apical surface of the epithelial cell layer, in direct contact with ventricular CSF.

The most likely candidate for the source of CSF serotonin activating the choroid plexus 5-HT$_{1C}$ receptor is the network of supraependymal serotonergic fibers lining the ventricular walls. We are currently testing whether denervation of these fibers by icv injections of 5,7 dihydroxytryptamine will promote regulatory changes in the levels of 5-HT$_{1C}$ receptors in the choroid plexus. Recent studies by Conn et al. (Brain Res. 400, 396 (87)) report supersensitivity of the 5-HT$_{1C}$-mediated phosphoinositide response following such treatments. The current studies will determine if this is accompanied by changes in 5-HT$_{1C}$ receptor levels.

342.9 2,5-DIMETHOXY-4-BROMOAMPHETAMINE (DOB) INTERACTIONS WITH 5-HT RECEPTOR SUBTYPES. S.S. Wang and S.J. Peroutka. Departments of Neurology and Pharmacology, Stanford University, Stanford, CA 94305.

2,5-dimethoxy-4-bromoamphetamine (DOB), a potent hallucinogen, has been suggested to be a highly specific ligand for the 5-HT_2 receptor through radioligand binding studies. Moreover, ^{3}H-DOB has been hypothesized to label a "high-affinity" form of the 5-HT_2 receptor (Lyon et al, Mol. Pharmacol. 31:194-199, 1987). The present study attempted to further characterize the interactions of DOB with brain 5-HT receptor subtypes.

Radioligand binding studies were carried out in rat brain membrane preparations with enantiomers of DOB and various serotonergic drugs. Both enantiomers of DOB display K_i values of approximately 3,000 nM for 3H-5-HT binding sites. No competition for 2 nM ^{3}H-5-HT binding could be detected at DOB concentrations below 100 nM.

Specific ^{3}H-spiperone binding in rat brain membranes is displaced by nM concentrations of DOB with Hill slopes of approximately 0.5-0.7. However, (+) and (-)DOB continue to compete for specific ^{3}H-spiperone binding below the level of "non-specific" binding as defined by 1 uM cinanserin. Similar data have been reported with agents which compete for the "spirodecanone" site labeled by ^{3}H-spiperone (Leysen and Gommeren, Life Sci. 23:447-452, 1978). These data show that DOB has complex interactions with ^{3}H-spiperone binding sites.

^{3}H-DOB was also used to label putative 5-HT_2 sites in rat frontal cortex. In agreement with the results of Lyon et al (1987), 5-HT, (+)DOB, (-)DOB, and d-LSD were all found to display nanomolar potency for these recognition sites. These results are not necessarily consistent with ^{3}H-DOB binding to a high-affinity form of the 5-HT_2 binding site. In addition, it is unlikely that ^{3}H-DOB binding sites represent a "hallucinogen" receptor since d-LSD, (-)DOB, and (+)DOB vary by two orders of magnitude in their human hallucinogenic activity, yet are equipotent agents at the ^{3}H-DOB binding site. Studies are currently under way to further characterize hallucinogenic radioligand binding in rat and human brain tissues.

342.10 **QUANTITATIVE AUTORADIOGRAPHIC MAPPING OF 5-HT_1 AND 5-HT_2 RECEPTORS IN FRONTAL, PARIETAL AND OCCIPITAL CORTEX OF ADULT RHESUS MONKEYS.**

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Serotonin receptors are present in the cerebral cortex and determination of their precise localization in different cytoarchitectonic areas may aid in the analysis of their role in cortical function. In the present study, the *in vitro* receptor autoradiographic technique was used to characterize the distribution of 5-HT_1 and 5-HT_2 receptors in Walker's areas 46, 9, 12, 25 and 4 and Brodmann's areas 1-2, 3, 5, 7, 17 and 18 of cerebral cortex in three adult rhesus monkeys. 5-HT_1 receptors were labeled with [^{3}H]5-HT while 5-HT_2 receptors were labeled with [^{3}H]ketanserin. Non-specific binding for [^{3}H]5-HT was determined using 8-OH-DPAT while methysergide was used as a non-specific displacer for [^{3}H]ketanserin. Total and non-specific binding were quantitatively analyzed on adjacent tissue sections by computer-aided image analysis. Evaluation of K_d values indicated that the affinity of each ligand for its receptor was uniform across the layers and sublayers and between cortical regions.

Both 5-HT_1 and 5-HT_2 receptors were present in all cortical layers in each cytoarchitectonic area. B_{max} for 5-HT_1 receptors varied from 55 200 fmol/mg of tissue and B_{max} for 5-HT_2 varied from 35 - 350 fmol/mg of tissue depending on area and layer. The pattern of 5-HT_1 receptors was similar in all areas except area 17. The basic pattern was that of high density in cortical layers I, II and in the most superficial part of layer III. In area 17, however, 5-HT_1 receptors were more uniformly distributed across the layers. The distribution of 5-HT_2 receptors was complimentary to that of 5-HT_1 receptors in all regions except areas 4 and 17. In areas 46, 9 and 12, the 5-HT_2 receptors were more dense throughout layers III and IV; in area 25 - throughout layer IV and sublayer Va and in areas 1-2, 3, 5, 7 and 18 - throughout layer III. In area 4, the 5-HT_2 receptors were prominent in layers I, II and in the most superficial part of layer III while in area 17, sublayer IVC_β contained the most label.

The high density of 5-HT_1 and 5-HT_2 receptors within the granular and supragranular layers, which contain the cells of origin and termination of cortico-cortical projections, implicates both types of receptors in the associative functions of the cerebral cortex. However, their complimentarity within these layers implies a differential contribution of each to these functions.

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342.11 1-(m-CHLOROPHENYL)PIPERAZINE (mCPP) INTERACTIONS WITH NEUROTRANSMITTER RECEPTORS IN HUMAN BRAIN. A. Hamik* and S.J. Peroutka. (SPON: P.B. Hicks) Departments of Neurology and Pharmacology; Stanford University Medical Center; Stanford, CA 94305.

1-(m-Chlorophenyl)piperazine (mCPP) is a breakdown product of the anti-depressant trazodone which elicits unique neuroendocrinological and behavioral effects in man. mCPP causes an increase in prolactin and cortisol levels in animals and humans. In humans, mCPP causes decreased self-rated scores for happiness and calmness, and increased scores for drowsiness, anxiousness, and feeling "high". Low doses do not cause hallucinations, but higher doses result in transient perceptual distortions and feelings of derealization (Mueller, E.A. et al, J. Clin. Endocrinol. Metab., 61: 1179, 1985; Charney, D.S. et al, Arch. Gen. Psych., in press, 1987). In general, mCPP has been considered an agonist at central 5-HT recptors, perhaps specifically with the 5-HT_{1B} subtype.

We determined the affinity of mCPP at 11 neurotransmitter receptor sites in human brain. mCPP is essentially equipotent at all 5-HT receptor subtypes (K_i values ranging from 250 - 870 nM). The drug displays similar affinity (K_i = 360 nM) for alpha2-adrenergic receptors and is only slightly less potent at $alpha_1$- and beta-adrenergic, dopamine, and muscarinic cholinergic receptors (K_i values = 1,1000 - 24,000 nM). mCPP is inactive at both benzodiazepine receptors and the 5-HT uptake sites at concentrations below 100,000 nM.

These data demonstrate that mCPP displays similar potency for multiple neurotransmitter receptors in human brain. The unique effects of mCPP cannot be attributed to interactions with a single receptor subtype. The differential neuroendocrine and psychological effects shown at different doses may be due to the affinity of mCPP for various neurotransmitter receptor sites. Low doses may selectively activate 5-HT and $alpha_2$-adrenergic sites, while slightly higher doses may interact with $alpha_1$-adrenergic, beta-adrenergic, dopamine and muscarinic cholinergic sites.

342.12 ULTRASTRUCTURAL RELATIONSHIPS OF SEROTONIN NERVE TERMINALS IN ADULT RAT FRONTAL CORTEX. Philippe Séguéla, Kenneth C. Watkins* and Laurent Descarries. Centre de recherche en sciences neurologiques (Dép. physiologie), Université de Montréal, Montréal (Qué.), Canada H3C 3J7.

To further elucidate the fine structural features and cellular relationships of serotonin (5-HT) afferents in adult rat cerebral cortex, an antiserum directed against 5-HT-glutaraldehyde-lysyl-protein complex (Patel et al., Histochemistry 85: 259, 1986) was used for immunocytochemical examination of identified 5-HT axon terminals (varicosities) in the superficial layers (I-II) of the frontal cortex (areas Fr1 and Fr2, according to Zilles, 1985). Tissue from 2 animals was fixed by perfusion with 3.5% glutaraldehyde, reduced with Na borohydride, immunostained using the peroxidase-anti-peroxidase technique, postfixed with osmium tetroxide, embedded in Epon, serially thin sectioned, stained with lead citrate and photographed at a magnification of X 14 500. More than 50 immunostained varicosities were analyzed for size and content, frequency and type of junctional complex, identity of synaptic targets and of other juxtaposed processes (microenvironment), and relative length of the different membrane appositions. The 5-HT immunoreactive varicosities measured 0.65 ± 0.17 µm (mean ± SEM) in average diameter. They usually exhibited small, round, clear synaptic vesicles associated with a few larger clear vesicles and mitochondria. In the whole population of 54 varicosities examined in series of 5 to 12 adjacent thin sections (mean of 7.3 sections per varicosity), the observed frequency of synaptic junction was 26%. The linear relationship between junctional frequency and the number of adjacent thin sections available for examination allowed to estimate the actual synaptic incidence at 35 to 40% of total. Most synaptic 5-HT varicosities (86%) showed symmetrical junctional complexes which were exclusively made with dendritic branchlets (57%) and spines (43%). The length of these synaptic contacts averaged 0.23 ± 0.06 µm. Preliminary quantitative comparisons between the microenvironments of synaptic and non synaptic 5-HT varicosities showed no significant difference in the identity and apposed lengths of surrounding processes. Thus, the cortical serotoninergic innervation appeared predominantly non junctional (60-65%) in the molecular layer of rat frontal cortex. It will be of interest to determine whether this synaptic incidence is different in other layers and other regions of rat cerebral cortex. Further analysis of the microenvironment of junctional versus non junctional 5-HT varicosities should provide clues as to possible determinants of their cellular relationships. (Supported by FRSQ and MRC grant MT-3544).

343.1 **ASSESSMENT OF CYTOSOLIC-FREE CALCIUM IN SYNAPTOSOMES WITH FURA-2 IN THE PRESENCE OF FURA-2AM: EFFECTS OF HYPOGLYCEMIA AND AGING.** T. Manger*, J. Bowers* and G. Gibson. Cornell Univ. Med. Coll. at the Burke Rehab. Ctr., White Plains, NY 10605.

Cytosolic-free calcium (Ca_i) is a regulator of many cellular processes. The development of calcium sensitive fluorescent probes has made Ca_i measurements feasible in intact cells. However, incomplete hydrolysis of the ester that was used in loading the cells leads to erroneous estimates of Ca_i. The described method estimates Ca_i with fura-2 even in the presence of varying amounts of the incompletely hydrolyzed fura-2-acetoxymethyl ester (FAM) that was used for loading. The method is based on the observation that in the presence of high calcium concentrations, the fluorescence ratio (excitation 340/380, emission 510 nm) predicts the mole fraction of FAM in the sample. With the appropriate fluorescence coefficients, this ratio allows the contribution of FAM to be subtracted from the signal. The subsequent calculation to estimate Ca_i paralliels the standard ratio method. With this approach, depolarization of synaptosomes from young CD-1 mice with 60 mM-KCl increased Ca_i from 179.4 $\pm$ 8.9 to 304.7 $\pm$ 26.4 nM.

Neuronal damage and increased spontaneous glutamate release accompany hypoxia, aging (Freeman and Gibson, 1987) and hypoglycemia (Wieloch, 1985). Elevated Ca_i possibly mediates the increase in glutamate release in each of these conditions. In histotoxic hypoxia, we previously demonstrated increased Ca_i (Gibson, Manger, Bowers, 1987). To see if alterations in Ca_i may also underlie the glutamate changes in aging and hypoglycemia, their effects on cytosolic calcium were determined. With decreased glucose, Ca_i (nM; mean $\pm$ SEM; n=7) increased from 190 $\pm$ 16 (1.5 mM-glucose) to 198 $\pm$ 17 (0.75 mM-glucose) to 286 $\pm$ 23 (0 glucose; $p<0.05$). With whole brain synaptosomes from BALB/C mice, no effect of aging on Ca_i was apparent. The basal concentration (nM; mean $\pm$ SEM, n=7) was 171 $\pm$ 7 (3 months), 164 $\pm$ 9 (10 months) and 163 $\pm$ 4 (30 months). The percent increase with potassium stimulation (15 mM and 60 mM) were similar at 3 months (46% and 70%), 10 months (46% and 54%) and 30 months (49% and 66%). Thus, increased Ca_i may underlie the elevated glutamate release in hypoxia and hypoglycemia, but does not appear to be altered during aging.

343.2 MEASUREMENT OF FREE INTRACELLULAR CALCIUM IN APLYSIA SENSORY NEURONS USING Fura-2. M.E. Spira*, H. Blumenfeld*, S. Schacher and S.A. Siegelbaum. (SPON: C. Kaufmann). HHMI, Columbia Univ., NY, NY 10032; and Dept. of Neurobiology, Hebrew University, Jerusalem, Israel.

Serotonin facilitates transmitter release from Aplysia sensory neuron terminals. This involves a closure of serotonin-sensitive K^+ channels, and is associated with an increase in the free intracellular Ca^{++} transient in response to long (0.5-1 sec) depolarizing voltage-clamp steps as measured by the transient Arsenazo signal (Boyle et al., PNAS 81, 7642, 1984). Here, we report experiments using the more sensitive Ca^{++} indicator, Fura-2, to investigate whether 5-HT 1) alters resting Ca^{++} levels in the cell bodies of sensory neurons, and 2) produces changes in Ca^{++} transients in response to more physiological stimuli.

Sensory neurons in culture were loaded with 100 uM Fura-2 using iontophoretic injection from microelectrodes containing 10 mM Fura-2 and 0.5 M KCl. For stimulation and recording from neurons, a 2.5 M KCl filled microelectrode was used. Fluorescence images of free Ca^{++} levels were obtained by digital video imaging using 350 nm and 380 nm excitation wave lengths (Williams et al., Nature, 318:558, 1985). Transient changes were monitored by a photomultiplier.

The resting level of free Ca^{++} in the sensory neurons is 90-100 nM. The region of the cell body corresponding to the nucleus showed a somewhat lower concentration. Short trains of 5-20 action potentials at 10-20 Hz produced a transient increase in the free Ca^{++} of about 20-40 nM, which lasts for 10-20 sec. Repeated trains of action potentials at intervals of 2-3 min did not change the steady state Ca^{++} levels and were not associated with changes in the recovery time course of the signal, indicating that under these conditions the Ca^{++} buffering systems are not affected. In some experiments, the Ca^{++} signal continued to increase even after termination of the train. This, and the observation of asynchronous Ca^{++} transients that sometimes occur after termination of the action potential train, raises the possibility that a component of the Ca^{++} transient may involve release of Ca^{++} from intracellular stores.

Serotonin (1-10 uM) did not produce marked changes in the steady state Ca^{++} level. In experiments in which serotonin application (1-10 uM) induced typical increases in sensory neuron excitability, the Ca^{++} transient (measured by the 380 nm Fura-2 signal) in response to an identical train of spikes (10-20 Hz) increased by 50-200% over the control Ca^{++} transient. However, as in the earlier study of Boyle et al., the increase in the Ca^{++} transient was delayed in respect to the onset of the increase in excitability, appeared gradually, and reached a maximum only 2-8 min after the onset of the membrane excitability changes. These experiments demonstrate that under conditions which do not involve overloading of the cell by Ca^{++}, 5-HT application increases the transient Ca^{++} concentration evoked by short trains of action potentials.

343.3 IONIC CURRENTS AT THE LIZARD NEUROMUSCULAR JUNCTION. C. A. Lindgren & J. W. Moore. Dept. of Physiology, Duke Univ. Med. Cntr., Durham, NC 27710.

We are interested in the role of calcium in synaptic transmission; its entry, its sub-membrane concentration transient and its effect on transmitter release. We have found the neuromuscular junction of the *ceratomandibularis* muscle in the lizard (*Anolis carolinensis*) to be a potentially useful preparation for studying these phenomena. The muscle is easy to dissect and is very thin and flat, making it possible to visualize the nerve endings with either Nomarski optics or by staining with the dye 4-(4-diethylaminostyryl)-N-methylpyridinium iodide (Magrassi, Lichtman & Purves, 1987, *J. Neuroscience 7* 1207). Sodium, potassium, calcium and calcium-activated potassium currents can be detected in the presynaptic nerve terminals with an extracellular electrode. The ability to isolate the calcium and calcium-activated potassium currents from the sodium and potassium currents, as described below, offers the possibility of elucidating calcium's role in activating transmitter release.

Ionic currents induced by nerve stimulation were measured at different positions along the motor nerve terminal by careful positioning of the heat-polished tip (~5 μm i.d.) of an extracellular electrode. Similar to what was observed at motor nerve endings of the mouse (Brigant & Mallart, 1982, *J. Physiol. 333*, 619), the net current flow is predominantly inward just distal to the end of the myelin, a region we refer to as "heminode", and outward over the remainder of the terminal. This shows that, as has been demonstrated in the mouse, the sodium and potassium channels are separated, with sodium the dominant current in the heminode and potassium in the terminal branches and boutons.

In all of the current patterns recorded, independent of the position of the electrode, there is an initial, brief outward current due to the discharging of the membrane capacitance as the action potential invades the terminal. In the heminode this initial positive phase is followed by two inward peaks. We think that the first of these represent active sodium current in the heminode membrane and the second a sodium sink (or return path) for the potassium current generated in the terminal branches and boutons. In contrast, we do not observe inward sodium current in distal portions of the terminal. Rather, the initial capacitative peak is followed immediately by an outward current, which was identified as potassium since it was reduced by increasing the potassium ion concentration in the bath ($[K]_o$) and blocked by adding 8 mM tetraethylammonium (TEA) or 0.1 mM 3,4-diaminopyridine (DAP).

Along with blocking the potassium current, application of TEA unmasked a small, prolonged current which was identified as a calcium current because it increased with increasing calcium concentration and was abolished when cobalt was substituted for calcium. In contrast, application of DAP revealed a slow, delayed current which was identified as a calcium-activated potassium current because it could be reduced by increasing $[K]_o$ and blocked by (a) replacing the calcium with cobalt, or adding (b) 8 mM TEA or (c) 50 nM Charybdotoxin.

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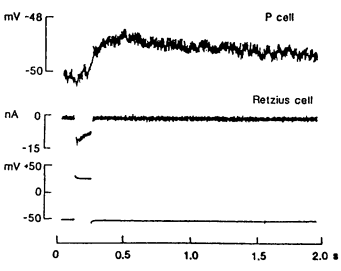
343.4 CORRELATION OF Ca, Ba, AND Sr ENTRY WITH 5-HT RELEASE AT RETZIUS - P SYNAPSES IN CULTURE. R.R. Stewart, J.G. Nicholls, W.B. Adams and R.J. Bookman*. Dept. of Pharmacology, Biocenter, CH-4056 Basel, Switzerland.

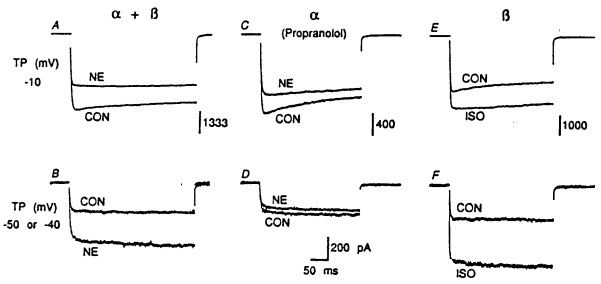
Pairs of Retzius cells and P sensory cells dissected from leech ganglia form chemical synapses in culture. The aim of these experiments is to determine the relationship between entry of divalent cations into the presynaptic Retzius cell and transmitter release. Cells were plated on poly-L-lysine, a substrate upon which synapses form without profuse outgrowth of neurites. Under voltage-clamp with Na and K currents reduced or blocked, depolarization of the Retzius cell gave rise to an inward current. This inward current carried by Ca, Ba or Sr was followed after a delay by release, as assessed by the synaptic potential recorded in the postsynaptic P cell. Fig. 1 shows an experiment in which a depolarizing pulse from -50mV to + 20 mV led to an inward Ba current of 13 nA (peak) and a synaptic potential of 1.5 mV (peak) in the P cell. These synaptic potentials were mediated by an increase in chloride conductance and were unaffected by low Na or high TEA in the bathing fluid. The amplitudes of the synaptic potentials were steeply dependent on the amplitudes and durations of the inward Ca-channel current. The efficacy of Ca, Ba and Sr in effecting release has been compared. Moreover by applying voltage clamp pulses of varying amplitude, duration and frequency, a quantitative analysis has been made of the relation between Ca currents and 5-HT release during facilitation and depression.

Supported by grants from the Swiss Nationalfonds and the US Navy.



343.5 **α-ADRENERGIC INHIBITION OF N-TYPE Ca CHANNELS AND ß-ADRENERGIC STIMULATION OF L-TYPE Ca CHANNELS IN FROG SYMPATHETIC NEURONS.** **D. Lipscombe* & R.W. Tsien, Department of Physiology, Yale University School of Medicine, New Haven, CT 06510.**

When norepinephrine (NE) is released from sympathetic neurons it affects its own release through negative feedback via presynaptic α-receptors and positive feedback via presynaptic ß-receptors. To determine which type(s) of Ca channel NE modulates, we have carried out whole cell and single channel recordings from frog sympathetic neurons. We found two types of Ca channels somewhat similar to L and N-type channels in chick DRG and rat sympathetic neurons. In whole cell recordings (2 mM Ba or 2-10 mM Ca, HP= -80 or -90 mV), nifedipine-sensitive L-type channels generated a sustained Ca current that was activated with weak depolarizations (*B,D,F*) and remained available even at relatively depolarized HPs. N-type Ca channels produced a slowly decaying current (*A,C,E*) that required negative holding potentials for repriming. Application of 1 μM NE inhibited Ca channel current evoked by strong depolarization (*A*) while it often increased the current produced by weak test pulses (*B*). The inhibition is due to α-adrenergic block of N current, since it is abolished by the α-blocker phentolamine or by depolarized HPs that inactivate N current (Lipscombe & Tsien, J. Physiol., in press). With the ß-blocker propranolol present, NE failed to enhance L current at weak test potentials (*D*), although it still inhibited the N current evoked by strong depolarizations (*C*). Conversely, selective ß-stimulation by isoproterenol increased Ca current seen with either strong (*E*) or weak test pulses (*F*). This whole cell response was paralleled by stimulation of unitary L-type Ca channel activity following application of NE or 8-bromocyclic AMP. Thus, it appears that NE inhibits N-type Ca channels via α-receptors and stimulates L-type Ca channels via ß-receptors. These actions may account for the opposing effects of NE on its own release.



343.6 INHIBITION BY SYNAPSIN I OF MEMBRANOUS ORGANELLE MOVEMENT IN ISOLATED SQUID AXOPLASM. T.L. McGuinness*, S.T. Brady, J.A. Gruner, M. Sugimori, R. Llinás, and P.Greengard (SPON: J.A. Grebb). Rockefeller Univ., New York, NY 10021; Dept. of Cell Biol., Univ. of Texas Health Science Ctr., Dallas, TX 75235; Dept. of Physiol. Biophys., New York Univ. Med. Ctr., New York, NY 10016; and Marine Biological Laboratory, Woods Hole, MA 02543.

Synapsin I, a synaptic vesicle-associated phosphoprotein, is thought to play an important role in synaptic vesicle function. Indeed, recent studies have shown that injection of synapsin I into the preterminal digit of the squid giant synapse inhibits neurotransmitter release in a phosphorylation-dependent manner (Llinás et al., PNAS, 82:3035, 1985). We considered the possibility that dephospho-synapsin I (d-synapsin I) might regulate release by restricting the ability of synaptic vesicles to move to or fuse with the plasma membrane. To test the possibility that d-synapsin I restricts synaptic vesicle mobility, we have begun using extruded axoplasm from the squid giant axon with video-enhanced contrast-differential interference contrast (VEC-DIC) microscopy. Axoplasm was extruded from the giant axon of the squid (Loligo pealii) onto a coverslip for viewing by VEC-DIC microscopy as previously described (Brady et al., Cell Motility 5:81, 1985) and was then bathed in buffer A (10 mM Hepes, pH 7.2/5 mM $MgCl_2$/150 mM K^+-aspartate/1 mM ATP) containing 0.1 mg/ml or 1.0 mg/ml d-synapsin I. D-synapsin I produced a dramatic decrease in the amount and rates of organelle movement along microtubules within the interior of the axoplasm. This inhibition was not observed along microtubules protruding from the edges of the axoplasm into the surrounding buffer, suggesting that d-synapsin I does not directly affect the fast axonal transport mechanism itself. Stoichiometric phosphorylation of synapsin I by either Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) or cAMP-dependent protein kinase abolished the inhibitory effect of the protein. Fluorescence monitoring of Texas Red-labeled synapsin I ensured that the difference between phospho- and dephospho-synapsin I was not due to differential penetration of the proteins into the axoplasm. Furthermore, when CaM kinase II was microinjected into a small region of the axoplasm, followed by bathing the axoplasm with buffer A containing d-synapsin I, Ca^{2+}, and CaM, organelle movement was inhibited in all regions except in the vicinity around the kinase injection. These results show that synapsin I inhibits directed organelle movement within axoplasm in a phosphorylation-dependent manner. In summary, these data are consistent with the possibility that d-synapsin I crosslinks synaptic vesicles to some component(s) of the cytoskeleton, thereby indirectly restricting the movement of all organelles within the axoplasm.

343.7 INTERACTION OF SYNAPSIN I WITH PHOSPHOLIPID VESICLES. F. Benfenati*, R. Jahn*, M. Bahler* and Paul Greengard. Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, NY 10021.

Synapsin I, a major neuron specific phosphoprotein, is localized on the cytoplasmic surface of brain small synaptic vesicles, to which it binds with a Kd of 10nM (at 40mM NaCl) and with a Bmax of approximately 750 fmol/μg vesicle protein or 250 fmol/μg vesicle phospholipid (PL). To characterize the binding of synapsin I to synaptic vesicles, we have analyzed the binding to integral vesicle proteins reconstituted into liposomes as well as to pure lipid vesicles. When lipid vesicles were prepared from cholesterol and PLs using a lipid composition (w/w) similar to that found in the native vesicle membrane (41% phosphatidylcholine [PC], 32% phosphatidylethanolamine [PE], 11% phosphatidylserine [PS], 6% phosphatidylinositol [PI] and 10% cholesterol), synapsin I bound with a Kd of 10 nM and a Bmax of about 180 fmol synapsin I/μg PL, corresponding to about 60 molecules of synapsin I/vesicle. As with native synaptic vesicles, increasing the ionic strength decreased the affinity without greatly affecting the maximal amount of synapsin I bound. In contrast to the data observed using native synaptic vesicles, changes in the phosphorylation state of synapsin I seemed not to affect its binding to PL vesicles. When pure PC or PC-PE vesicles were tested, it was not possible to detect a significant binding under any conditions tested. On the other hand, PC-PS and PC-PI liposomes bearing a net surface negative charge strongly interacted with synapsin I. The amount of synapsin I maximally bound was directly proportional to the percentage of acidic PL present in the liposome bilayer, whereas the Kd was not affected by varying the composition of the liposomes. The binding was virtually independent of the liposome size, since similar results were obtained using cholate-formed liposomes (mean diameter 30 nm) or octylglucoside-formed liposomes (mean diameter 300 nm). PC liposomes negatively charged by the inclusion of 10% (w/w) dicetyl phosphate bound synapsin I to a lesser extent and with a very low affinity (0.5-1 μM), providing evidence for the specificity of synapsin I binding to PS and PI. However the binding of synapsin I to small synaptic vesicles cannot be explained only as an interaction with endogenous acidic PLs. Synapsin I bound with high affinity to liposomes prepared from endogenous vesicle PLs. Nevertheless when more than 98% of the endogenous PLs were removed from detergent solubilized synaptic vesicles, over 50% of the synaptic vesicle binding was retained by the protein fraction reconstituted into pure PC liposomes. Thus, acidic PLs and specific protein(s) in the small synaptic vesicle membrane may both be involved in the specific association of synapsin I with small synaptic vesicles in vivo.

343.8 **DECREASE IN AVAILABLE QUANTA BY DEPHOSPHOSYNAPSIN I INJECTED PRESYNAPTICALLY INTO THE GOLDFISH MAUTHNER AXON.** **J.T. Hackett, S.L. Cochran, L.J. Greenfield, Jr., and T. Ueda. Dept. Physiol., Univ. Virginia Med. School, Charlottesville, VA 22908 and Univ. Mich. Mental Health Institute, Ann Arbor, MI 48109.**

Synapsin I is a neuron specific phosphoprotein (Ueda, Maeno, Greengard, 1973) associated with presynaptic vesicles (De Camilli et al., 1983; Hutter et al., 1983). Dephosphorylated synapsin I (dSI) has a high affinity for presynaptic vesicles and decreases synaptic transmission when injected into the squid presynaptic terminal (Llinás et al., 1985). The purpose of our investigation was to extend these observations to a vertebrate CNS synapse which can be analyzed at the quantal level.

Two microelectrodes were used to record simultaneously from pairs of neurons (presynaptic Mauthner axon, postsynaptic cranial relay neuron). The postsynaptic electrode was placed within 50 μm of the synapse and the presynaptic electrode then impaled the Mauthner axon 50 to 200 μm from the first electrode. Spontaneous (mEPSP) and evoked postsynaptic potentials were digitized and analysed by computer and their variance was calculated. Evoked release was triggered by action potentials at 2 to 5 Hz. Values of quantal content ("m"), available store ("n"), release probability ("p") and quantal size ("q") were obtained at steady states before and after dSI (0.2 mg/ml in 2 M K-acetate, about 100 picoliters, pH 7.2) or a control protein was pressure-injected into the presynaptic Mauthner axon.

In 6 experiments there was a reduction in the EPSP amplitude attributed to the action of dSI. The decrease in transmission began about 30 sec. after the injection, reached a plateau within 10 min., was reversible, and was dose dependent. Injection of dSI did not affect the frequency or the amplitude of mEPSPs, hence the decrease in transmission is due to a decrease in "m" with no effect on postsynaptic receptor sensitivity or the amount of transmitter per quanta. Further analysis based on the binomial model for quantal release revealed that dSI reduced the "n" without affecting "p". Other proteins injected in control experiments were found to be ineffective.

The most likely mechanism for the action of dSI is that it binds to presynaptic vesicles and removes transmitter quanta from a releaseable pool. The protein apparently does not affect the spontaneous mEPSPs or the probability that a quantum in the available pool will be released by an action potential. Since fatigue of transmission produced by repetitive stimulation at this synapse is also the result of a decrease in the store of available quanta, it is tempting to suggest that the phosphorylation of dSI and the resulting increase in available quanta is a rate limiting step important in the regulation of nerve terminal function.

(Supported by NSF Grant BNS 84-0629).

343.9 RECYCLING OF A SYNAPTIC VESICLE PROTEIN (P38)
F.Valtorta*, R.Fesce*, R.Jahn*, P.Greengard and B.Ceccarelli*
(SPON: J.Wang) The Rockefeller University, New York; Dept. of Medical Pharmacology, Univ. of Milan, Italy; and Max Planck Institut, Munich, FRG

Protein p38 (also called synaptophysin) has been shown to be an integral protein of the synaptic vesicle membrane (R.Jahn et al., PNAS 82:4137-4141, 1985; B,Wiedenmann and W.Franke, Cell 41:1017-1028, 1985). Using monoclonal antibodies raised against rat brain p38, we affinity purified p38 from frog brain. The frog protein appears to have a higher molecular weight than the mammalian protein and, like mammalian p38, can also exist in a dimeric form. The purified frog brain p38 was used to raise rabbit antisera and the specificity of the antisera obtained was verified by immunoblotting. When these antisera were used to stain frog nerve-muscle preparations by immunofluorescence, a bright specific signal was observed in the nerve-terminal region. The fluorescent image resembled that obtained using antisera against synapsin I, a phosphoprotein associated with the outer (cytoplasmic) side of the synaptic vesicle membrane. In both cases, permeabilization of the plasma membrane with detergent was required. Fluorescent end-plate images could be observed with p38 antisera without previous permeabilization in preparations treated for one hour with a low dose (0.2-0.4 μg/ml) of α-latrotoxin (αLTx) in Ca-free medium. Under this condition, α-LTx caused depletion of the quantal store of ACh and of synaptic vesicles, and swelling of the nerve terminal was observed as a result of the permanent incorporation of the vesicle membrane into the axolemma. These results support the validity of the vesicle hypothesis of neurotransmitter release and indicate that exocytosis leads to the exposure of antigenic determinants of p38 on the outer surface of the axolemma. After one hour treatment with the same doses of α-LTx in the presence of 1.8 mM Ca, immunofluorescent images were obtained only after permeabilization with detergents, and no swelling of the terminal was evident. Under this condition, the vesicle population was maintained by an active process of recycling and more than three times the initial store of quanta was secreted. Thus it seems that, in spite of the active vesicle and neurotransmitter turnover, no extensive intermixing occurs between vesicle and axolemma components.
(partially supported by MDA grant (B.C.) and by NIH grant NS 21550 (P.G.)

343.10 CURRENTS THROUGH THE FUSION PORE THAT FORMS DURING EXOCYTOSIS OF A SECRETORY VESICLE.
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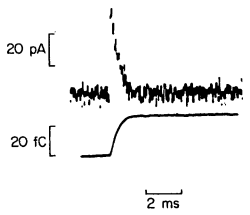
Early events in exocytosis of single secretory vesicles were captured by patch clamp measurements on mast cells of beige mice, a murine mutant with giant secretory vesicles. When exocytosis is induced by cytoplasmic $GTP_\gamma S$, membrane capacitance (C_m) is seen to increase in steps, each reporting the fusion of a single vesicle with the cell membrane or, more precisely, the making of an electric connection between vesicle lumen and cell exterior. Through this connection, termed the fusion pore, a capacitative current transient must flow to equalize the membrane potentials of vesicle and cell membranes. Indeed, the increase in C_m is preceded by current transients with a mean reversal potential (E_r) of -76 mV. The Fig. shows an example, recorded at 30 mV, along with its time integral. The transients have these properties: (1) They decline monotonically and (2) carry a charge Q that is linearly related to the final amplitude (^C) of the C_m step. (3) The ratio Q/^C depends linearly on holding potential with a slope of one, and intercepts the abscissa at E_r = -76 mV. (4) FCCP, a H^+-ionophore, shifts E_r to 0 mV. (1)-(4) are strong evidence that the current transients serve to charge the vesicle membrane through the fusion pore. E_r gives the membrane potential of the vesicle, 76 mV lumen positive to cytosol. The initial conductance of the fusion pore, g, is obtained either from the amplitude (I_o) of the transient ($g = I_o*^C/Q$) or from the time constant of decline ($g = \tau/^C$). Mean value is 230 pS, corresponding to a probable pore diameter of < 2 nm. Once the transient has declined, the further time course of g was reconstructed from the admittance (800 Hz voltage sinusoid) contributed by the fusing vesicle. Real and imaginary components of the admittance were sampled at 800 Hz with a synchronous-integration lock-in amplifier. We find that g rapidly grows to about 1 nS, as if the fusion pore rapidly dilated. The dilated fusion pore may persist for hundreds of ms and can repeatedly close and open again (C_m flicker) or expand further to complete membrane fusion. We conclude that membrane fusion starts with the sudden formation of an aqueous channel that connects the lumen of the exocytosing vesicle to the cell exterior. Its conductance is similar to that of a gap junction, the only other ion channel that spans two membranes. Supported by NIH AR-17803.



343.11 DISTRIBUTION OF SYNAPTIC NOISE IS A PREDICTABLE SUM OF PRESYNAPTIC BINOMIAL RELEASE FUNCTIONS.
H. Korn, Y. Burnod* D.S. Faber & J.R. Martin* Laboratoire de Neurobiologie Cellulaire, INSERM U261, Institut Pasteur, Paris, France, and SUNYAB, Buffalo, NY, USA.

We recently reported (H. Korn, et al., Proc. Natl. Acad. Sci. USA, in press) that quantal currents can be extracted from spontaneous synaptic noise in the voltage-clamped teleost Mauthner (M-) cell. Inhibitory postsynaptic currents, due to background firing of a well-defined population of presynaptic cells, were collected during depolarizing steps, to isolate them from excitatory ones. Their amplitude histograms exhibited multiple peaks, separated by a constant increment comparable to the basic quantal units derived by the simple binomial analysis of unitary postsynaptic potentials (H. Korn, et al., J. Neurophysiol. 1982, 48:679-707). However, the overall response distributions differed from binomial functions, with a predominance of single events and a regular sigmoidal-like decrease in the frequency of occurrence in successive peaks. This result is not surprizing since, unless parameters n and p are the same, one does not expect several binomial curves to sum up as a single one. Assuming the 42 presynaptic cells previously analyzed (H. Korn, et al., J. Neurophysiol. 1986, 55:402-421) were representative of the inhibitory network, the statistical behavior of responses to asynchronous activity in these neurons was modeled by 1) adding their corresponding binomial distributions (with n ranging from 3 to 52 and p from 0.17 to 0.65 for 1 Hz stimulation) and, 2) scaling all their release probabilities (ps) according to an exponentially decreasing function of presynaptic firing rate (H. Korn, et al., J. Neurosci. 1984, 4:125-130). In 8 experiments, the observed amplitude histograms, each of which included from 1200 to over 3000 events, were adequately fit by the predicted population curves, with the p values equal to 38±0.05% of their controls such as would occur when the presynaptic firing rate is superior to 33 Hz, and p reaches a minimum. Accordingly, with simultaneous recordings, high frequency presynaptic discharges have been observed in some inhibitory interneurons during depolarizing steps in the M-cells possibly due to accompanying outward currents. Therefore, synaptic noise brought about by activity in a given pool of presynaptic interneurons can be isolated and characterized by a unique function, which is here a limited series of single binomials, where the distribution of n (corresponding to the number of active sites) remains constant and p is the variable parameter.

343.12 A COMPARISON OF THE ACTIONS OF THE CLOSTRIDIUM BOTULINUM BINARY TOXIN ON THE STRUCTURE AND FUNCTION OF ADRENAL Y-1 CELLS AND MOUSE NEUROBLASTOMA N1E-115 CELLS.
Lance L. Simpson and Kevin S. Chinn*. Departments of Medicine and Pharmacology, Jefferson Medical College, Philadelphia, PA 19107.

The botulinum binary toxin is composed of two separate and independent polypeptide chains (Mr ~ 50,000 and 100,000). The heavy chain is a binding component that interacts with the plasma membrane of vulnerable cells; the light chain is an enzyme that possesses mono(ADP-ribosyl)ating activity. The intracellular substrate for the catalytic chain is G-actin. When studied on adrenal Y-1 cells, the toxin produces dose-dependent (10^{-11} to 10^{-9} M) morphological changes. These are characterized by rounding of the cell and by disruption of the cytoskeleton. The morphological changes do not lead to cell death, as indicated by: a) exclusion of impermeant ions, b.) continued incorporation of tritiated amino acids into protein, and c.) continued incorporation of purines into nucleic acids. The toxin does not alter basal levels of cyclic-AMP or cyclic-GMP, but it does produce a modest increase in steroid secretion. Equivalent studies were done on mouse neuroblastoma N1E-115 cells, with the addition of intracellular dialysis via patch clamp electrodes. When applied to the outside of cells, the holotoxin was needed to produce characteristic changes. When dialysed into individual cells, only the light chain was necessary. Morphological changes could be obtained prior to changes in leakage current. While morphological changes required approximately 30-45 minutes to occur when the holotoxin was bath applied (10^{-9} M), such changes occurred within 15-20 minutes after beginning cell dialysis (10^{-11} M light chain in the electrode). During the dialysis studies, a shrinking of the cell processes occurred before rounding of the cell soma. The data suggest that the binary toxin may be a powerful tool for studying the relationship between actin and various aspects of cell structure and function.

343.13 MOVEMENT OF Cl THROUGH DOUBLY RECTIFYING ELECTRICAL JUNCTIONS BETWEEN TOUCH CELLS IN THE CNS OF THE LEECH. S.E. Acklin* and J.G. Nicholls. Dept. of Pharmacology, Biocenter, CH-4056 Basel, Switzerland.

An unusual type of rectification occurs in the spread of current between touch (T) sensory neurons in the CNS of the leech. Depolarization spreads from one T cell to another in both directions, whereas hyperpolarization spreads in neither direction (Baylor, D.A. and Nicholls, J.G., J. Physiol. 203:591, 1969.) In principle such characteristics could be due to the properties of the electrical junctions themselves or to rectifying properties of membranes interposed between the cell somas and the junctions within the neuropil. The aim of the present experiments was to distinguish between these mechanisms and to characterize the permeability of the junctions to small ions. In one test, the membrane resistance and length constants of T cells were increased by substituting sucrose for Na. Under these conditions double rectification was still apparent, even though current spread through processes within the neuropil was increased. A more direct test was to stimulate one T cell repetitively. This activates the Na pump and gives rise to a hyperpolarization that spreads throughout the fine processes of the neuropil and the axons of the T cell (Van Essen, D.C., J. Physiol. 230:509, 1973). When one T cell was hyperpolarized in this way the IPSP's recorded in it became reversed. The other T cell showed no hyperpolarization and the IPSP's continued to be hyperpolarizing. These results indicate that hyperpolarization does not cross the electrical junctions. IPSP's also provided a reliable and sensitive assay for intracellular Cl. When Cl was injected into one T cell the IPSP's in it reversed within one minute. After a few minutes the IPSP's in the coupled T cell also became reversed, indicating that Cl can cross the junctions. (Supported by grants from the Swiss Nationalfonds and the US Navy).

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY IV

344.1 A CELLULAR ANALOG OF DEVELOPMENTAL VISUAL CORTICAL PLASTICITY. Y. Frégnac* and D. Shulz* (SPON: European Neuroscience Association). Lab. de Neuropharmacologie et Neurobiologie du Dévelopment. Univ. Paris XI, Bat 440, 91405, Orsay Cedex, France.

An electrophysiological paradigm has been developed to reproduce, during the time of recording, functional changes similar to those described during epigenesis of visual cortex. Ionophoretic pairing was used to control the temporal correlation between postsynaptic firing and given characteristics of the visual stimulus (Frégnac et al. Soc. Neurosc. Abstr. 10: 1078 (1984)). Four types of differential pairing procedures were applied: two test stimuli which could differ in 1) position within the receptive field (R.F.), 2) orientation, 3) ocular dominance or 4) interocular orientation disparity (I.O.D.), were paired respectively with an imposed increase and decrease in visual response. Generalisation effects for stimuli other than those used during pairing were looked for when possible.

429 cells were recorded in kitten area 17: 275 were used for control (including invariance of R.F. properties during non-differential pairing) and 154 were submitted to differential pairings. Significant changes in R.F. profile, orientation selectivity, ocular dominance and I.O.D., were found in 30 to 40% of "paired" neurones. They were largest in kittens between 4-8 weeks of age, but could be produced in the adult as well. Cells often retained the pattern of firing imposed during pairing. Extinction was eventually observed after several tens of minutes. Changes induced by I.O.D. pairing (using dichoptic stimulation) affected mainly binocular responses, and were not explainable by summed monocular responses. In spite of occasional global excitability changes, most modifications were interpretable in terms of competitive changes in responsiveness. Relative preference between the two test stimuli was displaced towards the stimulus paired with imposed increased visual responsiveness.

These changes were associative : no modifications were observed following ionophoretic action uncorrelated with visual stimulation (pseudopairing), or when control of activity was uneffective during pairing. Both imposed increases and decreases in postsynaptic activity appeared to be equally effective. If subsequent increase in synaptic efficiency is predicted by Hebbian schemes of plasticity (Hebb, 1949), forced repetitive failure of a given stimulus to evoke postsynaptic activation could result per se in a long-lasting decreased responsiveness for this stimulus. In favor of selective changes in synaptic efficiency is the finding that separate zones in the R.F. can be modified differentially. These data suggest that only sets of synapses active during control of postsynaptic firing will be susceptible of undergoing plastic changes.

This work was supported by C.N.R.S., M.I.R. and F.R.M. grants.

344.2 **A NOVEL EXPRESSION OF PLASTICITY IN KITTEN VISUAL CORTEX IN THE ABSENCE OF POSTSYNAPTIC SPIKE ACTIVITY.**
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Models of synaptic plasticity in the nervous system have conventionally assumed that spike activity in the postsynaptic cell enhances the efficacy of recently active presynaptic inputs. This contrasts with purely presynaptic models like those of classical conditioning in *Aplysia*, in which responses of cells were facilitated even while their somata were hyperpolarized by an intracellular microelectrode. The aim of the present experiments was to reveal whether, as postulated by Hebb, postsynaptic spike activity regulates the efficacy of synaptic transmission in cortex. We made use of the prompt and dramatic response of the visual cortex to occlusion of one eye during the critical period to test the requirement for postsynaptic discharges in ocular dominance plasticity. If postsynaptic spike activity were crucial, our expectation was that selectively blocking it should prevent plasticity.

We selectively blocked cortical cell discharges in 4 27-32-day old kittens with a continuous intracortical infusion of muscimol (10 mM, 0.5 µl/hr) during a 5-7 day period of monocular deprivation. Muscimol, an agonist of the principal inhibitory neurotransmitter in cerebral cortex, GABA, completely blocks cortical cell discharges with no apparent effect on the activity of their presynaptic inputs. The selective inhibition produced by muscimol was verified by microelectrode recordings. When the cortex was allowed to recover from muscimol-induced blockade, recordings from single cells revealed a consistent shift in the responsiveness of the visual cortex in favor of the less-active, **closed** eye. This surprising result was confirmed using a blind procedure in additional 4 kittens. Such an inhibition-coupled expression of plasticity in favor of the less-active, closed eye has never previously been reported and cannot be explained by a non-specific disruption of cortical function. The normal shift in favor of the more-active open eye was evident in control regions unaffected by muscimol treatment, as well as in border regions in which spike activity was profoundly attenuated.

The fact that identical presynaptic input activities can result in expressions of plasticity favoring either the less-active, closed eye or the more-active, open eye indicates (1) that the postsynaptic cell is crucially involved in plasticity of the visual cortex, (2) that the direction of cortical plasticity depends solely on postsynaptic membrane conductance or polarization and (3) that at least this novel expression of plasticity can occur in the absence of postsynaptic spike activity. The normal ocular dominance shift found in the border regions is consistent with the idea that excitation-coupled plasticity also does not require postsynaptic spikes. While these data provide direct evidence for Hebb's postulate of a crucial role for the postsynaptic element, they suggest that local responses rather than action potentials govern cortical plasticity.

Supported by grants from the NIH, McKnight Foundation and University of California Academic Senate.

344.3 OCULAR DOMINANCE SHIFT AFTER MONOCULAR EXPOSURE IN ANESTHETIZED AND IMMOBILIZED KITTENS: EFFECTS OF NORADRENALINE (NA) INFUSION.

K. Imamura and T. Kasamatsu. The Smith-Kettlewell Eye Research Foundation, 2232 Webster Street, San Francisco, CA 94115.

Monocular deprivation in early life induces profound changes in functional connection in the kitten visual cortex. At the peak of the sensitive period around 4-5 weeks of age, short-term deprivation for a day or less is sufficient to induce shift in ocular dominance towards the open eye. However, the changes do not occur if a kitten has been anesthetized and paralyzed during monocular exposure, suggesting that in addition to imbalance of visual input to the cortex, nonvisual afferent is necessary for inducing such modification. Recently we showed that in acutely anesthetized and paralyzed adult cats the ocular dominance distribution was arbitrarily altered in the NA-infused visual cortex by optical manipulations of the alignment of the visual axes. In the present study, we wanted to show that cortical NA infusion was sufficient to induce the shift in ocular dominance in response to brief monocular exposure in anestetized and paralyzed animals.

Five to seven week old kittens were used. Throughout the experiments, anesthesia was maintained with nitrous oxide (75% N_2O,22.5% O_2, 2.5% CO_2), supplemented with thiamylal sodium (1 mg/kg/hr or more), and paralysis was maintained with continous i.v. infusion of gallamine triethiodide (Flaxedil, 10 mg/kg/hr). Cut wounds and pressure points were infiltrated with 2% Xylocaine. One hemisphere of the visual cortex was locally infused with 0.5 mM NA using a cannula-osmotic minipump system. The contralateral eye was sutured shut and animals were continuously exposed to TV shows for 24 hr concommitantly with the NA infusion. The implanted cannula and minipump assembly was removed at the beginning of recordings. Single-unit recordings were carried out following the standard procedures in our laboratory. Thirty visually active cells were recorded, along a single recording track which was started near the cannulation site, with an intercellular spacing of about 100um. We obtained the shift in ocular dominance (binocularity, B=0.38; proprotion of group-7 cells, G7=0.47) in the NA-infused hemisphere, while no change was found, as expected, in the control hemisphere (B=0.63, G7=0.17) which do not receive the NA infusion. The difference was statistically significant. We conclude that in acutely anesthetized and paralyzed kittens the NA infusion was a sufficient condition to induce ocular dominance shift after brief monocular exposure.

(Supported by USPHS Grant EYO-5549).

344.4 **EFFECTS OF INTRACORTICAL INFUSION OF APV ON SYNAPTIC MODIFICATIONS IN KITTEN STRIATE CORTEX.**

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Depletion of cortical ACh and NE retards the ocular dominance (OD) shift in striate cortex that normally results from 10 days of monocular deprivation (MD, Bear and Singer, Nature 320: 172-176, 1986) Other visual response properties seem to be unaffected, suggesting that this treatment simply slows or prevents the process of synaptic modification. We have found that continuous intracortical infusion of 50 mM D,L-2-amino-5-phosphonovaleric acid (APV), a selective NMDA receptor antagonist, also prevents the OD shift (Kleinschmidt, Bear and Singer, Neurosci Lett. Suppl. 26:S58, 1986). However, unlike ACh + NE depletion, APV treatment also results in a striking loss of orientation selectivity and an overall reduction in visual responsiveness. Thus, it appears that synaptic modifications do occur under the influence of APV, although they may no longer be guided by retinal activity. To test this idea further, we asked whether APV infusion would prevent the recovery of cortical binocularity that normally occurs when a kitten is allowed binocular visual experience (BE) after a brief period of MD. Kittens were monocularly deprived by lid suture at approximately 4 weeks of age for 8-14 days. After this period of MD, the sutured eyelid was opened and the animals were given 7-13 days of BE, followed by a standard physiological assay of cortical OD. Most of the BE occured as one hemisphere was infused continuously with 50 mM APV at a rate of 1 µl per hour. We found no evidence of an ocular dominance shift in the experimental hemispheres even though they had received only 3-4 days of binocular vision without APV. This recovery of binocular connections is another example of plasticity in the presence of APV, and suggests that NMDA receptor activation is not a necessary condition for all synaptic modifications in striate cortex. The available data rather suggest that NMDA receptors may be involved specifically in the competitive strengthening/weakening of synapses that occurs during an OD shift or during the acquisition of orientation selectivity.

344.5 KETAMINE BUT NOT XYLAZINE IMPAIRS CONSOLIDATION OF PLASTIC CHANGES IN KITTEN VISUAL CORTEX.

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We have reported recently that ketamine-xylazine anaesthesia given to young kittens immediately after each of a series of 30 monocular exposures reliably prevents an ocular dominance shift in visual cortex (Rauschecker and Hahn, Nature, 326: 183-185, 1987). Using the same experimental protocol we have now tested the two drugs separately in order to find out whether ketamine, or xylazine, or both are responsible for the 'retrograde' impairment of cortical plasticity.

Nine kittens were reared in a totally dark room from three weeks of age. Every day they were taken out into the light for 20 minutes with one eye covered by a black contact occluder. During this time the kittens were alert and viewed a high-contrast visual environment. At the end of each exposure session they received an intramuscular injection of either ketamine (25 mg/kg; four kittens) or xylazine (10 mg/kg; five kittens) and were returned into the dark. Two of the xylazine-treated animals were given only single shots leading to a brief anaesthetic state of 15-20 minutes duration, in three xylazine kittens anaesthesia was prolonged to 60 minutes by increasing the dosage and/or giving additional injections. After 30 exposures, leading to a total of 10 hours monocular experience, a recording experiment in area 17 was performed.

All of the xylazine-treated kittens consistently showed a clear ocular dominance shift. In the ketamine-treated animals such a shift was absent or grossly reduced. In addition, the orientation tuning of cortical units in the ketamine kittens was broader than in the xylazine group, while responsiveness was the same. These results show that an impairment of synaptic consolidation in the visual cortex cannot be induced by any kind of reduced arousal. Since ketamine is known as a blocker of NMDA receptors for excitatory amino acids (Thomson et al., Nature, 313: 479-481, 1985), our results rather suggest that this type of receptor may be involved in cortical plasticity.

344.6 ENHANCED PHYSIOLOGICAL RECOVERY IN THE VISUAL CORTEX OF MONOCULARLY DEPRIVED KITTENS PROMOTED BY CERTAIN REGIMENS OF PART-TIME REVERSE OCCLUSION.

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After several weeks of monocular deprivation (MD) imposed on kittens, most cells in the visual cortex can be excited only by visual stimulation of the nondeprived eye. Such animals also appear blind when forced to use their deprived eye. Some recovery occurs subsequently if visual input is restored to the deprived eye sufficiently early. Until recently, only two conditions of recovery have been investigated, namely reverse occlusion where the nondeprived eye is occluded during recovery, and binocular recovery where both eyes are open during this period. However, neither of these conditions produces a satisfactory outcome for vision since the recovery is neither complete nor permanent, and frequently occurs at the expense of the vision of the nondeprived eye. We have found (Mitchell et al. Clin. Vis. Sci. 1:173, 1986) that certain regimens of part-time reverse occlusion could promote complete and seemingly permanent recovery of the vision of the deprived eye. We now report on the extent of physiological recovery in area 17 of 4 of these kittens.

After MD to 6 weeks of age the nondeprived eye was occluded for part of each day for 6 weeks by an opaque mask for either 3.5 (N=2) or 5 (N=2) hours each day after which the mask was removed to allow binocular visual input for the remainder of the short (7 hr) daily period of visual exposure. All 4 kittens eventually developed normal contrast sensitivity functions as well as vernier acuities in both eyes. Single cell recordings (478 cells) were made from area 17 of both hemispheres by conventional qualitative procedures. Quantitative measurements were made of the tuning for retinal disparity of about 20 of these cells in each animal. The animals that received 5 hrs of daily occlusion showed considerable physiological recovery with a substantial proportion (about 1/3) of cells dominated by the deprived eye. For one of these animals the proportion of binocular cells (80%) was similar to that observed in normal animals, but in the other animal the proportion was much lower, a finding that may be related to the presence of a strabismus in this animal. The physiological recovery observed in the 2 animals that received 3.5 hrs of daily occlusion was less impressive; only about 10% of cells were dominated by the deprived eye and less than half (45%) could be excited by both eyes. A number of the cells that were examined quantitatively in each animal exhibited reasonable tuning for retinal disparity, but most cells were quite unselective. Nevertheless it is possible that the former cells may be sufficient to provide a neural substrate for local stereopsis. These findings show that a relatively small proportion of cortical neurons may be sufficient to mediate behavioral recovery.

344.7 SPATIAL RECEPTIVE FIELD PROPERTIES OF STRIATE CORTICAL NEURONS IN CATS REARED WITH SURGICALLY INDUCED ESOTROPIA PAIRED WITH SAGITTAL TRANSECTION OF THE OPTIC CHIASM, Y.M. Chino, B. Timmney, W.L. Salinger, H. Wada*, G.A. Lesher*. Illinois College of Optometry, Chicago, IL 60616, University of North Carolina, Greensboro, NC 27412, and University of Western Ontario, London, Canada.

We have previously shown that rearing kittens with esotropia, surgically induced shortly after birth, leads to amblyopia of striate cortical neurons, i.e., a loss of spatial resolution and reduced contrast sensitivity (Chino et al. J. Neurophysiol. 50, 265-286, 1983). Our electrophysiological findings are corroborated with comparable results from behavioral testing performed on the same strabismic animals prior to our recordings (Holopigian and Blake, J. Neurophysiol. 50, 287-296, 1983). In an attempt to identify factors that may contribute to such deficits of our strabismic cats, we have raised strabismic kittens paired with a sagittal transection of the optic chiasm, thus eliminating direct binocular competition in the retino-geniculo-striate pathway during postnatal development. Surgeries were performed between the third and fourth postnatal week. Kittens' visual acuity was behaviorally measured with a jumping stand prior to electrophysiological recording. Extracellular single-cell recordings were made in the striate cortex with conventional methods.

We find that receptive fields of striate cortical neurons receiving inputs from the deviating eye were significantly enlarged as compared to those from the non-deviating eye or normally reared cats. Spatial modulation transfer functions, obtained with drifting high contrast sinusoidal gratings, show that the optimal spatial frequency and spatial resolutions of those units controlled by the deviating eye were significantly lower than those driven by the non-deviating eye. Interestingly, these values obtained with the non-deviating eye are slightly lower, but similar to optimal frequencies and resolutions measured in normal cats. In addition, the contrast threshold of the cells driven by the deviating eye was elevated as compared to the non-deviating eye. The threshold value for the non-deviating eye, however, was also higher than that of normal controls. These physiological findings are supported by our behavioral data; the acuity of the deviating eye was consistently lower than that of the non-deviating eye. Possible factors that may contribute to the present results will be discussed including a possible binocular competition through the corpus callosum.

Supported by NIH grant EY-03588 to YMC.

344.8 THE EFFECT OF NEONATAL VISUAL CORTEX DAMAGE ON VERNIER ACUITY AND RESOLUTION ACUITY IN CATS. S. Belleville and F. Wilkinson. Dept. of Psychology, McGill University, 1205 Dr. Penfield, Montreal, Que., CANADA H3A 1B1

The present study investigates the effect of neonatal lesions of visual cortex on two types of acuity: spatial resolution and vernier acuity. Eight kittens served as subjects in this study. In four, visual cortex (areas 17-18-19) was ablated bilateraly during the neonatal period (day 1-8); the remaining four animals served as littermate controls. Histological work and examination of the lesion extent have been completed in the four lesioned animals.Histological verification was based on the reconstructed borders of the lesion and the pattern of cell loss in the lateral geniculate nucleus and medial interlaminar nucleus. The lesions included most of area 17 and 18 and part of area 19.

Both types of threshold measurements were made using a two-choice discrimination procedure on a jumping-stand. Vernier acuity (detection of offsets in square-wave gratings) was tracked developmentally until asymptotic levels had been reached. Spatial acuity was then measured in adulthood in three of the lesioned animals and three littermate controls.

In the normal kittens, vernier acuity developed rapidly reaching asymptote at 3-5' by 90 days of age. At very early ages, vernier thresholds for the lesioned kittens were comparable to those of their normal littermates. However, their thresholds remained at that level (22-35' of arc) into adulthood.

Spatial resolution thresholds for the three normal cats tested on this task in adulthood were 3.6 c/deg, 4.1 c/deg and 4.5 c/deg. Thresholds in the lesioned animals were reduced only slightly (3.6 c/deg, 3.0 c/deg, 3.6 c/deg) in comparison to their respective littermates. These values all fall within the range of spatial acuities measured in this laboratory in intact adults cats. Consequently, whereas vernier acuity is severely decreased by neonatal damage to the visual cortex, resolution acuity remains relatively preserved. This provides further evidence that the sparing of function which has been claimed to occur following early brain damage is task-specific.

This research was supported by NSERC grant # 7551 to F.W.

344.9 OPTICAL STUDIES OF MACAQUE STRIATE CORTEX DURING DEVELOPMENT. L. Kiorpes* and G. Blasdel (SPON: J.A. Movshon). Dept. of Psychology and Center for Neural Science, New York University, New York, New York; Dept. of Medical Physiology, The University of Calgary, Calgary, Alberta, Canada.

As shown previously, optical techniques offer a powerful approach to the study of the functional organization of monkey striate cortex (Blasdel and Salama, 1986). Two features of these techniques make them particularly attractive for use in developmental studies: first, it is possible to study repeatedly the same cortical region in an individual animal; and second, it is thought that the optical signal derives from the upper cortical layers (laminae 2,3) where many of the neurons with projections to extrastriate cortex are located. Thus, the postnatal development of cortical organization in the upper layers, which remains largely unexplored, can be studied directly with these methods.

We have adapted one optical approach using the voltage sensitive dye NK2367 in conjunction with video imaging techniques to follow changes in the functional organization of striate cortex in individual infant monkeys during periods of normal and abnormal development. Our results to date show that high resolution optical images of neuronal activity can be obtained in animals as young as 7.5 weeks, and that the same patch of cortex can be studied at weekly intervals for at least 4 weeks in young monkeys. Results from one monkey, studied after 7.5 weeks of normal development reveal patterns of ocular dominance and orientation selectivity that are highly remeniscent of those in the adult. Visual stimulation with sine wave gratings of differing spatial frequencies, presented at all orientations, reveals a pattern of spots selective for low spatial frequencies that populate ocular dominance column centers and probably correspond to the cytochrome oxidase blobs seen histochemically (Tootell and Blasdel, 1987).

Monocular deprivation for 2 weeks following the period of initial study clearly altered the pattern of ocular dominance in the cortex. However, the topography of the ocular dominance pattern at this stage was not easily related to the normal topography. The ocular dominance pattern following a subsequent 2 week recovery period, during which the monkey was allowed binocular vision, revealed a shrinkage of the ocular dominance columns corresponding to the deprived eye, however, in addition, there appeared to be a break-up along the lengths of the columns as well.

344.10 ASSESSMENT OF SPATIAL VISION IN MONKEYS WITH EXPERIMENTALLY INDUCED APHAKIA. M. W. Quick*, C. D. O'Dell*, J. A. Gammon*, J. Wilson, M. Tigges, A. Fernandes*, and R. G. Boothe. Yerkes Regional Primate Research Center, Departments of Psychology, Ophthalmology, Anatomy and Cell Biology, Emory University, Atlanta, GA 30322.

To evaluate treatments of infantile monocular cataracts, neonatal monkeys were lensectomized unilaterally under conditions of general anesthesia and sterile surgery. Some animals were then optically corrected to a near point in the aphakic eye using extended-wear contact lenses. Visual input to the unoperated eye was either normal or blocked (part-time or full-time) using an occluder lens. Lens wear was monitored every 2 hours for compliance and lens power adjusted when necessary. Forced choice preferential looking (FPL) was used to assess acuity in the infants (O'Dell et al., IOVS Suppl. 28:216, 1987). Operant testing was used after 6 months, and final acuity was determined after 1 year of age. Final mean data for the various treatment groups were as follows: 1) uncorrected aphakia/no occlusion: .81 cpd/26.2 cpd; 2) corrected aphakia/no occlusion: 8.9 cpd/20.0 cpd; 3) corrected aphakia/full-time occlusion: 24.0 cpd/.025 cpd. One monkey did not wear any type of lens for one year after initial testing. When retested under original conditions no change in acuity was observed in either eye, indicating no acuity loss with lens removal after one year of age.

As evident in the above data, good vision is not maintained in the aphakic eye without occlusion of the unoperated eye. With occlusion, good acuity can be maintained (range 20-60 cpd). However, in the fully occluded eye, only one of six monkeys showed any pattern vision (.15 cpd); the others could distinguish only light-dark. We measured contrast sensitivity in the aphakic eye of one monkey from this group and found normal sensitivity across the entire spatial frequency range.

We are currently rearing several animals under conditions of corrected aphakia/part-time (6 hours) daily occlusion in an attempt to maintain good vision in both eyes. At 11 months, FPL measurements of acuity were the same in both eyes (14.2 cpd). One animal initially assigned to the corrected aphakia/full-time occlusion group learned to intermittently displace the occluder from in front of his pupil and maintained vision of 30 cpd in the unoperated eye; acuity in the aphakic eye was 20 cpd. Measures of contrast sensitivity in this animal showed an average sensitivity loss of 35% over the range of 1 cpd to the acuity point in the aphakic eye, and was normal in the unoperated eye. Furthermore, in a spatial phase discrimination task, threshold was normal in the unoperated eye but no phase discrimination ability was evident in the aphakic eye. We are currently measuring these and other (Landolt C acuity, vernier acuity) components of spatial vision to determine other possible deficits. NIH grants RR-00165 and EY-05975.

344.11 EFFECT OF PRENATAL MONOCULAR ENUCLEATION ON VERNIER HYPERACUITY IN RHESUS MONKEYS.

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Prenatal enucleation of one eye causes a set of dramatic changes in the pattern and number of neuronal connections in the primate visual system (Rakic, Science, **214**:928, 1981; Rakic & Riley, Nature, **305**:135, 1983). The remaining optic nerve has 30-40% more fibers than a normal optic nerve. Both lateral geniculate nuclei have normal numbers of neurons but only two layers (a magno- and a parvocellular layer). Striate cortex has a normal size and a normal complement of layers; however, it is uniformly innervated by the remaining eye, in marked contrast to the interdigitating ocular dominance columns of the normal striate cortex. To begin to understand how visual function is affected by these anatomical changes, we measured the vernier acuity in two adult (3-5 yrs) rhesus monkeys that were enucleated on embryonic days E63 and E91, respectively and were delivered at term (E165). The vernier task was chosen because it has been hypothesized that the hyperacuity ability assessed by this task depends critically upon the grain of the visual field representation on the granule cells of layer IVc (Barlow, Nature, **279**: 189, 1979) and thus might be improved by having all of the IVc granule cells devoted to only one eye.

Each experimental monkey was paired with an age and sex matched normal control and all subjects were tested on a detection version of the classic vernier acuity task in which they were rewarded for responding when two nearly abutting vertical lines were horizontally displaced. The method of constant stimuli was used. Threshold was taken as the displacement with 50% detection after correcting for guessing and fitting with a sigmoid curve. The detection thresholds for the two experimental monkeys were 0.002° and 0.0024° (e.g., 7.2 & 8.6 sec of arc), which were slightly better than the thresholds of 0.003° obtained for the two control monkeys tested binocularly. One control monkey was also tested monocularly; detection thresholds were 0.0032° and 0.0044° in the left and right eyes, respectively. One experimental monkey and one control were also tested in a forced-choice version of the vernier acuity task in which they had to indicate the direction of the vernier displacement. Their thresholds were approximately the same as in the detection version. We also tested three human subjects in this forced-choice version: their binocular thresholds were 0.001°, 0.0018°, and 0.002°.

We conclude that early monocular enucleation does not adversely affect vernier hyperacuity; in fact, acuity of the enucleated monkeys was slightly better than the acuity of the control monkeys. We further conclude that rhesus monkeys have hyperacuity abilities that are nearly as fine as those of humans.

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344.12 **"VISUAL" CORTEX OF HUMAN EARLY BLIND SUBJECTS MAPPED WITH POSITRON-EMISSION TOMOGRAPHY.**

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We studied glucose metabolism in the striate and prestriate cortex of 4 early blind and 4 normal sighted volunteers. The regional cerebral metabolic rate for glucose (rCMRGlc) was measured by positron emission tomography (PET) with [^{18}F]-2-deoxy-2-fluoro-D-glucose (^{18}FDG). In all experimental conditions, the sighted subjects were blindfolded. Each subject was involved in a tactile or in an auditory task during a PET study. The tactile task consisted either in silent braille reading or in manipulation of objects; the ears were plugged. The auditory task consisted in the mental localization of a sound source. The subjects remained seated during all these tasks. Furthermore, two blind and one control subjects took part in a second PET study performed in the resting state with the ears plugged. For each PET study, nine slices were collected at 10 mm interval, parallel to, and starting 20 mm above the suborbito-meatal plane. Neuroanatomic localization was performed by matching PET images with Magnetic Resonance Imaging (MRI) data obtained in the same planes, and by reference to a brain atlas. Relative rCMRGlc were calculated as the percentage of mean gray matter glucose metabolism defined over all gray structures in the forebrain.

MRI examinations revealed that the striate and prestriate cortices had a similar, normal appearance in blind as well as in normal subjects. In four PET studies of early blind volunteers performing auditory or tactile tasks, the maximal rCMRGlc was consistently found in striate or prestriate regions, irrespective of the type of task. When performing the same task, the relative rCMRGlc in the striate and prestriate cortices were always higher for the early blind subjects than for the blindfolded control subjects. In order to assess whether the high metabolic activity of the Blind's occipital cortex was induced by the task, two blind subjects were also studied in the resting state. As a result, rCMRGlc measured in striate and prestriate areas at rest or during tactile or auditory stimulation were just so high. A quantitative evaluation revealed that the relative rCMRGlc in the striate and prestriate cortices were significantly higher in the blind subjects than in the blindfolded sighted ones.

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CARDIOVASCULAR REGULATION: CNS PATHWAYS V

345.1 SPECIFICITY OF CONNECTIONS IN BRAIN STEM RETICULAR FORMATION: SYMPATHETIC CIRCUITS.

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A critical question concerning the organization of the brain stem reticular formation is whether its plan of connectivity is specific or nonspecific. The classic view is one of indiscriminate interconnnection of reticular neurons with axons directed to different targets. In this case, the reticular formation would exert uniform actions on all of its outputs under all conditions. An alternative view is that the plan of connectivity is highly ordered so that the discharges of reticular neurons with the same target are preferentially synchronized. In this case, the reticular formation would be comprised of a number of functionally distinct modules. The current study was designed to test these alternative views.

Crosscorrelation analysis was used to determine whether the discharges of adjacent neurons in the cat brain stem were synchronized on a time scale of a few milliseconds. Unit recordings were made in three regions of the brain stem reticular formation - lateral tegmental field (LTF) of dorsal medulla, rostral ventrolateral medulla (RVLM) and raphe (R) of medulla. Unit recordings also were made in the lateral hypothalamus (LH), a region whose architectonic design is similar to that of the LTF. Neuronal pairs were chosen so that at least one member had spontaneous activity temporally related to inferior cardiac postganglionic sympathetic nerve discharge (as demonstrated with spike-triggered averaging). When both members of a pair had sympathetic nerve-related activity, the pair was designated S-S. When only one member had sympathetic nerve-related activity, the pair was designated S-NS. Short time scale (a few milliseconds) interactions were demonstrated for 58, 65, 25 and 69% of S-S pairs in the LTF, RVLM, R and LH, respectively. The crosscorrelograms for these neuronal pairs contained either a sharp paracentral peak or a pericentral peak. The percentages of S-NS pairs showing short time scale interactions were considerably lower than for S-S pairs. They were 36, 17, 0 and 38% for S-NS pairs in the LTF, RVLM, R and LH, respectively. The data clearly point to a specific rather than a nonspecific plan of connectivity among the neurons in the regions studied. That is, our results support the view that the medullary reticular formation and LH are organized into functionally distinct modules, some of which direct their influences to sympathetic nerves. Short time scale synchronization of the discharges of members of some S-NS pairs may reflect weak coupling between sympathetic and nonsympathetic modules. The predominance of short time scale interactions for S-S pairs, however, clearly indicates that intramodular coupling was stronger than intermodular coupling under the conditions of our experiments. (Supported by HL13187 and HL33266.)

345.2 RAPHESPINAL SYMPATHOINHIBITORY NEURONS EMIT BRANCHES IN THE CERVICAL SPINAL CORD IN CATS.

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Morrison and Gebber (J. Neurophysiol. 53: 759-772, 1985) and Barman and Gebber (J. Neurophysiol. 53: 1551-1566, 1985) characterized cat raphe (R) and rostral ventrolateral medullary (VLM) neurons in sympathetic pathways. Spike-triggered averaging showed that the spontaneous activity of these neurons is temporally related to inferior cardiac postganglionic sympathetic nerve discharge (SND). Antidromic mapping of the upper thoracic spinal cord revealed that the axons of these neurons innervate the intermediolateral nucleus (IML). Since the firing rate of the R-spinal neurons increased during baroreceptor reflex activation, they likely subserve a sympathoinhibitory function. VLM-spinal neurons are considered to be sympathoexcitatory since their firing rate decreased during baroreceptor reflex activation. The current investigation was designed to test the hypothesis that the axons of R- and VLM-spinal sympathetic neurons emit branches in the cervical spinal cord. This hypothesis was considered since the studies of Kirchner et al. (Pfluegers Archiv. 357: 349-360, 1975) and Schramm and Livingston (Am. J. Physiol. 252: R514-R525, 1987) support the existence of cervical propriospinal neurons in sympathetic pathways. Such neurons may receive input from the same groups of medullospinal neurons that project directly to the thoracic IML. We microstimulated the third thoracic (T3) IML to antidromically activate 47 R-spinal sympathoinhibitory neurons and 15 VLM-spinal sympathoexcitatory neurons neurons in 22 cats. A second stimulating microelectrode was positioned in the gray matter of the cervical spinal cord (C3-C5). When a neuron was antidromically activated from both sites, we used a collision test to determine whether stimuli applied at C3-C5 activated an axonal branch or the main axon by current spread to the white matter. Specifically, we determined the maximum interval after a C3-C5 stimulus at which a T3 stimulus failed to elicit an antidromic response at the recording site. If the main axon is activated by C3-C5 stimulus, this collision interval (CI) equals the difference between the onset latencies of the antidromic responses elicited by thoracic and cervical stimuli ($L_T - L_C$) plus the axonal refractory period at the thoracic site (R_T); $CI = L_T - L_T + R_T$. When a cervical axonal branch is activated, $CI > L_T - L_C + R_T$. Conduction time in the axonal branch (t_B) = $1/2(CI - L_T + L_C - R_T)$. None of the 15 VLM neurons antidromically activated by stimulation of both C3-C5 and T3 IML emitted a branch in the cervical region. In contrast, stimulation of the C3-C5 gray matter activated an axonal branch of 12 of 47 R-spinal neurons. Conduction time in the axonal branch averaged 8.2±2.0 ms. The conduction velocity in the main axons of these R-spinal neurons averaged 2.9±0.4 m/s. Antidromic mapping revealed that the longest latency antidromic response was elicited with the least stimulus current when the microelectrode was positioned in lamina VII. It remains to be determined whether these cervical branches of R-spinal neurons innervate descending propriospinal neurons in sympathetic pathways. If so, R neurons may control SND by both an indirect and direct projection to the IML. (Supported by NIH grants HL13187 and HL33266.)

345.3 MIDLINE MEDULLA MEDIATES TONIC SYMPATHOINHIBITION AT THE LEVEL OF THE ROSTRAL VENTROLATERAL MEDULLA. Robert B. McCall. Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, MI. 49001.

Previous studies in our laboratory indicate that the midline medulla is heterogenous with respect to autonomic function containing both sympathoexcitatory and sympathoinhibitory elements. Sympathoexcitatory responses elicited by stimulation of the midline medulla are mediated by serotonin, while sympathoinhibitory responses are mediated by GABA. The purpose of the present study was to further characterize the nature of the sympathoinhibition elicited by stimulation of the midline medulla. Lesions of the midline medulla extending 2 to 7 mm rostral to the obex significantly increased inferior cardiac spontaneous sympathetic nerve discharge (SND) by 54± 11% in chloralose anesthetized cats (n=25). Lesions failed to affect baroreceptor-mediated inhibition of sympathetic activity. These data indicate that neuronal elements in the midline medulla tonically inhibit SND by a non-baroreceptor mechanism. The effects of midline stimulation on the discharge of sympathetic neurons in the rostral ventrolateral medulla were determined in order to test the hypothesis that the tonic sympathoinhibition was mediated at the level of sympathoexcitatory medullospinal neurons located in this area of the brain stem. Neurons in the rostral ventrolateral medulla were recorded extracellularly and classified as sympathoexcitatory if 1) they could be antidromically activated from the spinal cord, 2) they were inhibited during baroreceptor reflex activation and 3) their discharge was temporally correlated to SND. Electrical stimulation of the midline medulla (100 μA, 0.5 ms; 0.5 Hz) inhibited the discharges of sympathoexcitatory neurons in the rostral ventrolateral medulla. The mean onset latency of inhibition was 22 ms and the mean duration of inhibition was 175 ms. The onset of the raphe elicited sympathoinhibitory response recorded from the inferior cardiac nerve was equal to the sum of the onset latency of the sympathoexcitatory response elicited from the rostral ventrolateral medulla (76 ms) plus the conduction time (22 ms) in the raphe to rostral ventrolateral sympathoinhibitory pathway. A second group of neurons located in the immediate vicinity of the sympathoexcitatory neurons were excited by single shocks applied to the midline medulla. The onset latency of activation of these neurons ranged from 12 to 32 ms. These neurons fired spontaneously with a bursting pattern of discharge, were not affected during baroreceptor reflex activation and did not fire with a pattern that was obviously related to SND. Finally, bilateral microinjections of the GABA antagonist picrotoxin into the rostral ventrolateral medulla increased SND to an amount comparable to that observed following midline lesions. Baroreceptor inhibition of SND was not affected by the microinjection. These data are consistent with the possibility that non-baroreceptor neuronal elements in the midline medulla tonically inhibit sympathoexcitatory medullospinal neurons in the rostral ventrolateral medulla by activating closely adjacent GABAergic interneurons.

345.4 Non-adrenergic pace-maker neurons of rat rostral ventrolateral medulla. Miao-Kun Sun, John T. Hackett and Patrice G. Guyenet. Dept. Pharmacology, University of Virginia, Charlottesville, VA 22908.

Coronal slices of the rat rostral ventrolateral medulla (Sprague-Dawley, 100g) maintained "in vitro" (500 μm thick, interface method, temperature 31° or 37°C) were explored to characterize the properties of tonically active neurons. The majority of the latter consisted of cells with non-bursting rhythmic discharges (9 ± 0.3 spikes/sec N=84 at 31°C; 21.3 ± 2 spikes/sec, N=9 at 37°C) and positive going extracellular spikes. Stable intracellular recordings were obtained in 30 cases. All these cells displayed a typical pace-maker potential decreasing from -63 ± 0.2mV (N=18) after each action potential to -49 ± 1.1 mV (N=18) representing threshold for spike initiation. EPSP's were not detected even during negative current injection which resulted in a 10 ± 5 mV (N=15) hyperpolarization and complete silencing of the cell. Current-voltage relationship indicated a mean apparent resistance of 138 ± 10 MΩ (N=18) when measured around -60mV. Lucifer Yellow was iontophoresed into 15 pace-maker cells with resting potentials of -53mV or better and the tissue was processed for PNMT-immunohistochemistry (Texas-red labelled secondary AB). Ten cells were recovered in 40 μm sections, none of which contained PNMT-immunoreactivity. All ten cells were surrounded by adrenergic cells and were located in the retrofacial portion of nucleus paragigantocellularis lateralis (PGCL). Cells with similar characteristics were also extracellularly recorded within retrofacial PGCL in an "in vitro" preparation consisting of the entire medulla plus C_1 spinal segment (vascularly perfused through basilar artery, 31°C). Twenty-three neurons with pace-maker like discharges were recorded in this area (8 ± 2 s/sec mean firing rate). In four cases, the presence of a spinal axon could be detected. The rhythmic firing pattern of these neurons could be reset by orthodromic activation produced by focal stimulation of the caudal ventrolateral medulla. The rhythmic firing pattern of these cells was unaffected by 0.5-1mM of the glutamate-receptor antagonist kynurenic acid in the medium. This drug, however, completely abolished the excitatory effect produced by focal stimulation (N=6).

It is concluded that the rostral ventrolateral "pressor" area contains non-adrenergic pace-maker reticulospinal neurons. (HL 28785).

345.5 Sympathoexcitatory reticulospinal neurons of rostral ventrolateral medulla have intrinsic pace-maker properties. P.G. Guyenet and M.-K. Sun. University of Virginia, Dept. of Pharmacology, Charlottesville, VA.

The preceeding abstract described the presence of reticulospinal non-adrenergic pace-maker neurons in the retrofacial portion of nuc paragigantocellularis lateralis "in vitro" (PGCL, so called "ventrolateral medulla pressor" or "C_1" area). The aim of this second study is to provide evidence that these pace-maker neurons represent the reticulospinal sympathoexcitatory neurons previously recorded by us "in vivo" in the same brain area (PGCL-SE neurons, see Brown & Guyenet 1985, Circ. Res. 56: 359).

Single-unit recordings of PGCL-SE neurons were obtained in halothane-anesthetized 350 g Sprague-Dawley rats ventilated with room air. The lumbar sympathetic discharge (LSND) was recorded with bipolar electrodes (30-3000 Hz), full wave rectified, integrated and quantified with a 1 sec reset-time. The following sympathetic reflexes were investigated; baroreflex, vagal inhibitory and sympathoexcitatory responses, somatosympathetic reflexes, mixed excitatory-inhibitory responses produced by lateral hypothalamus stimulation.

Intracisternal injection of the glutamate-receptor antagonist kynurenic acid (KYN, 5 μmole) produced a sustained increase in AP and LSND provided that access of the drug to the spinal cord was minimized by head-down tilt. Under these conditions all sympathetic reflexes were abolished (N= 5-10) except somatosympathetic reflexes (75% reduction only, N=5). The inactive analog 8-OH kynurenic acid produced no effect. Following KYN, the discharge rate of PGCL-SE neurons stabilized around 22 spikes/second (N=22;SEM 1.6) and the cells became insensitive to arterial pressure. KYN had no effect on action potential size and configuration. After KYN the discharge rate of PGCL-SE neurons became extremely regular and pace-maker like. Antidromic activation by stimulating their spinal axon at T_3-T_4 resetted their rhythmic discharge (N=8) except when the spinal stimulus was triggered within the critical latency for collision extinction. When cord stimulation was delivered at random with regard to spontaneous spikes and below threshold for antidromic activation post-stimulus spike histograms were uniformly flat (N=8). Thus, after synaptic blockade with KYN, PGCL-SE neurons are the only tonically active cells of the rostral ventrolateral medulla and display a firing rate characteristic of pace-maker neurons which cannot be explained by the activity of recurrent or collateral networks. Since the spike configuration, location, discharge rate of PGCL-SE neurons after KYN is identical to that of the pace-maker cells recorded "in vitro", we conclude that they belong to the same non-adrenergic cell population. (HL 28785).

345.6 PARABRACHIAL STIMULATION INHIBITS CAROTID SINUS AFFERENT INPUT TO NUCLEUS TRACTUS SOLITARIUS. R.B. Felder, S.W. Mifflin* (SPON: E.A. Anderson). Cardiovascular Center, Department of Internal Medicine, University of Iowa, Iowa City, IA 52242.

The parabrachial nucleus (PBN) is a major projection nucleus for efferent neurons leaving nucleus tractus solitarius (NTS), the termination site for primary afferent fibers innervating reflexogenic areas of the circulation. We recently reported excitation of single neurons recorded extracellularly in and around NTS by electrical stimulation of PBN and ipsilateral or contralateral carotid sinus nerve (CSN), and demonstrated a potent inhibitory influence of the PBN stimulus on CSN input to the same neuron whether evoked by electrical or selective baroreceptor stimulation. The reciprocol interaction, though less pronounced, was also observed. In the present studies, we utilized intracellular recording techniques to further investigate the mechanisms underlying these inhibitory interactions.

In chloralose anesthetized, mechanically ventilated and paralyzed cats, we recorded in right NTS region from 37 neurons, which responded to electrical stimulation of both ipsilateral CSN and PBN. CSN stimulation evoked an EPSP in 19 units, an EPSP/IPSP in 14, and an IPSP in 4. The latency ranges for these PSPs were 3.5-26.4 msec, 3.1-10.0 msec and 10.9-15.3 msec, respectively. In these 37 cells the PBN stimulus (50-300 μA, 1-4 pulses) uniformly evoked an IPSP, which ranged in amplitude from 2.1 to 5.8 mV (mean 3.3±0.2 mV, x̄±SE) and in duration from 65 to 270 msec (mean 160±9 msec). The IPSP was reversed using negative DC current injection in 5 units. In 29 of the 37 cells the PBN evoked IPSP was preceded by an EPSP (onset latency 2.3-29.6 msec, < 5 msec in 16 cells). The latency of the PBN evoked EPSP was less than the CSN evoked PSP in 20 of 29 neurons. When CSN stimulation was preceded by PBN stimulation at a 50 to 70 msec interval (n=12), the CSN evoked PSP (EPSP or IPSP) was reduced in amplitude. In 5 cells tested, inhibition of the CSN PSP lasted for the duration of the PBN evoked IPSP. In contrast, the PBN evoked EPSP was inhibited by a prior CSN stimulus for intervals up to 150 msec with no change in membrane potential in 3 of 6 cells tested.

These findings demonstrate that parabrachial stimulation causes an early excitation followed by prolonged post-synaptic inhibition of NTS neurons also activated by CSN stimulation. This suggests a potential role for PBN in enhancing or inhibiting the responses of NTS neurons to sinus nerve input.

345.7 TIME DEPENDENT INHIBITORY INTERACTIONS BETWEEN CAROTID SINUS NERVE (CSN) AFFERENT INPUTS TO NUCLEUS TRACTUS SOLITARIUS (NTS).
S.W. Mifflin* and R.B. Felder (SPON: C.V. Gisolfi).
Cardiovascular Center, Dept. of Internal Medicine, Univ. of Iowa, Iowa City, IA 52242.

Previous studies have demonstrated inhibitory interactions between carotid sinus baro- and chemoreflexes and between ipsilaterally and contralaterally evoked carotid sinus baroreflexes. An earlier report from this laboratory demonstrated time dependent inhibitory interactions between CSN afferent inputs which independently excited neurons in NTS, the primary site of termination of CSN afferent fibers. Using a conditioning test paradigm, we electrically stimulated the CSNs to examine the mechanism for this phenomenon.

Intracellular recordings were made from cells in the NTS region in chloralose anesthetized, mechanically ventilated, paralyzed cats. Electrical stimulation of ipsilateral CSN evoked an epsp in 20 cells, an epsp/ipsp in 16 cells and an ipsp in 13 cells. In all cells, the CSN evoked psp was abolished or reduced in amplitude and duration by a conditioning stimulus consisting of a single pulse or train of pulses (2-5 at 200-500 Hz) to the same nerve at intervals of 50-70 msec. The inhibition could be graded by varying the number of conditioning stimuli and persisted for 150-450 msec in 19 cells tested. In cells receiving convergent input from both CSNs, the epsp (5 of 7 cells tested), epsp/ipsp (5 of 5), or ipsp (5 of 5) evoked by ipsilateral CSN was inhibited by conditioning stimuli to the opposite CSN. Similar inhibition was noted when the order for stimulating the two nerves was reversed. The inhibition occurred in the absence of changes in membrane potential or input resistance and persisted beyond the duration of CSN evoked ipsps (cells receiving an epsp/ipsp or an ipsp). These observations suggest distal dendritic inhibition and/or disfacilitation (epsps) or disinhibition (ipsps) as potential mechanisms for this phenomenon.

We conclude that the temporal pattern of converging inputs may profoundly influence cardiovascular afferent integration at NTS level.

345.8 POTASSIUM CURRENTS IN NEURONS ISOLATED FROM ADULT RAT SOLITARY TRACT NUCLEI INVOLVED IN CARDIOVASCULAR CONTROL
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Cardiovascular afferent fibers terminate in the the medial and dorsal medial nuclei of the solitary tract. A group of morphologically similar neurons from these nuclei has been enzymatically isolated for the purpose of characterization of the ionic currents which are responsible for their spontaneous activity and response to putative transmitters. A TTX sensitive sodium current and a calcium current with two distinct components have already been identified in all cells studied in this group (Kunze, D.L., AJP, 252:H867,1987). The present study examined the potassium currents in these cells. The gigaseal technique for whole cell recording was used for a voltage clamp analysis. To isolate potassium currents a bathing solution containing in mM 137 NaCl, 5.4 KCl, 1 $MgCl_2$, 10 Glucose, 10 Hepes, 10-5 TTX and 2x10-6 $CaCl_2$ was used. The pipette contained 140mM KAsp, 10mM Hepes, 5mM EGTA and 3 mM $MgCl_2$. Under these conditions an outwardly rectifying potassium current was present in all cells. This current was present at potentials more positive than -40mV, was slowly developing (20msec) and noninactivating during a 250msec depolarizing pulse. A transiently activated potassium current was also present in approximately 40% of the cells studied. When present it was seen with depolarizing steps to potentials positive to -40mV, it activated rapidly (<5 msec) and its inactivation was voltage dependent. It was inactivated at holding potentials more positive than -50mV. Of the ionic currents studied thus far, only this transient potassium current has been restricted to a subpopulation of cells from these nuclei. An inwardly rectifying potassium current was not present in any of the cells studied. These results will serve as a basis for examining the role of putative transmitters in modifying potassium currents and for interpreting the mechanism of spontaneous activity in this group of neurons.

345.9 MECHANISMS OF CARDIOVASCULAR AND ANTINOCICEPTIVE ACTIONS OF INTRATHECAL CLONIDINE.
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The α_2-adrenoceptor agonist clonidine produces antinociceptive effects following systemic or central administration and complex effects on blood pressure, including a transient pressor response followed by a prolonged depressor effect after bolus systemic administration, and depressor (at lesser doses) or pressor (at greater doses) effects after central administration. The present study was designed to characterize these effects of clonidine upon its intrathecal (i.t.) administration to the level of the lumbar enlargement. Rats were initially deeply anesthetized with pentobarbital (45 mg/kg i.p.) for placement of femoral arterial and venous and 7.5-8 cm i.t. catheters. Under light pentobarbital anesthesia (3-6 mg/kg/hr i.v.), the nociceptive tail-flick (TF) reflex was evoked by radiant heating of the tail. Mean arterial pressure (MAP) and heart rate (HR) were continuously recorded, and TF latencies were determined at 1-60 min after i.t. administration of single doses of clonidine (1-32 µg/7.5 µl). The effects of i.t. clonidine on MAP were also determined in conscious, freely-behaving rats. In both lightly anesthetized and conscious rats, lesser doses of clonidine (1-3.2 µg) decreased MAP, while greater doses (10-32 µg) produced marked pressor effects. In lightly anesthetized rats, antinociceptive and depressor effects produced by 3.2 µg clonidine were significantly attenuated by pretreatment with the α_2-adrenoceptor antagonist yohimbine (30 µg i.t.) but not by the α_1-adrenoceptor antagonist prazosin (30 µg i.t.) or by i.v. yohimbine (0.1 mg/kg). Thus, these effects of i.t. clonidine are mediated by spinal α_2-adrenoceptors. In contrast, the cardiovascular effects of the greatest dose of i.t. clonidine (32 µg; maximal ↑ in MAP of 53.3±3.3 mm Hg within 5 min, maximal ↓ in HR of -72±8 bpm) were similar to those effects observed upon injection of this dose either i.v., or i.t. to the cervical level, and were not significantly altered by pretreatment with yohimbine (30 µg i.t. or 0.1 mg/kg i.v.), prazosin (30 µg i.t. or 0.1 mg/kg i.v.) or the vasopressin antagonist [1-(β-mercapto-β,β-cyclopentamethylene propionic acid), 2-(o-methyl) tyrosine] Arg^8-vasopressin (10 µg/kg i.v.), nor by adrenal demedullation. The ganglionic blocking agent chlorisondamine (2.5 mg/kg i.v.) significantly attenuated the bradycardia, but potentiated the pressor response, whereas the non-selective α-adrenoceptor antagonist phentolamine (2 mg/kg i.v.) abolished the pressor response but had no significant effect on the bradycardia. Thus, the pressor effects of i.t. clonidine are not mediated by activation of spinal α-adrenoceptors or spinal sympathetic preganglionic neurons. Rather, these effects appear to result from activation of peripheral α-adrenoceptors, suggesting a rapid systemic redistribution of large doses of i.t. clonidine. Supported by T32 GM 07069 and DA 02879.

345.10 **TONIC CONTROL OF ARTERIAL PRESSURE AND REGIONAL HEMODYNAMICS BY PONTINE REGIONS.**
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We have previously reported on the role of the rostral ventrolateral medulla (RVLM) in the maintenance of vasomotor tone in rats. In earlier studies, the microinjection of lidocaine (LIDO) into the lateral aspect of the RVLM resulted in a depressor response and a fall in resistance in the renal, mesenteric, and hindquarter vascular beds. Reduction of tidal volume (TV) from 2.5cc to 1.5cc resulted in an attenuation of the depressor response and the fall in resistance in the three vascular beds. However, under conditions of reduced TV, the level of sympathetic nerve activity remained the same or was slightly elevated. Thus, some other region must assume the role of maintaining vasomotor tone under conditions of reduced TV.

In this study, we examined the role of pontine sites in the tonic control of vasomotor tone. 200nl of a 4% solution of LIDO was microinjected into the pons at the level of the Kolliker-Fuse nucleus (KFN). These injections were made 0.9 and 2.5mm lateral to midline and 6,7,8 and 9mm below dura. For reference, the injection 2.5mm lateral to midline and 7mm below dura centered on the KFN nucleus. First, the inactivation of the region 2.5mm lateral to midline and 6mm below dura resulted in an insignificant (-4±7mmHg) fall in arterial pressure when the animal was respired at a 2.5cc TV. A significant depressor response to LIDO was seen when TV was reduced to 1.5cc (-14±3mmHg). Resistance was lower in each vascular bed. No significant effects were noted with LIDO injection at the site 0.9mm lateral to midline. The injection of LIDO into the site 2.5mm lateral to midline and 7mm below dura (i.e. in KFN) resulted in a depressor effect at 2.5cc (-14±4mmHg) and at 1.5cc (-14±4mmHg) TVs. These effects were mediated primarily by a fall in hindquarter vascular resistance. Injection of LIDO 0.9mm lateral to midline (in the region of the ventral tegmental nucleus) resulted in a slight increase in arterial pressure at both TVs. Injection of LIDO into the region 1mm below KFN yielded similar results. At the lateral site, depressor responses were seen at both the 2.5 and 1.5cc TVs (-17±4 and -13±3mmHg respectively). Again, these effects were mediated primarily by a fall in resistance in the hindquarter vascular bed. LIDO in the medial site produced a pressor response at both the 2.5 and 1.5cc TVs (14±4 and 14±4mmHg respectively). These increases in arterial pressure were mediated by an increase in resistance in all three vascular beds. Injection of LIDO 9mm below dura (most rostral pole of the A_5 region) had no significant effect on arterial pressure regardless of site or TV. This work illustrates a role for supra-medullary cell bodies or fibers of passage in the tonic control of vasomotor tone. (Supported by HLB-14388 and GM-07069.)

345.11 SPINAL CORD ADMINISTRATION OF A THYROTROPIN-RELEASING HORMONE (TRH) ANALOG, MK-771, INCREASES SYMPATHETIC OUTFLOW TO THE CARDIOVASCULAR SYSTEM. C.J. Helke and E.T. Phillips*, Dept. of Pharmacology Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20814.

TRH increased mean arterial pressure (MAP) and sympathetic activity when administered into the brain ventricles (J. Pharmacol. Exp. Ther. 238:232, 1986), however sympathetically mediated spinal cord actions of TRH have not been investigated. This is of interest because TRH binding sites and TRH-immunoreactive nerve terminals are present in the intermediolateral cell column (IML), the origin of sympathetic preganglionic neurons. Recent studies showed that the TRH innervation of the IML arises from a region in the ventral medulla (Brain Res. 381:1, 1986) which plays an important role in regulating sympathetic activity to the cardiovascular system. We studied the effects of intrathecal (i.t.) administration of the stable TRH analog, MK-771, on mean arterial blood pressure (MAP) and heart rate (HR) in chloralose/urethane anesthetized, artificially ventilated rats. MK-771 (0.01-5.0 µg i.t.) caused dose-related increases in MAP and HR. A 1 µg dose of MK-771 increased MAP 33±5 mmHg and HR 80±21 bpm. The onset of action was rapid and the peak response occurred 5-10 min after administration. Similar doses of MK-771 administered i.v. were without effect. TRH (i.t.) produced cardiovascular effects similar to those seen with MK-771 but was less potent. TRH-free acid produced no cardiovascular effects in doses up to 1000 µg (i.t.) That the MK-771 effects were due to activiation of the sympathetic nervous system was shown by blockade of the MAP effects of MK-771 (5 µg i.t.) with phentolamine (10 mg/kg i.v.) and both MAP and HR effects with pentolinium (10 mg/kg i.v.). Assessment of changes in regional blood flow and vascular resistance caused by MK-771 (1 µg i.t.) were done with the radioactive microsphere technique. MK-771 increased total peripheral resistance due to vasoconstriction in cutaneous, skeletal muscle, and renal vascular beds. MK-771 had no effect on vascular resistance locally, in the spinal cord region of the i.t. injection. Cardiac output was also unchanged by MK-771 (1 µg i.t.).

These data show that TRH receptor activation in the thoracic spinal cord, presumably in the IML, increases sympathetic activity to the peripheral vasculature. Assessment of the role of TRH-innervation of the IML on maintenance of sympathetic tone to the vasculature awaits development of TRH receptor antagonists. (Supported by NIH grant #NS24876).

OPIATES, ENDORPHINS AND ENKEPHALINS: ANATOMY AND CHEMISTRY II

346.1 IDENTIFICATION OF PRO-DYNORPHIN HIGH MOLECULAR WEIGHT INTERMEDIATES IN THE RAT ANTERIOR PITUITARY. R. Day* and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan, 48109.

The post-translational processing of pro-dynorphin (Pro-DYN) is not well understood. The anterior pituitary is an interesting tissue to examine this question since it is well known that dynorphin-immunoreactivity (DYN-ir) is contained in high molecular weight intermediates and not as free products such as DYN A 1-17 (1). A 6 Kd product containing DYN A and DYN B was identified as well as an 8 Kd product containing alpha-neo-endorphin (α-NE). The aim of this study is to fully characterize these products with the aid of a series of antibodies directed to various regions of Pro-DYN including non-opioid regions. These included antibodies to DYN A 1-17, DYN B, α-NE, Bridge peptide (the amino acid sequence between α-NE and DYN A), C-peptide (the carboxyl terminal region of Pro-DYN), and Pro-DYN mid-amino terminal peptide (an 11 amino acid sequence midway between the amino terminal of Pro-DYN and α-NE). Rat anterior pituitary extracts were submitted to G-50 gel filtration chromatography. Fractions were analyzed by radioimmunoassay. Various fractions were pooled and submitted to HPLC analysis. Also immunoaffinity columns were used to complement the above information. Four major products containing Dyn A-ir were observed with MWs ranging from 4 Kd to 9 Kd. Two of these products contained C-peptide-ir while the other two contained only DYN B-ir. Furthermore two high MW α-NE-ir products were observed with respective masses of 16 Kd and 4 Kd. The 16 Kd molecule was also immunoreactive for Pro-DYN mid-amino terminal peptide. Based on these data we conclude that Pro-DYN post-translational processing in the anterior pituitary can occur by multiple pathways. A scheme for the processing of Pro-DYN will be proposed.

(1) Seizinger, B.R., Hollt, V., Herz, A. Endocrinology, 115, 662, 1984.

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346.2 DEVELOPMENTAL CHANGES IN STEADY STATE LEVELS OF FORMS OF PRO-DYNORPHIN IN THE RAT SUBSTANTIA NIGRA. C.A. Sei and R.M. Dores, Department of Biological Sciences, University of Denver, Denver, CO 80208.

In the rat brain the substantia nigra is a rich terminal field area for the pro-dynorphin end products: dynorphin A, dynorphin B, and alpha-neo-endorphin. Although there have been extensive studies on the pro-dynorphin system in the substantia nigra of the adult rat, there is relatively little information on the forms of pro-dynorphin end products in this terminal field during post-natal development. In this study the steady state levels of pro-dynorphin end products were analyzed in the substantia nigra of post-natal day 0, post-natal day 7, and adult animals.

Acid extracts of pools of tissue were separately fractionated by gel filtration chromatography on a Sephadex G-50 column and analyzed with radioimmunoassays specific for dynorphin A(1-17) and dynorphin A(1-8). The identity of each immunoreactive peak was confirmed by reverse phase HPLC analysis. In the adult substantia nigra the molar ratio of dynorphin A(1-17) to dynorphin A(1-8) is approximately 1:15. However, at post-natal day 0 the molar ratio of these forms in the substantia nigra is approximately 0.6:1. By post-natal day 7 the adult ratio of dynorphin A(1-17) to dynorphin A(1-8) has been reached. These observations for the substantia nigra are in contrast to the ontogeny of the pro-dynorphin system in the posterior pituitary as reported by Seizinger et al. (Neuroendocrinology, 39: 414-422, 1984). In the latter terminal field the adult molar ratio of dynorphin A-related products is not reached until post-natal day 21.

Experiments are currently in progress to determine the steady state levels of pro-dynorphin end products at pre-natal time points. Additionally, a parallel study is being conducted on pro-dynorphin products in the striatum. This research was supported by NIMH Grant MH41000 and BRSG Grant RR07138 from the NIH.

346.3 PHOSPHORYLATION OF PROENKEPHALIN-DERIVED PEPTIDES. N.B.D' Souza* and I. Lindberg, Dept. of Biochemistry and Molecular Biology, L.S.U. Medical Center, New Orleans, LA 70112

The opioid peptide precursor proopiomelanocortin has been shown to undergo several biologically important posttranslational modifications during processing, such as phosphorylation, glycosylation, acetylation, and amidation. We are currently studying the biosynthesis of peptides derived from another opioid peptide precursor, proenkephalin. In an effort to determine whether similar post-translational modifications occur within the proenkephalin system, we have investigated the phosphorylation of proenkephalin-derived peptides in bovine adrenal medulla.

Cultured chromaffin cells were incubated with (^{32}P)orthophosphate for 24 h. Cells were then washed and extracted with acid. The acid extract was dried, resuspended in buffer, and centrifuged. Labelled peptides were immunoprecipitated from the supernatant using antiserum to Peptide B (the carboxyl terminal fragment of proenkephalin) and subjected to SDS polyacrylamide electrophoresis in a 15% gel. Two labelled bands of M_r 30 kDa (minor) and 4-6 kDa (major) were observed following autoradiography. Thin layer electrophoresis of the hydrolysis products of labelled peptides (immunoprecipitated and then size-fractionated) demonstrated the presence of labelled phosphoserine rather than phosphotyrosine or phosphothreonine.

We have also analyzed size-fractionated extracts of bovine adrenal medulla using thin layer isoelectric focusing (IEF; pH range 2.5-5.0) in order to detect possible charge differences in endogenous Peptide B-immunoreactive peptides. More than half of total Peptide B-ir peptides were present as more acidic species than Peptide B. Treatment of the adrenal extract with alkaline phosphatase prior to IEF resulted in a shift of immunoreactivity of more acidic to less acidic immunoreactive peptides. Multiple peaks of immunoreactive Peptide B were also observed following HPLC of size-fractionated medullary extracts.

Taken together, the above results provide strong evidence that proenkephalin is phosphorylated at serine residue(s) within its carboxyl terminal portion, Peptide B. Given the importance of phosphorylation as a regulatory event in a large number of biochemical processes, it will be extremely interesting to investigate the control of the phosphorylation of this opioid peptide precursor. (Supported by DK 35199)

346.4 RAT ADRENAL MEDULLARY ENKEPHALINS AND mRNA ARE INCREASED BY DEXAMETHASONE. C.E. Inturrisi, R.D. Howells, S.O. Franklin*, J.R. Shapiro*, S.E. Calvano* and B.C. Yoburn. Dept. of Pharmacology, Cornell Univ. Med. Coll., New York, NY 10021.

The rat adrenal medulla contains low levels of enkephalin-containing (EC) peptides. However, if depolarizing influences are removed by surgical or pharmacological denervation or by culturing medullary explants, there is a dramatic increase in prepro-enkephalin mRNA and EC peptide biosynthesis. In a previous study (Yoburn et al., Life Sci. in press 1987) we found that the denervation-induced rise in rat adrenal medullary EC peptides is blocked by hypophysectomy and partially reinstated by corticosterone, dexamethasone or ACTH treatment. We now report the effect of dexamethasone on EC peptides and preproenkephalin mRNA in adrenal medullary explants (glands) from sham and hypophysectomized (hypox) rats. Culture for 4 days in serum-free medium without dexamethasone resulted in a 13 and 4-fold increase in EC peptide levels in sham and hypox glands, respectively. The addition of dexamethasone (10^{-5}M) produced a 20-26 fold increase in EC peptides in sham and hypox glands to approximately 9 pmole/gland. In serum free medium, hypox glands show a concentration dependent increase in EC peptides with the ED50 for dexamethasone equal to 5.7×10^{-7}M. Since the glucocorticoid antagonist RU486 partially blocked the rise in EC peptides in sham glands, it appears that the increase in EC peptides in sham glands in the absence of dexamethasone is a result of the higher concentration of endogenous corticosterone in sham (27 ng/gld) compared to hypox glands (0.2 ng/gld). Dexamethasone results in an increase in preproenkephalin mRNA in hypox glands after 2 days in culture that is approximately proportional to the increase in EC peptides seen at 4 days. In serum free medium progesterone, testosterone and the mineralcorticoid, deoxycorticosterone failed to increase EC peptides. Enzymatic digestion (trypsin and carboxypeptidase B) and size exclusion chromatography of the extracts from rat adrenal medullary explants indicate that a high molecular weight (18,000) peptide with a (Met)/(Leu)-enkephalin ratio of approximately 6 (i.e., proenkephalin) is the major component of the glucocorticoid-induced increase in EC peptides. These results indicate that a loss of depolarizing stimuli initiates a pretranslational event wich requires glucocorticoid for proenkephalin gene expression and EC peptide biosynthesis. The physiological response of the adrenal medulla to stress includes increases in both transsynaptic impulse activity and glucocorticoid levels. The recognition that both of these factors are capable of regulating EC peptides adds significantly to our understanding of the physiological response to stress and may point the way to the sites of action and therapeutic utility of EC peptides. Supported in part by NIDA Grant DA-01457 and NIGMS Grant GM-34021.

346.5 LOCALIZATION OF POMC mRNA AND β-ENDORPHIN IN THE PRIMATE PINEAL ORGAN. O.K. Ronnekleiv, J. Douglass*, J.P. Adelman*, I. Maslar*. Dept. of Physiology and IABR, OHSU, and Oregon Regional Primate Research Center, Portland, OR 97201

Previous studies have described the presence of α-MSH immunoreactivity in rat pineal extracts (Eskay et al., Brain Res. 178:55, 1979). Recently, we have observed nerve fibers containing vasopressin in the macaque pineal, but we were unable to find nerve fibers containing α-MSH. This would indicate that α-MSH is synthesized in the pineal organ. Therefore, initial studies were designed to determine if the precursor to α-MSH, proopiomelanocortin (POMC), is synthesized in the primate pineal. Pineals from rhesus macaques (N=3) and baboons (N=2), obtained at necropsy, were fixed in 4% paraformaldehyde, sectioned at 10 μm and mounted on poly-L-lysine coated slides. Six additional pineals (4 rhesus and 2 baboons) were enzymatically dispersed and the cells cultured for 3-6 days, at which time the cells were fixed in paraformaldehyde. Tissue sections and cultured pinealocytes were first reacted immunohistochemically for β-endorphin (β-End) using the Avidin Biotin method. Next, cells and tissue sections were hybridized with ^{35}S-labelled POMC mRNA full length probe for 20 h at 55°C and then washed to a final stringency of 0.25 X SSC at 55°C. Emulsion autoradiography revealed specific hybridization of the probe to POMC mRNA in the cytoplasm of cultured pinealocytes. The same cells were also immunoreactive to β-End. On pineal tissue sections clusters of cells had high concentrations of silver grains over the cytoplasm indicating the presence of POMC mRNA. These studies indicate that a subpopulation of pinealocytes within the pineal organ of primates contains mRNA coding for POMC. We are investigating the processing of POMC in the primate pineal.
(Supported by PHS grants HD 16793 and RR 00163)

346.6 REGULATION OF ENKEPHALIN AND SUBSTANCE P mRNA'S BY EXOGENOUS OPIOIDS A. Tempel, J.A. Kessler and R.S. Zukin. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Long-term blockade of brain opioid receptors by the opiate antagonist naltrexone produces marked increases in Met-enkephalin in the striatum and nucleus accumbens (94% and 45%, respectively). We determined by RNA blot analysis that these changes in peptide levels were the result of increased peptide synthesis. Levels of proenkephalin mRNA in striata of chronic antagonist-treated rats were increased approximately 25-fold relative to that of control animals. In order to determine the regional specificity of this increase, levels of proenkephalin mRNA in antagonist-treated and control rat brain regions were determined by RNA blot analysis. The mRNA corresponding to preproenkephalin was identified using a riboprobe made using the rat cDNA hybridization probe complementary to preproenkephalin mRNA (J. Roberts, Columbia Univ.) and RNA blot analysis. The level of proenkepahlin mRNA in the hypothalamus of chronic antagonist-treated rats was increased approximately 3-fold relative to that of control animals. No significant changes were found in the hippocampus or frontal cortex. The time course of the increase in proenkephalin mRNA levels was examined. Large increases in message levels were seen as early as 24 hours following antagonist treatment. This is in contrast to changes in opioid receptor density which take 3-4 days to reach half maximal upregulation following chronic antagonist treatment. Recent evidence has suggested that substance P is regulated by opioid peptides. In order to determine substance P mRNA levels in striata of control and drug-treated animals, the mRNA corresponding to the precursor for substance P was identified using a probe for exon-7 of the preprotachykinin gene (T. Bonner, NIMH). The level of substance P mRNA in the striatum was increased 14-fold after chronic antagonist treatment relative to that in the striatum of control animals. By contrast, no statistically significant change was observed in striatal mRNA for actin. In summary, these data suggest that chronic blockade of brain opioid receptors leads to the increased synthesis of both enkephalin and substance P in the striatum, and that these changes are relatively specific. The finding of a significant change in substance P levels supports the concept of a role for enkephalin in the regulation of substance P. (Supported by NSF grant BNS 8308634; NIH grants DA01843, DA00069, and NS21973).

346.7 DISTRIBUTION OF OPIOID PEPTIDES AND mRNA IN GUINEA PIG ADRENAL MEDULLA. K.L. Valentino, J. Hunter*, L. Tecott, C.J. Evans and J.D. Barchas. Nancy Pritzker Laboratory, Department of Psychiatry and Behavioral Sciences, Stanford University, Stanford, California 94305.

Peptides from both proenkephalin and prodynorphin precursors have been shown to coexist at the cellular level in guinea pig adrenal medulla. The two precursors are differentially regulated by dexamethasone treatment: proenkephalin mRNA levels increased two-fold, with a slight increase in peptide levels, and prodynorphin mRNA and peptide levels showed no change (Eberwine et al., Soc. Neurosci. Abstr. 11:857, 1985).

We decided to further investigate this system using the techniques of immuno-electron microscopy and in situ hybridization. Adrenals from either normal or dexamethasone treated (100 mcg/kg i.p.) male Hartley Albino guinea pigs were removed, fixed by immersion and either processed through LR White or Epon resins for electron microscopy or frozen and sectioned on a cryostat for in situ hybridization and immunohistochemistry. Antibodies were raised in rabbits and mice against the proenkephalin peptide met-enk-arg-gly-leu (MeRGL) and the prodynorphin peptide dynorphin B. Secondary antibodies were conjugated to 5 nm and 15 nm gold (Janssen Pharmaceutica). Oligonucleotide probes for proenkephalin and prodynorphin mRNAs were synthesized (BioSearch) and labeled with ^{32}P or ^{35}S.

The distribution of the two peptides in normal adrenal medulla was as follows: dynorphin B immunoreactivity was present in the vast majority of medullary cells, whereas MeRGL immunoreactivity was found in a smaller proportion of cells. MeRGL cells usually contained dynorphin B as well, and this colocalization occurred at the secretory granule level. We then set about answering the following questions: Does the distribution of proenkephalin and prodynorphin mRNA parallel the distribution of the peptides and does this distribution change after dexamethasone treatment? Is there any change in the ultrastructural localization of MeRGL following dexamethasone treatment?

Preliminary EM experiments have shown ultrastructural changes in the dexamethasone-treated adrenal medulla. Some cells appear to be depleted of granules and contain large membrane-bound structures. Other cells seem to have larger amounts of MeRGL in secretory granules than normal, although the number of MeRGL containing cells appears the same. Amounts of dynorphin B immunoreactivity appear similar to normal. A small percentage of cells have very dark, condensed cytoplasm and appear to be dying. Further EM studies and in situ hybridization experiments are in progress to confirm these results.

Supported by NIDA grant DA 01207.

346.8 PROOPIOMELANOCORTIN GENE EXPRESSION IN RAT PITUITARY DURING DEVELOPMENT: AN IN SITU HYBRIDIZATION STUDY. H. Khachaturian[1], S.P. Kwak*, M.K.-H. Schafer*, and S.J. Watson. Mental Health Res. Inst., Univ. Michigan, Ann Arbor, MI 48109; [1]Dept. Anatomy & Neurobiology, Univ. Tennessee, Health Sci. Ctr., Memphis, TN 38163.

Proopiomelanocortin (POMC) codes for several bioactive peptides which are important not only in the adult neuroendocrine function, but also during development. Specifically, during embryogenesis POMC peptide immunoreactivity can be detected in pituitary corticotrophs from day E15 and in intermediate lobe cells from E16. The existence of POMC mRNA has also been shown in rat pituitary as early as E17 (Lugo and Pintar, Soc. Neurosci. Abstr. 11:142, 1985). In the present study, we have further addressed the question of early POMC mRNA detection in rat pituitary during pre- and postnatal development.

Embryonic (E) and postnatal (P) rats from days E14, E15, E17, E19, P1, P7, P14 and P21 were decapitated and the pituitaries were postfixed in 4% formaldehyde. All tissues were frozen in liquid nitrogen. Ten micron cryostat sections were mounted onto polylysine-coated slides. Some sections were processed for PAP immunocytochemistry using an antiserum to ACTH. Other sections were processed for in situ hybridization. Riboprobes for mouse POMC (cRNA, a generous gift from J. Roberts, Columbia Univ.) were prepared in SP6 plasmid. The plasmid was transcribed in the presence of [^{35}S]UTP yielding a probe length of 460 bases, which was used for in situ hybridization and autoradiography as described (Lewis et al., Proc. Natl. Acad. Sci. 83:5419, 1986).

The results were as follows: no autoradiographic labelling was observed on day E14, but grains were seen over anterior lobe corticotrophs on E15. This observation corresponds well with the earliest detection of ACTH immunoreactivity in anterior lobe corticotrophs on E15. At E17 and E19 labelling was seen in both the anterior and intermediate lobes of the pituitary corresponding with the pattern of ACTH immunoreactivity in both lobes. From P1 to P21, labelling was progressively "diluted" in both lobes of the pituitary, and ACTH staining was likewise progressively weaker in later postnatal stages. This progressive diminution can best be explained by the fact that the pituitary expands rapidly during development, and thus labelling per unit area drops as a result of expansion. Finally, the observation that both in situ hybridization and immunocytochemistry detect mRNA and peptide labelling, respectively, on the same day (i.e., E15), suggests that POMC gene transcription and subsequent translation occur within a very short time in the pituitary during embryogenesis.

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BLOOD-BRAIN BARRIER II

347.1 CENTRAL NERVOUS SYSTEM UPTAKE OF Ca-45 IN RATS FED CALCIUM DEFICIENT DIET. V. A. Murphy and S. I. Rapoport. Laboratory of Neurosciences, NIA, NIH, Bethesda, MD 20892.

Brain and cerebrospinal fluid (CSF) [Ca]'s decrease far less than concurrent decreases in plasma [Ca]'s in developing rats fed Ca deficient diets (Murphy, V.A. et al., J. Neurochem., 47:1735, 1986). Because plasma [Ca] is reduced for at least 4 weeks in such animals, stability of central nervous system [Ca] suggests an active regulatory mechanism. We therefore decided to measure the unidirectional transfer coefficients for Ca-45 from plasma to brain and to CSF, to see if regulation of Ca transfer into the central nervous system is responsible for Ca homeostasis.

Male Fischer-344 rats, 3 weeks old, were divided into 3 groups: (1), fed diet containing 0.01% w/w Ca (LOCA); (2), fed diet containing 0.67% Ca (CONT); and (3), fed reduced amounts of CONT diet to match the diet intake of anorexic LOCA rats (RCONT). After 8 weeks on the diets, mean weights were LOCA, 105 g; CONT, 221 g; and RCONT, 114 g. Plasma ionized [Ca] was significantly less in LOCA rats (0.80 mmol/l), than in CONT (1.32) or RCONT (1.28) rats.

The right femoral artery and vein were catheterized under ketamine anesthesia and the rats were allowed to recover for 5 h under partial restraint. Ca-45 and Cl-36 were injected i.v. and allowed to circulate for 10 min before killing the animal. H-3 raffinose was injected i.v. 3 min before death to determine blood volume. Serial blood samples were taken over the 10 min period and centrifuged. Transfer coefficients were calculated by dividing dpm/g CSF or brain (corrected for residual blood) by integrated plasma radioactivity times ionized fraction of isotope in plasma.

CSF transfer coefficients for both Ca-45 and Cl-36 in LOCA (419 and 528 nl/g/s) and RCONT (274 and 474) rats were significantly higher than CONT (226 and 412) rats ($p<0.05$). The LOCA value for Ca-45 was also significantly above the RCONT value. Brain regions close to the ventricles (eg, hippocampus) had elevated transfer coefficents for Ca-45 in LOCA (48.0) compared to CONT (34.3) and RCONT (29.1) rats, but more distant brain regions (eg, parietal cortex) had transfer coefficients that were not significantly different among the groups (8.3, 8.3, and 9.0, respectively). Brain transfer coefficients for Cl-36 did not differ among the groups.

The results indicate that the transfer coefficient for Ca-45 into CSF is elevated in LOCA, and that this elevation contributes to stability of both CSF and brain [Ca]'s. The increased transfer coefficient for Cl-36 into CSF was probably due to diminution of CSF volume rather than to increased uptake because only very small volumes (about 0.015 ml) could be obtained from LOCA animals. It thus appears that the choroid plexus is the major site contributing to Ca homeostasis of the central nervous system during chronic hypocalcemia.

347.2 THE GLUCOSE TRANSPORTER OF THE BLOOD-BRAIN BARRIER IN EXPERIMENTAL DIABETES MELLITUS. S.I. Harik, S.A. Gravina* and R.N. Kalaria, Department of Neurology, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Recent evidence suggests that hyperglycemia has deleterious effects on ischemic brain injury in man and experimental animals, possibly due to the production of high levels of brain lactic acid and the resultant tissue acidosis. However, there is controversy as to whether sustained hyperglycemia in man or animals is associated with a change in the transport capacity (Tmax) or affinity (Km) of blood-to-brain glucose transport. We assessed this question directly by measuring the number of glucose transporter molecules in the endothelium of brain microvessels, which constitutes the blood-brain barrier, using specific [3H]cytochalasin B binding to cerebral microvessels obtained from streptozotocin-treated rats and from normoglycemic controls.

Male Wistar rats were used in all studies. Diabetes mellitus was induced in the experimental group by intravenous injection of 60 mg/kg of streptozotocin. Control rats received the vehicle solution. The streptozotocin-treated rats developed polyuria and hyperglycemia (mean plasma glucose ~ 32 mM) and they failed to gain weight. Control rats had mean plasma glucose of ~ 9 mM. Rats were killed 2 weeks later and their cerebral microvessels harvested from the cortical mantles by bulk separation. Specific [3H]cytochalasin B binding to brain microvessels was estimated by subtracting nonspecific binding in the presence of 0.3 M D-glucose from total binding in the presence of 0.3 M L-glucose. D-glucose-displaceable [3H]cytochalasin B binding to microvessels from both groups was saturable. Scatchard analyses yielded linear plots indicating a single class of binding sites. The maximal number of binding sites (Bmax) in the control group was 61 pmol/mg protein while that of streptozotocin-treated diabetic rats was 76 pmol/mg protein; a 20 % increase in the number of glucose transporters in brain microvessels of diabetic rats. The dissociation constant of binding (Kd) was similar in both groups at ~ 0.7 μM.

These results support our earlier in vivo findings that blood-to-brain glucose transport is not decreased in chronic hyperglycemia (Harik S.I. et al., Soc. Neurosci. Abstr. 12:1260, 1986).

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347.3 THE GLUCOSE TRANSPORTER OF THE HUMAN BLOOD-BRAIN BARRIER.
R.N. Kalaria, S.A. Gravina*, J.W. Schmidley* and S.I. Harik, Dept. of Neurology, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106.

We used cytochalasin B as a ligand to identify and characterize the glucose transporter moiety in preparations of human cerebral microvessels, choroid plexus and P2 fractions of the frontal cerebral cortex. Tissues were obtained at autopsy from victims of motor accidents, and microvessels obtained by bulk isolation. Specific [3H]cytochalasin B binding to tissues was estimated by subtracting non-specific binding in the presence of 300 mM D-glucose from total binding in the presence of 300 mM L-glucose. D-glucose-displaceable [3H]cytochalasin B binding was saturable. Scatchard analysis yielded linear plots indicating a single class of noninteracting binding sites. Maximal binding (Bmax) of microvessels, choroid plexus and P2 fractions were 42.3 ± 4.2, 26.7 ± 2.6 and 7.0 ± 0.8 pmol/mg of tissue protein (means ± SEM). The dissociation constant of binding (Kd) to all tissues was similar at about 0.4 μM. Irreversible D-glucose-displaceable [3H]cytochalasin B binding after UV photoactivation, followed by SDS-PAGE enabled us to estimate the apparent molecular weight of the glucose transporter. The peak of radioactivity appeared between 55-50,000 daltons.

We also used monoclonal antibodies (IgM) to the glucose transporter of human erythrocyte ghosts to localize the glucose transporter in human frontal cerebral cortex. These immunocytochemical studies corroborated our binding studies by revealing a very high density of the reaction product in brain capillaries and larger blood vessels, with only little background reaction. In separate studies, we also demonstrated that the monoclonal antibody abolished specific cytochalasin B binding to cerebral microvessels by more than 90 %. Studies are now in progress to localize the glucose transporter in brain microvessels at the ultrastructural level.

These studies corroborate and extend our previous findings in experimental animals which showed that brain capillary endothelium, which consistutes the blood-brain barrier, is richly endowed with the glucose transporter (Dick, A.P.K. et al, PNAS 81:7233-7237, 1984; Dick, A.P.K. and Harik, S.I., J. Neurochem. 46:1406-1411, 1986.). This is reasonable in view of the fact that endothelial cells, which constitute less than 0.1 % of the brain weight, have to transport glucose for the whole brain.

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347.4 RECOVERY OF $5HT_2$ BINDING SITES IN AGED BRAIN BY EXPOSURE OF HUMAN BRAIN MEMBRANE FRAGMENTS TO 0.1 M GLYCINE.
A.C. Andorn and M.A. Pappolla* Dept. of Psychiatry, Case Western Reserve Univ. Schl. Med., Cleve. Metro. Gen. Hosp., Cleve. OH 44109 and Dept. of Pathology, FDR Veterans Admin. Hosp., New York Medical College, Montrose, N.Y. 10548.

Serotonin$_2$ ($5HT_2$) receptor density is decreased in aged human brain (Reynolds, G.P. et al., Neurosci. Letts. 44:47, 1984). We reported that serum proteins inhibit ligand binding at human brain cortical neurotransmitter binding sites including $5HT_2$ as labeled with [^{3}H]spiroperidol (SP) (Andorn, A.C., Synapse, 1:82, 1986). We also showed that serum proteins can be seen immunocytochemically in aged, but not young, brain (ibid). We hypothesized that the decrease in receptor density observed in aging was secondary to leakage of serum proteins through a compromised blood brain barrier (BBB). As a first test of this hypothesis, we exposed tissue sections of brain staining positive for serum proteins, to 0.1 M glycine (ph 2.5 at 40C) for up to 3 hrs at room temperature (RT). Immunocytochemical studies showed that serum proteins were effectively removed from the tissue sections by this method (not shown). This method is also valid for the removal of proteins bound with high affinity L(antibody reactions). We also exposed membrane fragment preparations (MFP) from young and aged brain to the glycine buffer for 60 min at RT prior to use in the standard assay of [^{3}H]SP (0.2 nM) binding in cortex (Andorn, A.C., Life Sci., 38:1251, 1986). Specific binding (SB) in MFP preincubated in glycine was compared to SB in MFP preincubated in assay buffer for 60 min at RT. Cortical MFP from 4 brains (aged 18-41) preincubated in assay buffer had 80.8 +/-9.9 fmol/mg protein SB (N=5). The SB in the companion MFP preincubated in the glycine buffer was 38.6 +/- 18.7 fmol/mg. The decrease in SB was accounted for by both an increase in non-specific binding (NSB) as determined in the presence of 10 uM haloperidol, and by a decrease in total binding. In contrast, 3 aged brains (ages 78-80) demonstrated 49.8 +/- 18.1 fmol/mg SB after exposure to assay buffer in 71..9 +/- 39.0 fmol/mg SB after exposure to glycine buffer (N=8). NSB in young and aged brain was the same, as determined by Student's t-test, within each condition. The statistically significant increase in SB in aged brain ($p=0.05$) was most likely due to an increased B_{max} since the K_D of the multiple [^{3}H]SP binding sites in cortex are not affected by age or glycine preincubation (not shown). These findings are consistent with the hypothesis that the apparent decline in receptor density seen in aging may be due in part, to a masking effect of putative serum or other proteins which are abnormally present in aged brains due to a compromise of the BBB.

347.5 ALLOGRAFTS OF CNS TISSUE DO EXHIBIT A BLOOD-BRAIN BARRIER TO PROTEIN.
R. Broadwell, M. Charlton*, M. Salcman, and R. Schlegel*. University of Maryland School of Medicine, Balto., MD, 21201.

A recent article in **SCIENCE** (235:772, 1987) suggested that neonatal rat cortex transplanted into the IVth ventricle or near the cortical surface of adult rats failed to exhibit a blood-brain barrier (BBB) to blood-borne endogenous and exogenous proteins. If this observation is correct, absence of a BBB in transplanted brain tissue, coupled with a host immune response for tissue rejection, could complicate potential clinical application of CNS transplants in the treatment of neuro-degenerative disorders. Although the mammalian BBB is not absolute to circulating, non-lipid soluble macromolecules, results from our laboratory strongly advocate the presence of a BBB to blood-borne protein within intracerebral allografts of the mammalian CNS. Brains of fetal day 13 to term mice possess a well-developed blood supply and a BBB to intravenously injected HRP. Blocks of the parietal cortex or basal forebrain from fetal/neonatal mice were placed stereotaxically into the IIIrd ventricle or basal ganglia deep to the cortical surface of adult mice for 1 day to 6 months. Systemically administered HRP failed to enter the parenchyma of CNS allografts or that of the host brain from blood vessels within allografts. Blood-borne HRP exiting fenestrated vessels supplying the hypothalamic median eminence of the host spread extracellularly into the ventralmost portion of all allografts positioned within the IIIrd ventricle. Blood vessels intrinsic to the CNS allografts were sustained and anastomosed with host blood vessels at the allograft-host interface for perfusion with host blood (red cells) and blood-borne HRP between 7-10 days post-transplantation. Ultrastructural inspection of allograft blood vessels at all post-transplantation times confirmed that the endothelial cells exhibit BBB characteristics (e.g, interendothelial tight junctions, absence of transendothelial vesicular transport, lysosomes, etc.). Adult pituitary tissue, which contains fenestrated capillaries that normally are leaky to blood-borne HRP, transplanted intracerebrally in our animals did not develop a BBB and permitted HRP delivered systemically to the host to enter the pituitary allograft and surrounding host brain parenchyma. The results suggest that blood vessels indigenous to solid allografts of fetal, neonatal, or adult mammalian tissue, whether neural or non-neural, sustain their normal characteristics and eventually connect with host blood vessels. The mammalian CNS can be expected to possess a BBB to circulating protein when grafted as solid tissue within or outside the CNS. Supported by NIH/NINCDS Grant #NS 18030.

347.6 TRANSCYTOSIS OF PROTEIN THROUGH THE CEREBRAL ENDOTHELIUM AND CHOROID PLEXUS EPITHELIUM.
B. Balin*, R. Broadwell, M. Salcman. University of Maryland School of Medicine, Balto., MD, 21201.

Transcytosis (endocytosis,transport, exocytosis) of protein through cerebral endothelia and choroid plexus epithelia was investigated in mice injected intravenously or into the cerebral ventricles with native HRP or HRP conjugated to the lectin wheat germ agglutinin (WGA); post-injection survival times were 5mins-24hrs. Native HRP enters cells indiscriminately by fluid phase endocytosis, whereas WGA-HRP binds to oligosaccharides on the cell surface and is internalized by adsorptive endocytosis. Blood vessels throughout the cerebral vascular tree were visible with the blood-borne tracers at 5mins. Native HRP-labeled organelles within the endothelium included endocytic vesicles, endosomes, tubules, multivesicular and dense bodies. Acid phosphatase cytochemistry revealed that a population of the tubules, multivesicular and dense bodies are representative of secondary lysosomes. Transcytosis of native HRP from luminal to abluminal sides of the endothelium did not occur, and the peroxidase-positive tubules did not form para-junctional channels. Blood-borne WGA-HRP, in addition to labeling the same organelles as those seen with native HRP, appeared within the Golgi complex at 3hrs. By 6-24hrs, WGA-HRP was observed on the abluminal surface of the endothelium and within the perivascular clefts and pericytes. Pericyte organelles sequestering WGA-HRP were identical to those in the endothelium. Cerebral endothelia exposed abluminally to native HRP and WGA-HRP delivered into the ventricles failed to internalize the tracer proteins significantly through 24hrs, suggesting that endocytosis at the abluminal plasmalemma is not demonstrable. Choroid plexus epithelia exposed to blood-borne native HRP and WGA-HRP for 5mins-24hrs contained peroxidase-positive endocytic vesicles, endosomes, tubules, multivesicular and dense bodies; the latter three types of organelles were acid phosphatase-positive cytochemically. Transcytosis of the two blood-borne tracers through the choroid epithelia was not observed. Similar results were obtained with native peroxidase injected intraventricularly. Conversely, transcytosis of cerebrospinal fluid-borne WGA-HRP through the choroid epithelia from the microvillus border to the basolateral surfaces occurred within 10mins post-injection. This transcytosis of WGA-HRP appeared at a time prior to WGA-HRP labeling of the Golgi complex. The results suggest that blood-borne protein entering the endothelium by the process of adsorptive endocytosis can undergo transcytosis through the intact blood-brain barrier without compromising the integrity of this barrier; the cerebral endothelium appears to be polarized with regard to the internalization of its cell surface membrane. Whether or not the choroid epithelium is polarized concerning the transcytosis of protein entering the cell by adsorptive endocytosis remains equivocal at this time. Supported by NINCDS #NS 18030.

347.7 FREE FATTY ACIDS EFFECT ON CEREBROMICROVASCULAR ENDOTHELIAL AND SMOOTH MUSCLE CELL PERMEABILITY: IN VITRO STUDIES. A. Villacara, O. Kempski, J. Bembry and M. Spatz. LNNS, NIH, Bethesda, Md. 20892

The release of free fatty acids from cellular membranes has been implicated in attenuating the blood-brain barrier (BBB) permeability under pathological conditions. Recently we adopted a new in vitro system consisting of cerebromicrovascular endothelium grown on dextran microcarriers to study their properties related to the function of BBB under controlled conditions (Kempski and Spatz in press 1987). This method utilizes the fact that cell covered beads prevent accumulation of dyes like trypan blue(TB) in the microcarriers. Any increase in cellular permeability to TB results in reduced level of TB in the supernatant since TB that passes through the cellular layer binds tightly to the dextran beads.

The aim of this investigation has been to use this model for evaluating the effects of free fatty acids on the permeability of separately cultured endothelial and smooth muscle cells derived from cerebral microvessels (Spatz et al Brain Res, 191:577-582, 1980; 280:387-391, 1983).

Washed samples of endothelium or smooth muscle cells grown on microcarriers as well as empty beads were incubated with various concentration of palmitic, linoleic or arachidonic acid (20-100μM) in phosphate buffer (PBS) containing serum albumin and glucose (0.1%) while the controls were exposed to the solvent solution in the same buffer at pH 7.4 for 30 min at 37°C. Thereafter each sample was equilibrated with TB albumin [serum albumin fatty acid free (0.2% TB)] phosphate buffer and glucose (0.1%) at pH 7.4. Aliquots of supernatant were removed at short intervals (15 sec - 19 min). The TB concentration in the supernatant was measured with the use of spectrophotometer at a wavelength of 580nm.

Both cell types excluded TB equally. There were no significantly different changes between the TB permeability of endothelium and smooth muscle cells exposed to either arachidonic or linoleic acid. Arachidonic acid leads to a 5-fold while linoleic acid leads to a 4-fold increase in cellular permeability to TB. On the other hand, palmitic acid caused little change but TB permeability of the smooth muscle appeared greater than that of the endothelial cells. Each of these changes could be reversed when the cells were washed after their incubation with free fatty acids but prior to the trypan blue albumin exposure.

These results support the contention that free fatty acids can alter the permeability of cerebromicrovascular cellular elements and thus the function of BBB.

347.8 ALKALINE PHOSPHATASE STAINING OF LEAKY BLOOD VESSELS IN HUMAN POST MORTEM AREA POSTREMA AND CHOROID PLEXUS. M.A. BELL, D.M. MOODY‡ J.N. ANGELO‡ V.R. CHALLA* AND T.C. JOHNSTON‡ Departments of Radiology and Pathology, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

In a study of post mortem human brain sections (100-500 um celloidin and 2 um glycol methacrylate) we have observed strong Gomori alkaline phosphatase staining of the endothelial lining of area postrema and choroid plexus vessels. Alkaline phosphatase is thought to assist transmural transport of nutrients, metabolites or ions. It appears in small exchanging vessels (arterioles and capillaries) throughout the brain, so its appearance in these two areas would not be remarkable except for previous postulates that the enzyme is associated with blood-brain barrier (BBB) functions; the absence of a BBB in these two specialized (leaky) vascular beds distinguishes them from the tight junction vessels characteristic of most other brain regions.

Our observation and other arguments suggest that endothelial BBB and alkaline phosphatase functions may coexist, but are not strongly interdependent or identically distributed along the microvasculature, to wit: 1) alkaline phosphatase activity has here been demonstrated in leaky area postrema and choroid plexus vessels; 2) it has been demonstrated (by one of us, MB, and others) in human or mammalian capillaries of peripheral nerve (a less restrictive barrier system than the brain's), skeletal muscle (no specialized barrier), and dorsal root ganglia (leaky fenestrated vessels); 3) most brain venules, while possessed of a BBB, do not stain for alkaline phosphatase; 4) rabbit brain vessels, while presumably tight, do not stain for alkaline phosphatase at all. Perhaps barrier and phosphatase transport functions are cooperatively rather than intrinsically related.

The discrepancy between our phosphatase positive vessels in area postrema and choroid plexus and the phosphatase negative ones reported there in detailed EM studies by Vorbrodt et al (Acta neuropathol. (Berl.), Suppl. VIII: 43, 1983; Dev. Neurosci., 8:1, 1986) is puzzling. Species differences, pathological conditions, post mortem effects, or methodological differences might be invoked.

347.9 WIDESPREAD DISTRIBUTION OF TRANSFERRIN IMMUNOREACTIVITY IN HUMAN BRAIN. A.J. Dwork*, E.A. Schon*, and J. Herbert* (SPON: S. DiMauro). College of Physicians and Surgeons of Columbia Univ., New York, NY 10032

Non-pathological sections from five adult autopsy brains were examined immunohistochemically for transferrin (Tf) using the avidin-biotin-peroxidase complex technique. Specificity of primary antibody was confirmed by immunoblot. Intensity of staining and number of stained cells varied from case to case, but the anatomical distribution was similar in all cases. Neuropil stained most heavily in iron-rich areas: globus pallidus, substantia nigra, dentate nucleus, and to a lesser extent, corpus striatum. Virtually all neurons of cranial nerve nuclei, substantia nigra, locus ceruleus, and nucleus basalis were intensely positive for Tf, compared to a moderate number in hippocampus and few or none in neocortical areas. Staining of Purkinje cells was quite common. Selected astrocytes, oligodendrocytes, ependymal cells, and choriod plexus epithelial cells were strongly immunoreactive. Subependymal astrocytes stained with particular frequency and intensity. Staining of neurons and glia generally involved cell bodies and processes, but not nuclei. Whether this widespread distribution of Tf represents local synthesis or uptake from plasma or CSF remains to be determined.

Our results show immunoreactivity that is more abundant and involves more cell types than that which has been reported in fetal and newborn humans and that differs in cellular distribution from that reported in adult rat. Ontogenetic and phylogenetic differences evidently exist.

347.10 ANIMAL SUSCEPTIBILITY TO 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE NEUROTOXICITY VARIES INVERSELY WITH MONOAMINE OXIDASE ACTIVITY IN THEIR BRAIN MICROVESSELS. N. J. Riachi*, S. I. Harik, R. N. Kalaria and L. M. Sayre* (SPON: R. B. Daroff), Departments of Neurology and Chemistry, Case Western Reserve University, Cleveland, OH 44106.

Systemic 1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP) causes parkinsonism in man and other primates, but not in rats; while mice are intermediate in their susceptibility which varies among strains. Since MPTP selectively lesions dopamine neurons when infused directly into rat substantia nigra (Harik, S. I. et al, JPET, 1987), we suspected that the rat's resistance to systemic MPTP is due to inability of MPTP to reach the brain, possibly because of unique capability of the rat "biochemical" blood-brain barrier (BBB). We then discovered that rat brain microvessels, which constitute the BBB, have unusually high monoamine oxidase (MAO) activity while human brain microvessels were deficient and mice microvessels had intermediate MAO activity (Kalaria, R. N. et al, PNAS, 1987). We therefore hypothesized that the rat's resistance to systemic MPTP toxicity is likely due to the rapid metabolism in brain capillaries of MPTP to MPP+, a water-soluble cation that would have difficulty in traversing biological membranes.

We tested this hypothesis by: (i) studying MPTP metabolism, in vitro, by human, rat and mouse brain microvessels, and (ii) following MPTP metabolism, in vivo, in the brain and liver of Wistar rats and 2 strains of mice known to react differently to systemic MPTP. We find that rat brain microvessels are extremely efficient at converting MPTP to MPP+ and that this conversion is totally inhibited by pargyline. Microvessels from C57 black mice, which are more sensitive to MPTP toxicity than white CFl mice, were less capable of metabolizing MPTP to MPP+. Human microvessels were least capable of producing MPP+.

In vivo metabolism of MPTP in Wistar rats and the 2 strains of mice showed that 1 hour after MPTP injection, rats had the least amount of MPTP and its metabolites in their striata and that white mice were intermediate between rats and black mice, with black mice having the highest concentration of MPTP and its metabolites. On the other hand, liver metabolism of MPTP by the 3 groups showed highest levels of MPTP metabolites in rats and lowest levels in black mice.

These results correlate differences in susceptibility to systemic MPTP neurotoxicity to the ability of the BBB and other tissues like the liver to metabolize MPTP and prevent it from reaching its sites of toxicity within the brain.

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348.1 ABNORMAL NEURONAL MORPHOLOGY IN THE BRAIN OF AN AIDS-INFECTED CHILD: A GOLGI STUDY. Ronald F. Mervis[1], Carl Boesel*[1], Marc Rosenblum*[2], and Richard W. Price*[2] (SPON: A.J. Yates). [1]Div. of Neuropathology, The Ohio State Univ. Med. Ctr., Columbus, OH 43210, and [2]Memorial Sloan-Kettering Med. Ctr., New York, NY 10021.

Infants and adults with the acquired immune deficiency syndrome (AIDS) frequently develop a progressive encephalopathy which is accompanied by neurological, motor, and behavioral dysfunction. Although secondary opportunistic infections accompanying AIDS may be responsible for some of the findings, there is substantial evidence that HTLV-III infection of the CNS is a causative agent. The neuroanatomical basis of the disorder is poorly understood. The virus itself has not been clearly shown to infect neurons directly, and only on rare occasion has evidence implicated its presence in astrocytes or oligodendroglia. Here, we report the effects of confirmed AIDS infection of the CNS on the neuronal morphology of a two year-old infant with severe developmental disabilities who died of the disorder.

He was born full term and was initially normal until 7 months-old when he failed to reach normal developmental milestones. At two years he was shown by CT scan to have cerebral atrophy. At autopsy, neuropathology tissue sections stained with Nissl and hematoxylin and eosin showed gliosis and multinucleated giant cells in the brain. Golgi staining of cerebellum showed Purkinje cells that were generally poorly branched and sparsely spined. Purkinje cells that were atrophic or had distorted soma were frequently seen. In the neocortex, the dentritic trees and soma of pyramidal cells were similarly affected. Dendritic branches were swollen or otherwise distorted with varicosities. Spines were generally fewer in number and often grossly enlarged or misshapen. These observations suggest that neurological and behavioral dysfunction in this AIDS-infected infant are accompanied by widespread abnormalities of neuronal structure. In view of the apparent absence of secondary opportunistic infection, the findings described here could be attributed to AIDS infection of the brain.

(Supported by the Department of Pathology of the Ohio State University College of Medicine.)

348.2 Gamma-Interferon (G-IFN) May Mediate Central Nervous System (CNS) Disease in HIV-1 Infection

Sheremata WA, Bohn N,* Resnick L,* Berger J,* Sazant A.*

The pathogenesis of CNS disease complicating HIV-1 infection is not explained. As alternative to direct infection of CNS cells, we have hypothesized that g-IFN may mediate such disease. G-IFN (an immuno-modulatory lymphokine) is a potent activator of macrophages, can induce Class I and II MHC antigens and enhance a variety of immune responses. We studied paired serum and CSF of 8 normals, 10 with HIV sero-conversion, and 10 other CNS disorders including 4 with myelopathies. Using a double antibody assay radio-immune assay (RIA) modified for detection of very low g-IFN levels we found very low level serum antibody in 7/10 HIV sero-converters (confirmed by Western blot) and 1/2 tropical spastic paraplegia patients, but failed to find detectable levels in normals serum. Low cerebrospinal (CSF) levels were found in all subjects, however. Values ranged from 0.18 to 0.42 U/ml in normals and from 0.24 to 1.40 U/ml in HIV + patients. Values were higher in those with absent or minimal neurological disease. Two HIV + patients had increased levels of serum alpha-IFN and 2 others had increased alpha in CSF as compared with normals and controls. This study provides preliminary evidence that CNS disease with HIV infection may be mediated by inappropriate g-IFN production early in disease. However, a prospective longitudinal study of sero-negative subjects and early sero-converters will be needed to further evaluate the role of g-IFN in HIV related CNS disease.

348.3 GLYCOLIPID MODIFICATION OF THE SCRAPIE PRION PROTEIN. N. Stahl*, D. R. Borchelt*, K. K. Hsiao, and S. B. Prusiner (SPON: B. Libet). Dept. of Neurology, University of California, San Francisco, CA 94143-0518.

We are investigating posttranslational modifications of the scrapie prion protein that may play a role in scrapie pathogenesis and explain the differences in physical properties between PrP^{C} and PrP^{Sc}. Several lines of evidence indicate that the scrapie prion protein is posttranslationally modified with a phosphatidylinositol-containing glycolipid that shares many properties with the glycolipids found on the carboxy termini of trypanosome variant surface glycoprotein (VSG), Thy-1, and acetylcholinesterase. We have used GC/MS to identify ethanolamine, P_i, inositol, and stearic acid in acid hydrolysates of purified PrP 27-30, the protein that is derived from PrP^{Sc} by limited proteolysis. These compounds are found associated with PrP 27-30 despite boiling the protein in SDS followed by repeated extraction with chloroform/methanol, or purification of PrP 27-30 from gel slices after SDS-PAGE. Gel-purified PrP 27-30 contains 2-3 moles of ethanolamine per mole of protein when analyzed by reverse-phase HPLC of acid hydrolysates after derivatization with phenyl isothiocyanate. GC analysis of trimethylsilylated fatty acids released from purified PrP 27-30 by 1 N NaOH at 65°C after extraction with chloroform/methanol indicates the presence of 5 different species of fatty acids dominated by palmitate. However, gel-purified PrP 27-30 contains mostly stearic acid, suggesting that the other fatty acid species are attached to a contaminating component that purifies with PrP 27-30. Furthermore, the amount of stearic acid observed decreases after digestion of purified PrP 27-30 with a bacterial phosphatidylinositol-specific phospholipase C (PI-PLC), while the amount of palmitate does not change. This suggests that the PrP glycolipid contains only stearic acid. After PI-PLC digestion, PrP 27-30 crossreacts on Western blots with antisera raised against the PI-PLC treated glycolipid present on VSG. Exposure of primary cell cultures derived from neonatal hamster brain to PI-PLC results in the release of >90% of their PrP^{C} into the media when measured by Western blotting or immunoprecipitation of [^{35}S]-labeled protein. This release by PI-PLC is not proteolytic because the media contains less than 10 [^{35}S]-labeled bands by SDS-PAGE. Upon release by PI-PLC, PrP^{C} migrates more slowly in SDS-PAGE; similar results have been observed previously with other glycolipidated proteins. Incubation of neuroblastoma N2a cells with PI-PLC also results in a substantial decrease in cell surface indirect immunofluorescence when the cells are stained with PrP antisera. These results show that nearly all of the PrP^{C} present in primary cell cultures are anchored to the cell surface by a glycolipid.

348.4 C-11-METHIONINE ACCUMULATION IN NORMAL AND TUMOR-CONTAINING HUMAN BRAIN: QUANTITATIVE IMAGING -- COMPARISON OF LINEAR AND NON-LINEAR MODELS. L.A. O'Tuama*, K.H. Douglass*, R.F. Dannals*, N.D. LaFrance* and H.N. Wagner, Jr.* (SPON: E. Matthew). Division of Nuclear Medicine, The Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Several groups (e.g., Huebner et al., 1982; Bergstrom et al., 1987) have shown that 11-C-L-Methionine (MET) accumulation is a sensitive method for detection and delineation of extent of brain tumors. Further development of this technique will require improved quantification of data. We have compared two mathematical models for evaluation of k (accumulation rate constant). Patients were injected with 11-C-L-MET (18 to 21 mCi) with or without a pre-tracer dose of L-phenylalanine (100 mgs/kg) to separate saturable transport from diffusion. Dynamic PET imaging was performed for up to 25 minutes after tracer injection. Input functions to the model consisted of (1) the "arterialized" plasma time-radioactivity curve, and (2) the time-activity curve of 11-C-L-MET assessed in regions of interest (15-20 pixels of.08 cm^2 per pixel) placed over normal brain or center of the tumor, respectively. In the linear method, k was defined as the slope of the graph of the regional brain activity/plasma activity versus integrated plasma activity/plasma activity. In the non-linear least squares method, a sum of convolution integrals, equivalent to a three compartment model, was fitted to the data. When the linear method was restricted to the later data points, and the non-linear method included an estimate for k-4, the rate constants agreed (R=.99). Agreement between the models for other stages of the acquisition was in the range of R=.75 to .8. We conclude that there are complementary advantages and disadvantages to the linear and non-linear methods of analysis, and that best results are obtained when both are used.

348.5 SUSTAINED SPONTANEOUS ACTIVITY IN COMPRESSED SCIATIC NERVE OF CATS AND RATS. M.H. Bennett. Dept. Neurosurgery, Univ. of Pittsburgh Sch. of Med. Pittsburgh, PA 15261.

It has long been thought that static compression of nerve is ineffective in producing sustained activity. In contrast, compression of nerve has been implicated in many pathological states in which sustained activity has been hypothesized. It was thought that the different findings might be due to the mode of force application experimentally. In attempting to mimic the clinical situation, low-level forces were slowly applied to the nerve and maintained for hours. Due to the viscoelastic properties of nerve a diminishing rate of advance of the compressor was required. Nerve function was tested by measurement of compound action potentials in response to nerve or foot stimulation. EMG monitoring was performed with pre- and postaxial needle electrodes placed at mid-tibial levels. Exposed nerve was covered with oil or saline soaked cotton pledgets. Uncompressed nerve was maintained in the experimental arrangement for up to 4 hrs. with no sign of spontaneous activity.

Spontaneous EMG activity was first seen with a force of 4-10 gr. Maintenance of this level of compression produced an increase of activity over the next 30 to 60 min. Prior to this increase, removal of compression resulted in a loss of spontaneous activity. Once activity was well established, compressor advance was stopped and spontaneous activity was seen for up to 12 hrs. After 2 to 3 hrs. of activity, removal of compression produced a declining level of activity which disappeared within 30 to 40 min. After 6 hrs. of activity, removal of compression produced a slightly lower leve of activity which continued for the remaining 4 hrs. of the experiment. At times, waxing and waning of activity was seen with both silent periods and bursting activity. These findings were repeated in experiments in which the spinal cord was transected at T6 and/or the sciatic nerve was transected at the notch. Xylocaine applied to the nerve just distal to the compressor reversibly abolished spontaneous activity but the site of xylocaine effect moved distally with time.

Direct nerve recording in paralyzed animals also demonstrated compression induced activity. With compression, a decreased threshold at the compressor site was also seen. Evidence of ephaptic action at the compressor site was obtained using action potential collision techniques.

Further descriptions of methods and results, suggestions of possible mechanisms and a discussion of clinical implications of these findings will be presented.

348.6 MEASUREMENT OF SERIAL CHANGES IN LACTATE AND PYRUVATE IN SPINAL CORD ISCHEMIA AND REPERFUSION USING MICRODIALYSIS AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. J.C. Goodman, C.S. Robertson*, and R.G Grossman. Departments of Pathology and Neurosurgery, Baylor College of Medicine, Houston, Tx 77030

Measurement of changes in metabolites in central nervous system tissue during ischemia and reperfusion has classically required the sacrifice of animals at discrete times during the experiment, measurement of the metabolites, and collation of results of many experiments to reconstruct the time course of metabolic changes. We have developed a method of measuring serial changes in lactate and pyruvate in the extracellular space of the spinal cord in an individual rabbit with spinal cord ischemia caused by intra-aortic balloon inflation. Prior to inflation of the balloon, a microdialysis probe is placed in the spinal cord at the level of the fifth lumbar vertebra and perfused with artificial CSF at a rate of 10 µl/min. Specimens are collected every 15 minutes for 1 hour. The balloon is then inflated, and metabolic failure of the cord is confirmed by abolition of spinal sensory evoked potentials (Cheng M.K., et al, J.Neurosurg. 60:786, 1984). Ischemia is maintained for 20 minutes during which time dialysate is collected every 5 minutes. The balloon is then deflated restoring circulation to the cord, and dialysate is collected every 15 minutes for 3 hours. The animal is sacrificed and the dialysate is stored at -70° C. until analysis.

The dialysate is analyzed for lactate and pyruvate by direct injection of 25 µl onto a BioRad Aminex HPX-87H cation exchange column with 5 mmol/L sulfuric acid mobile phase, and detection by absorbance at 210 nm at 0.005 AUFS. Excellent baseline separation of the organic acids is obtained, and quantitation by peak height measurement is linear over the applicable range. In vitro studies reveal an 8% recovery of organic acids in the dialysate relative to calibration solution at the flow rate used.

During ischemia the lactate levels increase and pyruvate levels decrease resulting in marked elevations of the lactate/pyruvate ratio reflecting increased anaerobic metabolism. During reperfusion, the pyruvate level rebounds dramatically, lactate levels decline though not to baseline levels, and the lactate/pyruvate ratio drops toward normal. These changes in extracellular concentrations of these metabolites parallel changes in tissue levels determined by classical methods.

This method of measuring metabolites during experimental manipulation of the spinal cord requires far fewer animals to acquire comparable data, and adds a new dimension to studies of therapeutic agents for CNS ischemia. Biochemical studies are by no means confined to organic acids; indeed, analysis of amino acids and neurotransmitters is extensively described in the microdialysis literature. Additionally, sample acquistion by microdialysis often results in a sample suitable for direct injection into the the liquid chromatograph, raising the possibility of directly coupled on-line microdialysis HPLC analysis.

(This work was supported by the William H. Lane Fund for Neurological Research. This work was reviewed and is in compliance with institutional and NIH guidelines for experimental procedures.)

348.7 INCREASED VULNERABILITY OF THE MILDLY TRAUMATIZED RAT BRAIN TO CEREBRAL ISCHEMIA: THE USE OF A CONTROLLED SECOND INSULT AS A RESEARCH TOOL. L. W. Jenkins*, W. Lewelt*, K. Moszynski*, B. G. Lyeth, R. L. Hayes, D. S. DeWitt*, S. E. Robinson, A. Allen*, J. Opoku*, A. Marmarou* and H. F. Young* (SPON: Richard J. Krieg). Div. of Neurosurgery, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298-0001.

Mild mechanical brain injury alone does not usually result in overt cell death. However, we hypothesized that certain pathophysiological mechanisms initiated during mechanical injury may ultimately result in cell death when post-traumatic steady state conditions are challenged. The present study compared the response of rodent brains, initially subjected to mild mechanical injury, and subsequently challenged with a "subletha1" ischemic insult, to the response of the normal untraumatized brain subjected to an identical ischemic insult. The ultimate goal of this approach is to describe in detail common, or different, pathophysiological mechanisms accompanying mechanical and ischemic brain injury.

Fasted male Wistar rats were intubated and maintained under halothane/nitrous oxide anesthesia and subjected to either: a 1.0 ATM fluid pulse (Group 1, N=6), 6 minutes of transient forebrain ischemia (Group 2, N=5), or a 1.0 ATM fluid pulse followed 1 hour later by 6 minutes of forebrain ischemia (Group 3, N=6). EEG and evoked potentials were recorded prior to, during, 1 hour after, and 7 days after injury. Morphological analysis was performed on all rats after 7 days post-injury survival.

All animals survived 7 days post-insult and in all three groups complete qualitative recovery of electrical activity was observed. No overt neuronal cell loss was seen in either trauma alone or ischemia alone, but bilateral CA1 pyramidal cell loss was found in the rostral third of the dorsal hippocampi in the combined insult group.

Employing neuronal death as an endpoint in the present paradigm, we conclude that mild mechanical injury potentiates ischemic selective vulnerability rather than ischemia potentiating typical traumatic brainstem vulnerability. Acute studies in the cat also support this conclusion (Mechanisms of Secondary Brain Damage, A. Baethmann (Ed.), Plenum Press, 1986, p. 273). These data suggest that mild mechanical trauma decreases the threshold of the brain to lethal ischemic injury. Delayed ischemic hippocampal neuronal death has been linked to an excitotoxic induced pathological calcium signal. A similar but sublethal excitotoxic process after mild trauma may occur, which is then potentiated to lethality by a second excitotoxic induced pathological calcium signal. (Supported by NS19550.)

348.8 ENDURING SHORT-TERM MEMORY DEFICITS IN THE ABSENCE OF HIPPOCAMPAL CELL DEATH FOLLOWING MODERATE HEAD INJURY IN THE RAT. B. G. Lyeth, L. W. Jenkins, R. J. Hamm, S. E. Robinson, C. E. Dixon, M. L. Geibel,* A. Allen,* M. Schaeffer,* L. Oleniak,* J. Opoku,* R. G. Chapouris,* A. Marmarou,* H. F. Young* and R. L. Hayes. Division of Neurosurgery, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298-0001.

Enduring neurological dysfunction that results from an insult to the brain is often equated with irreversible structural CNS damage such as loss of neurons or axonal degeneration. Following diffuse cerebral ischemia, the loss of CA_1 hippocampal neurons has been directly associated with enduring deficits in radial maze memory performance (Stroke 15:558, 1984). The present study examined the relationship between hippocampal cell death and enduring memory deficits resulting from concussive mechanical brain injury.

Male Sprague-Dawley rats (300-400g) were maintained at 85% of their free-feeding weight and trained to criteria on an 8-arm radial maze prior to sham injury or moderate fluid percussion brain injury (2.1-2.3 atm). After daily assessments for 25 days, animals were perfusion fixed, processed for paraffin, and serially sectioned at 8 µm. Cell counts from consistent coronal sections of the rostral-dorsal hippocampus were made and all other brain regions were assessed qualitatively. Sham-injured controls were compared to injured animals with enduring maze deficits.

Animals manifesting maze deficits at sacrifice averaged 2.4 short-term errors/session compared to 1.1 errors/session for sham-injured animals. No overt evidence of cell death was observed in any brain region. Preliminary cell counts were expressed as the number of pyramidal or granule neurons per unit area for the intrinsic rostral third of the dorsal hippocampus as follows: 1) the dentate gyrus, 2) sectors CA_2-CA_4, and 3) CA_1-subiculum). Mean sham control values for region 1 (3704.5 neurons/10^6 µm^2), region 2 (1296.9 neurons/10^6 µm^2) and region 3 (1748 neurons/10^6 µm^2) were not significantly different from means in region 1 (3457.6 neurons/10^6 µm^2), region 2 (1206.2 neurons/10^6 µm^2) or region 3 (1842 neurons/10^6 µm^2) from injured animals with maze deficits.

These data suggest that enduring short-term memory deficits may exist in the rodent following moderate head injury without quantitative neuronal cell death within representative samples of the intrinsic hippocampi or overt cell death in other associated brain regions. While axonal and/or dendritic field injury cannot yet be eliminated, a sublethal neurochemical basis for these persisting deficits appears probable.

Supported by NIH grants NS21458 and NS19550 and the Richard Roland Reynolds Neurosurgical Research Laboratories.

348.9 PRETREATMENT WITH PHENCYCLIDINE (PCP) ATTENUATES LONG-TERM BEHAVIORAL DEFICITS FOLLOWING CONCUSSIVE BRAIN INJURY IN THE RAT. R. L. Hayes, R. Chapouris*, B. G. Lyeth, L. Jenkins*, S. E. Robinson, H. F. Young* and A. Marmarou*. Div. of Neurosurgery and Department of Pharmacology and Toxicology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA 23298-0001.

A number of lines of evidence suggest that excitatory information flow pathways participate in injury processes associated with CNS insults such as seizures, hypoxia/ischemia and hypoglycemia. These brain injuries may share information flow abnormalities mediated by agonist-receptor interactions at excitatory amino acid/NMDA receptors. The present study examined the possibility that similar processes may contribute to behavior deficits following mechanical brain injury. We administered varying doses of PCP, an NMDA receptor antagonist, and evaluated the drug's effects on durations of transient behavior suppression (i.e. "unconsciousness") and longer-term behavioral deficits.

Rats were surgically prepared under Nembutal 24 hours prior to injury by placing a hollow tube epidurally over a central craniotomy. Rats were administered either saline or PCP (1.0, 2.0 or 4.0 mg/kg, i.p.) 15 minutes, and methoxyflurane, 5 minutes prior to 2.35-2.45 atm fluid percussion injury. Rats were ventilated as necessary following injury. The duration of acute suppression of several reflexes (pinna, corneal, righting, and flexion) and responses (escape, head support, and spontaneous locomotion) was recorded for up to 60 minutes after trauma. Longer-term behavioral assessments (beam walking, spontaneous activity and body weight) were made for up to 10 days after trauma. PCP did not significantly alter the duration of acute behavioral suppression. Rats pretreated with 1.0 mg/kg PCP lost significantly ($p<.05$) less weight than saline-treated rats through the first eight days after injury. Saline-treated rats showed significant ($p<.05$) decreases in spontaneous locomotion for 10 days after injury while no significant changes were seen in rats pretreated with 1.0 mg/kg of PCP. In contrast, while PCP significantly ($p<.05$) reduced deficits in the beam-walking task, maximal protection was provided by the 4.0 mg/kg dose. Sixty-five percent of saline-treated animals died within 10 days after injury. For rats pretreated with 1.0, 2.0 and 4.0 mg/kg of PCP, 40%, 20%, and 33% died, respectively.

While the possibility of effects mediated by other neurotransmitter systems cannot be excluded, these data suggest that NMDA agonist-receptor interactions contribute to the pathophysiology of brain injury. In addition, neural mechanisms mediating transient unconsciousness following moderate levels of head injury may differ from mechanisms mediating more persistent neurological deficits. (Supported by NIH Grant #NS21458.)

349.10 THE EFFECT OF NALOXONE PRETREATMENT ON BEHAVIORAL RESPONSES TO CONCUSSIVE BRAIN INJURY IN THE RAT. S.E. Robinson, B.G. Lyeth, L.W. Jenkins*, T.K. McIntosh, H.F. Young*, R.G. Chapouris* and R.L. Hayes. Department of Pharmacology and Toxicology and Division of Neurosurgery, Medical College of Virginia, Richmond, VA 23298

Data from our laboratory suggest that endogenous opiates may contribute to certain physiological responses observed after experimental brain injury (Hayes et al. J. Neurosurg. 58: 720, 1983). The present study examined the effect of naloxone pretreatment on transient behavioral suppression (e.e. "unconsciousness") and more enduring behavioral deficits associated with experimental concussion in the rat.

Rats (male Sprague-Dawley, 300-350g, n = 10 per group) were surgically prepared under Nembutal 24 hours prior to injury by placing a hollow tube epidurally over a central craniotomy. Rats were administered either saline or naloxone (0.1, 1 or 20 mg/kg, i.p.) 15 min and methoxyflurane 5 min prior to 2.15-2.25 atm fluid percussion injury. Rats were ventilated as necessary following injury. The duration of suppression of several reflexes (pinna, corneal, righting, and flexion) and responses (escape, head support, and spontaneous locomotion) was recorded for up to 60 min after trauma. Longer-term behavioral measures (beam balance, beam walking, and body weight) were measured up to 10 days after trauma. The lower doses of naloxone tended to exacerbate the transient behavioral suppression produced by experimental head trauma, with the lowest dose (0.1 mg/kg) significantly increasing the duration of suppression of the righting, corneal and escape reflexes. The two lower doses of naloxone (0.1 mg/k/g and 1 mg/kg) significantly exacerbated deficits in beam balance and beam walking. On the other hand, the highest dose of naloxone (20 mg/kg) tended to reduce the deficits in beam balance and beam walking.

In low concentrations naloxone preferentially binds to mu receptors; however, at higher concentrations it also binds to additional opiate receptors (Wood, Neuromethods Vol. 4, p. 329, Humana Press, 1986). These findings suggest that endogenous opiates acting on mu receptors may attenuate transient behavioral suppression and exert a protective effect on longer term behavioral deficits observed after brain injury. On the other hand, endogenous opiates, possibly acting at additional receptors, may contribute to the production of longer-term behavioral deficits. It is possible that the high dose of naloxone will be found to significantly protect against long term behavioral deficits after higher levels of injury. Future research will include studies with higher injury levels and will include measurement of additional physiological parameters. (Supported by NIH Grants #NS 21458 and NS24413).

349.11 SYSTEMIC PHYSIOLOGICAL AND CINE-RADIOGRAPHIC CHARACTERIZATION OF A NEW FLUID-PERCUSSION BRAIN INJURY TECHNIQUE IN RATS. C.E. Dixon, J.W. Lighthall, and T.E. Anderson. General Motors Research Labs, Biomedical Science Dept., Warren, MI 48090.

The fluid-percussion (FP) technique produces brain injury through brief deformation of neural tissue associated with injection of small volumes of saline. We have developed a new FP technique that may assist in delineating biomechanical components of brain injury. This report demonstrates comparability with previous techniques at comparable pressure pulse parameters and characterizes: 1) systemic physiologic and histopathologic responses, and 2) the fluid dynamics associated with FP brain injury through use of cine-radiography.

For physiological characterization, rats (n=14) were anesthetized with Ketamine (87mg/kg) plus Xylazine (13mg/kg), tracheostomized and ventilated. A tube was fastened over a central craniotomy. Blood pressure, ECG, EEG, and blood gases were recorded for 60 minutes post injury. Offline, EEG magnitude (μV) was computed for Delta (1-4 Hz), Theta (5-8 Hz), Alpha (9-14 Hz), and Beta (15-25 Hz) frequency bands. The FP device consists of a gas accelerated piston which drives a chambered valve spool through a valve body to rapidly open and close a connection between a pressurized saline reservoir and the intracranial cavity. Rats received either a low (1.5 atm) or high (3.1 atm) severity fluid pressure pulse, both with 20msec duration. The new technique produces changes in blood pressure similar to previous techniques; however bradycardia was not observed. Increases in heart rate were produced by both injury levels, and were more prolonged at the high level of injury severity. Both magnitudes of injury produced decreases in EEG amplitude immediately post-injury, but high severity injury produced a greater decrease in delta activity than did low severity injury. Both levels produced hemorrhage at the site of injury, thalamus, corpus collosum, hippocampus and fimbria hippocampus similar to previous techniques. Higher severities produced more extensive cerebral hemorrhage and greater spinal involvement. This data demonstrates that our new FP technique is comparable to other techniques for comparable pressure pulse parameters, while providing full control of pulse parameters to enable biomechanical characterization.

For biomechanical characterization, cine-radiographic images were made in a separate group of rats (n=7) at coronal and sagittal orientations during the fluid pressure pulse. Intracranial fluid movement was characterized by rapid lateral and anterior/posterior epidural flow. This suggests that the distributed nature of FP induced pathology and dysfunction reflects, in part, a diffuse mechanical loading of brain tissue.

349.12 CONTROLLED CORTICAL IMPACT: A NEW MECHANICAL BRAIN INJURY MODEL. James W. Lighthall, Biomedical Science, General Motors Research Laboratories, Warren MI, 48090-9055.

A new experimental model of mechanical brain injury was produced in the laboratory ferret using a stroke-constrained pneumatic impactor. This model for the first time allows the independent control of the impact contact velocity and the level of forced compression used to produce the brain injury. The purpose of this study was to design a model which reliably produced graded cortical contusion and subcortical injury by precisely controlled brain deformations. Ferrets were anesthetized with Ketamine HCL (25mg/kg) and Xylazine (2mg/kg) I.M. Changes in blood pressure (BP), heart rate (HR) and respiration were monitored continuously. Cortical impacts were performed through a craniotomy centered between bregma and lambda with contact velocities of 2.0 to 4.0 m/s and deformation levels of 2.0 to 5.0 mm. The impactor tip measured 1.25cm in diameter with a spherical interface. Stability of the skull and level of deformation were verified radiographically. Low velocity (2.0 m/s) impacts at deformations <3.5mm produced no discernible morphologic changes. Medium velocity (3.0 m/s) impacts produced a range of morphopathologic alterations including immediate subdural hematoma, extensive subarachnoid hemorrhage, and production of graded cortical contusion. High velocity (4.0 m/s) impacts consistently produced contusion of the cerebellum, brainstem and spinal cord. High velocity impacts at deformations >3.5mm were fatal. Histologic examination revealed graded severity injury including petechial and intraparenchymal hemorrhage in the cortex, subcortical white matter, hippocampus, cerebellum, brainstem and spinal cord. Axonal beading and retraction balls were observed in the subcortical white matter underlying the impact site when survival times were 10-12hrs. Cortical impact produced changes in the HR, SABP and respiration similar to those described in published mechanical brain injury models. The severity of the injury response was dependent on both contact velocity and the level of cortical deformation. Several aspects of the new model are unique: a) graded severity cortical contusions can be produced in conjunction with subcortical reactive axonal injury and vascular damage; b) independent control of the impact contact velocity and level of cortical deformation is possible, thus allowing physical and analytical modelling of the impact dynamics to be performed; c) this model utilizes a colony-bred laboratory mammal which possesses a convoluted cerebral cortex.

The model described here will form the basis for further studies which address the influence of velocity and forced deformation on the severity of brain injury.

349.1 **BODYWALL MUSCLE FIBERS OF *DROSOPHILA* LARVAE ARE INNERVATED BY BOTH GLUTAMATE AND ASPARTATE IMMUNOREACTIVE NERVE TERMINALS.** J. Johansen, M.E. Halpern*, K.M. Johansen*, and H. Keshishian, Department of Biology, Yale University, New Haven, CT 06511.

Glutamate, classically, has been thought of as the principal excitatory transmitter at the neuromuscular junction in dipteran larvae (Jan & Jan, *J. Physiol.*, 1976). However, physiological evidence suggests that aspartate may play a role as well (Irving & Miller, *J. Comp. Physiol.*, 1980). We have investigated bodywall muscle innervation in *Drosophila* third instar larvae by applying immunocytochemical techniques. Using polyclonal antisera (Toomim et al., *Soc. Neurosci. Abstr.*, 1986) we have mapped the distribution and characterized the morphology of glutamate and aspartate immunoreactive nerve terminals on the bodywall muscles. Immunoblot analysis and preincubation experiments with BSA-conjugated aspartate and glutamate showed that the two antibodies used were specific for the respective transmitters and that they did not have any cross-reactivity.

The bodywall of *Drosophila* larvae is a particularly favorable preparation for analysis of motor innervation since each hemisegment is composed of less than 30 discrete muscle fibers which are segmentally repeated in a stereotyped pattern. Immunocytochemical staining with the two antibodies revealed that every bodywall muscle fiber was innervated by both aspartate and glutamate positive nerve fibers. The innervation was in most cases initiated at the middle of each individual muscle fiber from where multiple axons branched over the inner surface of the muscle fibers. However, in some instances, as exemplified by the longitudinal muscles 6 and 7, two muscle fibers were innervated simultaneously by the same axon. These axons were running longitudinally in the cleft between the muscles giving off alternating terminals to the two muscle fibers. Consequently, muscle 6 and 7 may constitute a functional unit. The innervating axons were beaded in appearance with regularly spaced varicosities of from 1-5 μm in diameter. Immuno-electron microscopy with peroxidase labeled second antibody showed that the varicosities were surrounded by a subsynaptic reticulum, that they contained immunoreactive vesicles of about 30-50 nm, and thus probably represent synaptic release sites.

We could not discern any differences in the innervation pattern and morphology between the glutamate and aspartate positive nerve fibers. However, double labeling experiments with the aspartate and glutamate antibodies using different fluorescent conjugated second antibodies showed that the aspartate and glutamate positive nerve terminals were separate and belonged to different axons. Thus, our experiments suggest that every larval bodywall muscle fiber in *Drosophila* is dually innervated by at least one aspartatergic and one glutamatergic axon. A direct physiological excitatory transmitter role for aspartate, in addition to that of glutamate, has been demonstrated in *Musca* (Irving & Miller, *J. Comp. Physiol.*, 1980) and similar experiments to detemine whether this is also the case for aspartate in *Drosophila* are in progress.

We thank Drs. C. Toomim and P. Petrusz for their generous gift of glutamate and aspartate antibodies.

349.2 PHARMACOLOGICAL STUDIES OF AN IDENTIFIED MONOSYNAPTIC CHOLINERGIC SENSORY-TO-MOTOR CONNECTION IN THE NICOTINE-RESISTANT INSECT MANDUCA SEXTA. B.A. Trimmer* and J.C. Weeks (SPON: D. Bentley). Dept. of Entomological Sci. Univ. of California, Berkeley, CA 94720

Sensory hair afferents on the prolegs of the tobacco hornworm Manduca sexta make monosynaptic excitatory connections with an identified motorneuron, PPR. This motorneuron forms the efferent part of a reflex arc which results in proleg withdrawal. As in other insect sensory systems, the afferents appear to release acetylcholine (ACh) (Weeks & Jacobs, J. comp. Physiol. 160:315, 1987). This synapse provides a unique system in which to study the pharmacological basis of Manduca's remarkable tolerance to the ACh agonist nicotine, which is present in high levels in its natural diet.

Single sensory neurons were stimulated at 1 Hz by current pulses through a suction electrode applied to the epidermal surface of a hair socket. The responses of PPR were recorded intracellularly while perfusing the desheathed ganglion with various cholinergic agents. The evoked unitary EPSP was found to be relatively stable over the course of a two hour experiment. PPR's resting potential, input resistance and signal-averaged EPSP were measured during drug application. The potent nicotinic antagonist mecamylamine reversibly blocked the EPSP with the half maximal effect (V_{50}) at 5 x 10^{-5}M. Higher concentrations of tubocurarine and atropine also blocked the synapse. During perfusion with the agonists carbachol or tetramethylammonium, PPR rapidly and reversibly depolarized with a corresponding decrease in input resistance. The potency of these effects is equivalent to that seen in the cockroach (David & Sattelle, J. exp. Biol. 108:119, 1984). Nicotine, which also depolarized PPR, is much less potent than on cockroach motorneurons (V_{50} = 1 x 10^{-4}M compared to V_{50} = 2.3 x 10^{-6}M). The rapidity and reversibility of these agonists' actions would suggest that in addition to the cellular protection mechanisms described previously (Morris, J. Insect. Physiol. 29:807, 1983) Manduca's insensitivity to nicotine results from a specific resistance of the ACh receptors themselves.

We have also observed potent effects of several muscarinic agents. Oxotremorine completely suppress the EPSP at doses as low as 2 x 10^{-8}M. In addition it caused long-lasting oscillations of the resting potential and evoked bursts of activity in the ganglion. These effects were antagonized by atropine. At low concentrations, atropine and scopolamine-methyl-bromide caused a potentiation of the EPSP and a slight hyperpolarization of PPR. In insects there is no known role for the numerous muscarinic binding sites identified with radioligands. We are at present using physiological methods to determine if some of these muscarinic effects are mediated by receptors presynaptic to PPR. Supported by a research grant from the Whitehall Foundation.

349.3 REGULATION OF BEHAVIORAL SENSITIVITY TO THE NEUROPEPTIDE, ECLOSION HORMONE, IS DIFFERENT IN THE ADULT AND PUPAL STAGES OF THE TOBACCO HORNWORM, MANDUCA SEXTA. D.B. Morton and J.W. Truman (SPON. K.A. Mesce), Department of Zoology, University of Washington, Seattle, WA 98195.

The neuropeptide, eclosion hormone (EH), triggers ecdysis behavior at the end of each molt in the tobacco hornworm, Manduca sexta. Previous studies have shown that at pupal ecdysis, EH acts directly on the CNS via cGMP to phosphorylate two proteins, termed the EGPs (Eclosion hormone and cGMP regulated Phosphoproteins).

At each developmental stage, the animals will only respond to EH during the 6-8 hrs immediately preceding the expected time of ecdysis. We have previously shown, for pupal ecdysis, that the onset of this window of behavioral sensitivity coincides with, and is believed to be due to, the appearance of the EGPs in the CNS.

The present report describes the presence of the EGPs in the CNS of both larvae and adults. During the last larval molt, the EGPs are first seen 6 hrs before the expected time of ecdysis, the same time as when behavioral sensitivity is first seen. Immediately after ecdysis, the EGPs will no longer accept ^{32}P from ATP, most probably because they have already been phosphorylated by unlabeled phosphate as a result of the action of endogenously released EH. Thus, larval ecdysis is similar to pupal ecdysis in that EH acts via the phosphorylation of the EGPs. Also, the appearance of the EGPs at larval ecdysis coincides with the appearance of sensitivity to EH.

The EGPs are also present prior to adult ecdysis and, as with the larval and pupal ecdyses, will no longer accept labeled phosphate after ecdysis. This suggests that EH acts via the EGPs at each ecdysis. Unlike larval and pupal ecdysis, however, the EGPs are first present 6 days before the animals will first respond to EH. Furthermore, they can also be phosphorylated by the action of EH prior to behavioral sensitivity. This implies that at adult ecdysis, the regulation of sensitivity to EH is at a step distal to the phosphorylation of the EGPs.

Supported by NIH grant NS-13079.

349.4 CLONALLY RELATED NEURONS IN MANDUCA SEXTA EXPRESS DIFFERENT PUTATIVE TRANSMITTERS. J.L. Witten and J.W. Truman. Dept. of Zoology, U. Washington, Seattle, WA 98195.

We have been studying the neurochemical differentiation of identified clusters of neurons born during the larval life of the moth, Manduca sexta. Using immunohistochemical methods, we have mapped the distribution of gamma-aminobutyric acid (GABA), an octopamine-like compound and a peptide that is similar to molluscan small cardioactive peptide$_b$ (SCP$_b$) in the segmental ganglia of this insect. We have localized these three putative neurotransmitters to one identified cluster of clonally related postembryonic neurons (PEN's).

During the larval stages arrays of identifiable neuroblasts generate discrete clusters of neurons. These nests are in stereotypic positions in the ganglia and can be studied as identified neuron clusters. After birth the cells in each cluster are arrested at an early stage of differentiation. At the onset of metamorphosis, ecdysone triggers the resumption of their development. The strength of this system is that each cluster represents a unique, identified lineage of a single stem cell and we can follow its neurochemical differentiation throughout the postembryonic development of the insect.

Antisera to each putative transmitter stains a distinctive subpopulation of the PEN clusters. The SCP$_b$-like and octopamine-like immunoreactivity (IR) are restricted to 2 of the 22 paired clusters in the thoracic ganglia. The subpopulation of PEN's that show GABA-IR is the largest (80% of the clusters staining). Only a subgroup of the neurons within each cluster are immunoreactive for each antigen. This heterogeneity in phenotype may indicate that both lineage position and extrinsic factors are involved in regulating transmitter expression. All three antisera stain subsets of neurons within one identifiable cluster in the thoracic ganglia (F cluster). Birth-dating and cell lesion techniques are being applied to neurons of the F cluster to investigate the interaction of cell lineage with steroid hormones in the regulation of transmitter expression.

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349.5 NEUROPEPTIDE BIOSYNTHESIS: MODEL SYSTEM IN AN INSECT

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We have investigated the biosynthesis of two homologous neuropeptides in the insect Schistocerca gregaria (Desert locust). The two neuropeptides are adipokinetic hormones called AKH I (pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2, Stone et al., Nature 263, 1976) and AKH II (pGlu-Leu-Asn-Phe-Ser-Thr-Gly-Trp-NH_2, Siegert et al., Biol. Chem. Hoppe Seyler 366, 1985). We have shown (Hekimi and O'Shea, 1987, J. Neurosci. in press) that both are synthesised by the neurosecretory cells of the glandular lobe of the corpus cardiacum (CC), a major neurosecretory structure in the insect head. The AKH peptides regulate a variety of physiological processes during long-duration (migratory) flight.

Using an in vitro organ culture system we are able to study the biosynthesis of AKH I and II by monitoring the incorporation of ^{3}H-labeled Trp into proteins and peptides. The products of the neurosecretory cells are purified by size exclusion and reverse-phase HPLC and can be identified by radioimmunoassay (RIA). To achieve the latter, analogs of AKH I and II were designed for the production of antisera which discriminate between AKH I and II. We have also generated, using a non-amidated analog of AKH I, an antiserum which recognises the two primary precursor polypeptides, P1 and P2, of the AKH peptides (Hekimi and O'Shea, 1987, J. Neurosci. in press). Using the in vitro system and by combining HPLC purification with RIA detection we have identified polypeptides intermediate in size between P1/P2 and the two hormones. We believe these peptides are intermediates in AKH biosynthesis. In order to characterize enzymatic steps involved in precursor processing we incubated the neurosecretory cells with two non-protein amino acids, L-canavanin (analog of arginine) and S^2-amino-ethyl cystein (an analog of lysine). These analogs are incorporated into the P1 and P2 precursors in place of Arg and Lys. Processing of the newly made precursors (P1 and P2) is inhibited, suggesting trypsin-like enzymes are involved in precursor processing. The culture system we have developed also has allowed us to study the regulation of AKH biosynthesis during postembryonic development. This study shows that the rate of synthesis of both precursors and neuropeptides increases dramatically in late postembryonic development and during adult maturation, just prior to the first appearance of flight behavior. We are currently investigating whether this increase in synthesis rate is hormonally induced.

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349.6 **PURIFICATION AND CHARACTERIZATION OF THE FMRFamide RELATED PEPTIDE OF DROSOPHILA MELANOGASTER.**

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The amidated tetrapeptide FMRFamide has been shown to exhibit a wide range of activities on target neurons and muscle cells in both vertebrate and invertebrate preparations. In addition, FMRFamide immunoreactivity has been observed in a variety of organisms and several structurally related peptides have been identified. The FMRFamide gene has been cloned from Aplysia californica, and encodes a precursor which contains 28 copies of FMRFamide and a single copy of FLRFamide. In Drosophila melanogaster, K. White et al. (J. Comp. Neurol., 247:430-438, 1986) have demonstrated that a stereotypic pattern of neurons and processes in the larval and adult CNS stain specifically with FMRFamide antibodies, implying a neurosecretory function for this material. Interestingly, FMRFamide staining was observed to overlap the staining patterns of antisera against two other neuropeptides, CCK and Substance P.

In order to determine the precise nature of the FMRFamide immunoreactive material, we employed ion exchange chromatography, gel filtration, and HPLC techniques in conjunction with a FMRFamide radioimmunoassay to purify the FMRFamide immunoreactive substance from adult Drosophila melanogaster. Amino acid composition analysis as well as FAB mass spectrometry, and protein sequencing methods indicated that the most abundant immunoreactive substance is a 9 amino acid peptide with a molecular weight of 1182, and a sequence of DPKQDFMRFamide. The peptide thus contains a FMRFamide core but is extended amino-terminally and is distinct from all other known FMRFamide related peptides. Studies are currently in progress to determine the physiological activities of this peptide, and also, to isolate the corresponding gene sequences. It is hoped that application of the powerful molecular and genetic techniques available in Drosophila will permit evaluation of the actions and importance of this molecule in the fly's physiology and development.

349.7 RFamide NEUROPEPTIDES IN THE HYDROMEDUSA POLYORCHIS PENICILLATUS; LOCALIZATION, ISOLATION AND ELECTROPHYSIOLOGY.

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Within the nervous systems of all cnidarians, examined to date, there are populations of neurons which are immunoreactive to antisera raised against RFamide and FMRFamide. These immunoreactive neuronal populations share several characteristics. They form diffuse nerve-nets closely associated with smooth muscles and many somas have a neurosensory appearance. In addition they may be found close to regions of neuronal condensation, such as the nerve-rings of medusae.

Using a radioimmunoassay for RFamide, large quantities (3.4 nmol/g wet weight) of immunoreactive material were found in an acetic acid extract of the bell margins of the hydromedusa, Polyorchis penicillatus. After desalting and partial purification using Sep-pak cartridges, further purification using cation-exchange chromatography on CM-Sephadex C-25 and reversed-phase HPLC, gave a peptide which was subsequently analysed and sequenced. This Polyorchis peptide has the sequence <Glu-Leu-Leu-Gly-Gly-Arg-Phe-NH_2. The chromatographic behavior of the purified peptide was compared with that of synthetic peptide produced by Bachem Ltd. (Switzerland). No differences were found. Antisera raised against this peptide gave strong neuronal staining, particularly of a nerve-net in the manubrium, with lighter staining of neurons in tentacles and the marginal nerve-rings.

Applications of RFamide, FMRFamide, FLRFamide and <Glu-Gly-Arg-Phe-amide (an anthozoan neuropeptide) to the partially isolated motor nerve-net (SMN network) of Polyorchis produced long duration (2 min.) depolarizations with an associated increase in firing frequency. The anthozoan peptide was active at the lowest concentration (approx. 10^{-7}M). Unlike the above peptides, the Polyorchis RFamide peptide does not appear to produce electrophysiological effects in the swimming motor nerve-net. Preliminary data on the effect of the Polyorchis RFamide peptide on other identified neurons and epitheliomuscular cells in primary cultures will be described. Primary cultures are produced by exposing the marginal nerve-ring to hypotonic shock (20% ASW) to remove the epithelium and then subsequent treatment in divalent-cation free seawater followed by digestion in 1000 U/ml collagenase.

The purification and sequencing of the Polyorchis RFamide peptide were carried out by CJPG and MH in Heidelberg; electrophysiological experiments were performed by ANS and JP in Edmonton and the Bamfield Marine Station. Supported in part by the Deutsche Forschungsgemeinschaft (Gr 762/4-3, Gr 76217-1) and NSERC, Canada (A0419).

349.8 FMRFAMIDE AND RELATED PEPTIDES SWITCH A CRUSTACEAN MUSCLE INTO AN OSCILLATORY MODE.

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The pyloric dilator (cpv) muscles of the shrimp, Palaemon serratus, are capable of producing myogenic contractions, which can be entrained by rhythmic excitatory synaptic input from the Pyloric Dilator (PD) motor neurons found in the stomatogastric ganglion (STG) (Meyrand & Moulins, J Comp Physiol A,158:489-503, 1986). Previous work showed that bath-application of dopamine can transform the cpv muscle from a non-oscillatory state to an oscillatory state. We now find that a FMRFamide-like peptide is present in the motor nerves innervating the cpv muscle, and that FMRFamide and related peptides can evoke rhythmic movements of the cpv muscle in the absence of excitatory synaptic input.

Cpv muscles were isolated with the nerve attached, but all ganglia removed. Movement was monitored with a force-displacement transducer, membrane potential monitored intracellularly, and peptides were applied through a continuously flowing superfusion system. FMRFamide (10^{-6}M) and Helix peptide (pGluAspProPheLeuArgPheamide) (10^{-7}M) elicited strong rhythmic (0.75-1 Hz) contractions of the cpv muscle. This frequency is similar to those of the pyloric rhythm generated by the stomatogastric ganglion. Peptide-evoked contractions were as much as 7-fold larger than those produced by stimulation of the nerve in the absence of the peptide. The rhythmic contractions elicited by peptide could be entrained by synaptic stimulation.

The presence of a FMRFamide-like peptide in the shrimp stomatogastric nervous system was demonstrated by whole mount immunocytochemistry. As in lobsters and crabs, (Marder, Calabrese, Nusbaum, & Trimmer, J Comp Neurol, 259: 150-163, 1987), FMRFamide-like staining was seen in fibers in the stomatogastric nerve (STN), and in neuropilar ramifications in the STG. Unlike lobsters and crabs, two stained somata were seen in the STG. Stained fibers were seen in the motor nerves of the stomatogastric nervous system, including those eventually terminating on the cpv muscles.

These data suggest that a FMRFamide-like peptide may be neurally released onto or in the vicinity of the cpv muscles. This peptide may function to switch the muscle from a "follower" mode to one in which endogenously produced rhythmic contractions are entrained by neural inputs, as seen in the leech heartbeat system (Kuhlman, Li, & Calabrese, J Neurosci, 5:2310-2317, 1986).

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349.9 PEPTIDE F_1: A MYOACTIVE LOBSTER PEPTIDE RELATED TO FMRF-AMIDE. *E.A. Kravitz, L. Kobierski*, B.A. Trimmer*, and M.F. Goy**. Neurobiology Dept., Harvard Med. Sch., Boston, MA 02115.

The tetrapeptide FMRFamide, originally isolated in 1977 by Price and Greenberg as a molluscan cardioexcitatory peptide, is the first isolated member of a large family of related peptides found in both invertebrate and vertebrate nervous systems. Considerable interest has focused on these peptides and their structural relationship to the vertebrate opioid peptides, a relationship strengthened recently by genetic analysis, which shows considerable sequence homology between the gene coding for FMRFamide peptides in Aplysia and the genes coding for several members of the vertebrate opioid family of peptides (Taussig and Scheller, 1986).

A large family of FMRFamide-like peptides also has been found in the lobster, Homarus americanus. Two members of this family have been purified and sequenced (Peptides F_1 and F_2, Trimmer et al., in press) and one has been synthesized (peptide F_1: Thr-Asn-Arg-Asn-Phe-Leu-Arg-Phe-NH_2). FMRFamide-like peptides have been localized in about 350 neurons in the lobster nervous system. The morphological localization has suggested possible roles of these peptides in integration of visual and olfactory information, in regulation of the anterior and posterior gut, and in the control of exoskeletal muscles (Kobierski et al., in press). In addition, a dense nerve terminal plexus containing these peptides covers the connective tissue sheath surrounding much of the ventral nerve cord, suggesting an important neurosecretory role for these substances.

The functional roles have been detailed further by examining the actions of synthetic peptide F_1 on several different types of lobster muscle and comparing these actions to the effects of FMRFamide, proctolin and the amines serotonin and octopamine. Peptide F_1 has significant long duration potentiating actions on all preparations that we have tested. These include the heart, the opener muscle of the dactyl of the walking leg and the oesophagus. The thresholds for F_1 actions on these tissues is in the 10^{-10}-10^{-9}M range, about 3-4 orders of magnitude lower than the thresholds for FMRFamide on the same preparations. F_1 increases the rate and strength of the heart beat, increases transmitter release and induces a contracture in exoskeletal muscles, and generates rhythmic contractions of the gut. These changes are seen with little or no change in cAMP levels in these tissues. Qualitative and quantitative differences exist between the actions of F_1 and the other modulator substances. Peptide F_1 therefore seems to be a potent myoactive agent in lobsters: its mechanism of action is under active investigation. (Supported by NIH).

349.10 PURIFICATION AND PROPERTIES OF PEPTIDE G_1, A PUTATIVE HORMONE THAT ALTERS CYCLIC GMP METABOLISM IN LOBSTER TISSUES. *Michael F. Goy*, Mark H-F. Kuo*, Michael Pavloff*, and Edward A. Kravitz.* (SPON: K. Dunlap). Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Levels of cyclic GMP, of cyclic GMP-dependent protein kinase, and of phosphoprotein substrates for the kinase are unusually high in invertebrates, like the American lobster. In an effort to understand the physiological role of this cyclic nucleotide, we have tried to identify hormones that affect its metabolism in lobster tissues. As part of this search, we have found that extracts of the sinus gland, a well-known crustacean neurohemal organ, potently stimulate cyclic GMP levels in lobster skeletal muscle, heart, gut, ventral nerve cord, and other tissues.

Fractionation of the sinus gland extract reveals three independent peaks of cyclic GMP-promoting activity. Two of these are relatively minor components, and have not yet been characterized. The main peak, which comprises about 80% of the total, is a 7-8,000 dalton peptide. We have purified it more than 10^6-fold by sequential ion exchange and reverse phase HPLC. Preliminary amino acid analysis, coupled with information about its chromatographic properties and relative abundance (about 1 pmol per sinus gland), suggests that this is a novel peptide. We have named it peptide G_1, because it is the first lobster peptide to be purified solely on the basis of its ability to affect cyclic GMP metabolism.

We have produced highly enriched fractions of peptide G_1 by gel filtration and ion exchange chromatography, and tested these fractions for physiological effects at the lobster neuromuscular junction. Lobster muscle is an important target for this peptide, showing up to 100-fold increases in cyclic GMP content after treatment with the peptide. Along with these biochemical changes, the enriched fractions cause a slow, reversible suppression of muscle contractility. The time course is particularly striking, with onset occuring over 30-60 min and washout requiring many hours. Contractile responses to nerve stimulation and potassium depolarization are both reduced, approximately equally. In contrast, the amplitudes of synaptic potentials during stimulation of the excitatory motor axon are slightly increased. This suggests a possible disruption of excitation-contraction coupling in the muscle fiber. Current efforts are focused on large-scale purification, in order to verify these observations with peptide that has been purified to homogeneity. (Supported by NIH grant NS21290).

349.11 **PRESENCE AND RELEASE OF A CCK/GASTRIN-LIKE MOLECULE IN THE LOBSTER STOMATOGASTRIC GANGLION.** G. Turrigiano* and A.I. Selverston. Dept. of Biology, Univ. of Calif., San Diego; La Jolla, CA 92093.

The stomatogastric ganglion (STG) of the lobster contains two central pattern generators (CPGs), the pyloric and the gastric mill CPGs. Each produces a characteristic pattern in the absence of sensory input, but recent work from several labs suggests that modulatory inputs can alter properties of CPG neurons to select among different stable patterns. Bath application of monoamines and of neuropeptides (FMRFamide and proctolin) can modulate the output of the STG, and immunohistochemistry has shown that these substances are present in the neuropil of the STG, and in soma in the commisural ganglia (CGs), which are connected to the STG by the stomatogastric nerve (STN). Recently cck/gastrin-like immunoreactivity has been reported in tissues of several invertebrates, including arthropods (B. Larson and S. Vigna, *Gen. Comp. Endocrin.* 50:469-475,1983), and a related peptide, leucosulfakinin, has been isolated and sequenced from cockroach heads (R. Nachman et al., *Science* 234:71-73,1976). This abstract reports the identification of cck/gastrin-like immunoreactivity in the STG nervous system of the lobster *Panulirus interruptus*, and suggests that a cck/gastrin-like molecule may be released into, and modulate the output of the STG.

Immunohistochemical localization of neurons and fibers was accomplished using a polyclonal antibody raised against the terminal sequence of cck (Immuno Nuclear Corp.). At least four cell bodies in the CGs show cck-like immunoreactivity, and fibers which appear to originate in the brain have neuropil in the CGs. Positively staining fibers were seen in the STN, which branched out into densely staining neuropil throughout the STG. None of the STG neurons themselves stained, and no fibers were seen leaving the ganglion. We tested the crossreactivity of this antibody using a sensitive immunoblot assay, and found that it crossreacted with peptides with the same terminal sequence as cck, but not with other small peptides such as proctolin and FMRFamide. The pattern of staining was very similar in *Panulirus* and *Procambarus clarkii*.

CCK and the structurally related caerulein peptide could modulate the output of the STG. When bath was applied at high concentrations (10^{-4} M) these peptides reliably turned on a pyloric rhythm in quiescent preparations, and in some preparations started up a gastric rhythm as well. These effects were reversible and took up to two hours to wash out.

In addition, we used our immunoblot assay to demonstrate that crude extracts of lobster stomachs and nervous systems contained cck/gastrin-like immunoreactivity, and that stimulation of the STN produced release of a cck/gastrin-like molecule into the STG. A small vaseline well was built around the STG, and the desheathed ganglion was bathed in 5 µl of saline with the protease inhibitor pmsf. The STN was electrically stimulated for ten minutes, and the saline collected and assayed for cck/gastrin-like immunoreactivity. The stimulated condition showed a many-fold stronger reaction than unstimulated controls. We are currently attempting to quantify this release using an ELISA assay.

Taken together, these results suggest that a cck-like molecule has a functional role in the STG. The high concentrations needed to produce a modulatory effect suggests that the endogenous substance is related to, but distinct from, mammalian cck. We are currently attempting to isolate and characterize the endogenous substance. (Supported by NIH NS09322 and NSF BNS81-18756 to A.I.S.)

349.12 A NEWLY IDENTIFIED MODULATORY PROCTOLIN-CONTAINING NEURON (MP NEURON) IN THE STOMATOGASTRIC NERVOUS SYSTEM OF THE CRAB, CANCER BOREALIS. Michael P. Nusbaum and Eve Marder. Dept. of Biology, Brandeis Univ., Waltham, MA 02254.

The pyloric rhythm of decapod crustaceans is generated by a well-defined neural network located in the stomatogastric ganglion (STG), one of the ganglia of the stomatogastric nervous system (The Crustacean Stomatogastric System, eds. Selverston & Moulins, 1987, Springer-Verlag). The neuropeptide proctolin is found in neuropilar processes in the STG, fibers projecting into the STG from more central ganglia and subsets of neurons in the oesophageal ganglion (OG) and the commissural ganglia (CGs) (Hooper & Marder, Brain Res., 305:186-191,1984; Marder et al., J. Comp. Neurol., 243:454-467,1986). Bath application of synthetic proctolin to the isolated STG causes an excitatory modulation of the pyloric rhythm (Hooper & Marder, 1984; Marder et al., 1986).

In the crab Cancer borealis we have identified a modulatory proctolin neuron (MPN). The MPN has a 15 micron soma and is found in the region of the OG. Intracellular Lucifer-yellow (LY) fills indicate that the MPN extends processes towards both CGs and towards the STG. The proctolin content of MPN is revealed by double labeling the neuron using intracellular LY dye filling and subsequent processing for proctolin immunoreactivity (anti-proctolin antibody courtesy of M. O'Shea & C. Bishop), visualized with a rhodamine conjugated secondary antibody.

Intracellular stimulation of MPN increases the pyloric frequency of slowly cycling preparations. The effect on pyloric rhythm frequency was proportional to the frequency of MPN firing. MPN stimulation also produces an increase in the amplitude of the slow wave oscillations and the number of impulses per burst in some of the pyloric circuit neurons, including the VD and LP neurons. For example, the number of impulses per burst in the LP neuron increases up to 70%. During prolonged MPN stimulation the effects increase during the first 3-5 seconds, then attain a peak action which persists for the duration of the MPN activity. The effects decline slowly during the 5-10 seconds after the cessation of MPN activity. No discrete postsynaptic potentials time-locked with MPN impulses have been seen in any pyloric network neuron. These data suggest a modulatory type of synaptic action for the MPN. The effects of MPN stimulation on pyloric cycle frequency and the number of LP neuron impulses per burst are statistically indistinguishable from those of bath application of synthetic proctolin (10^{-6}M).

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350.1 TRANSNEURONAL CHANGES IN COCHLEAR RADIAL AFFERENT FIBERS FOLLOWING DESTRUCTION OF LATERAL OLIVOCOCHLEAR NEURONS. K.M. Spangler and W.B. Warr. Dept. of Anatomy, Creighton University School of Medicine, and Center for Hearing Research, Boys Town National Institute, Omaha, NE 68178.

Lateral olivocochlear (OC) neurons, which are found in or near the lateral superior olivary nucleus (LSO) are the major, if not exclusive, source of the efferent synapses found on the peripheral processes (radial afferent fibers) of the Type I spiral ganglion cells. One of the few observations relating to the possible functional role of the lateral OC neurons comes from a study on patas monkeys by Bodian and Gucer (*J. Comp. Neurol. 192*:785-796, 1980), in which they severed the entire OC bundle within the vestibular nerve and examined the ultrastructure of the organ of Corti. They found a dramatic trans-synaptic effect on the radial afferent fibers consisting of an increase in volume and engorgement with microtubules, neurofilaments, vesicles, and other organelles. This phenomenon was re-examined during the course of an EM degeneration study of the inner spiral bundle. The rat was chosen as a model because all lateral OC neurons projecting to one cochlea are located within the ipsilateral LSO and can be lesioned selectively. Six days after unilateral electrolytic lesions of the LSO, animals were perfused with aldehyde fixatives. Serial frozen sections (40 μm) of the auditory brainstem were mounted on glass slides. Alternate sections were stained for Nissl or acetylcholinesterase for evaluation of the extent of the lesion and possible interruption of the OC bundle. Additional fixative was perfused through the cochlea via the round and oval windows and the cochleas were prepared for EM. In two cases with nearly total ablation of the LSO, the inner spiral bundles from part of the middle turn of the cochleas were examined in detail. The two main preliminary findings were 1) that there was a substantial, but subtotal, reduction in the number of thin fibers (presumably efferents) in the inner spiral bundle, and 2) that morphological changes, like those described by Bodian and Gucer, were evident in a number of radial afferent fibers. These findings confirm the observations in the monkey, but additionally demonstrate that a lesion of the lateral OC neurons is a sufficient condition for the effect. Further investigations are in progress to explore the time course of this trans-synaptic phenomenon and its possible effects on the physiology of cochlear nerve fibers. Supported by the Deafness Research Foundation and NIH Grant # 1 R01 NS24060.

350.2 THE CENTRAL PROJECTIONS OF TYPE-II SPIRAL GANGLION CELLS IN RODENTS. M.C. Brown, A.M. Berglund and D.K. Ryugo. Departments of Otolaryngology, Physiology, and Anatomy and Cellular Biology, Harvard Medical School; and Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA. 02114.

The mammalian spiral ganglion contains two populations of neurons: type-I cells contacting inner hair cells and type-II cells contacting outer hair cells. Although the central projections of the more numerous type-I cells have been extensively studied, the central projections of type-II cells have not. We have labeled the central axons of both types of cells using focal extracellular injections of horseradish peroxidase (HRP) into the peripheral edge of the spiral ganglion. The tissue was processed using diaminobenzidine. Typically, these injections label fewer than 100 ganglion cells from a discrete region in the cochlear basal turn. Using light microscopy, we traced the central axons through serial sections to their terminations in the ipsilateral cochlear nucleus. It has been difficult to mark the entire extent of these neurons in cats and guinea pigs so smaller mammals (gerbils and mice) were used in order to minimize the distance from injection site to termination.

In agreement with previous labeling studies in cats (Kiang, N.Y.S. et al., *Science* 217:175-177, 1982) and guinea pigs (Brown, M.C., *Soc. Neurosci. Abstr.* 12:1264, 1986), type-II cells in gerbils can be separated from type-I cells since they usually have smaller cell bodies and project centrally with thinner (less than 1 μm diam.) axons. In contrast to type-I axons, the thinner type-II axons lack obvious constrictions and areas of darker labeling associated with nodes of Ranvier; thus our observations are consistent with the hypothesis that the type-II central axons are unmyelinated.

In gerbils, HRP-labeled thin axons from three type-II somata were reconstructed from the spiral ganglion to their terminals in the cochlear nucleus with no apparent fading of any branches. In 5 other instances, the somata were obscured, but the thin axons were fully reconstructed within the cochlear nerve and nucleus. Other thin fibers, some traced from type-II somata, were partially reconstructed. Most of these thin fibers, however, were consistent with the following description: they coursed with the thicker type-I axons in the nerve and bifurcated in a similar region of the interstitial portion of the cochlear nucleus, formed ascending and descending branches, and emitted terminals in the anterior (AVCN), posterior (PVCN) and the dorsal (DCN) subdivisions of the cochlear nucleus. Although the type-II axons gave off fewer branches than type-I axons, the axon varicosities and terminal swellings of type-II fibers were as widely distributed throughout the cochlear nucleus as the type-I endings labeled in the same focal injections. However, in two specific areas, type-II terminations were found where type-I terminations were usually absent: 1) the superficial granule cell region of the AVCN dorso-medial to the type-I endings, and 2) layer II (the granule or pyramidal cell layer) of the DCN, superficial to the type-I endings. Preliminary results in mice are consistent with the gerbil results.

These results suggest that afferent information from outer hair cells, carried by thin type-II central axons, reaches all three major subdivisions of the cochlear nucleus. Type-I and type-II cells adjacent in the spiral ganglion have a topographical overlap for a portion of their cochlear nucleus endings, and a striking spatial segregation of the furthest central portion of the their arborizations, where the type-II endings are in areas having a high density of granule cells. Since branches from medial olivocochlear efferents also terminate in the superficial granule cell region of AVCN (Liberman, M.C., and Brown, M.C., *Hearing Res.* 24:17-36, 1986), efferent and type-II fibers associated peripherally with outer hair cells may converge centrally in a heretofore undescribed neural system. (Supported by NIH grants NS23508, NS20156, NS13126, GM07258, and the F.V. Hunt fellowship, Acoustical Society of America.)

350.3 AXONS OF PRESUMPTIVE TYPE-II SPIRAL GANGLION NEURONS SYNAPSE WITH GRANULE CELLS OF THE CAT COCHLEAR NUCLEUS. T.E. Benson and D.K. Ryugo. Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA 02115 and Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA 02114.

Type-II spiral ganglion neurons contact outer hair cells (OHCs) whereas type-I neurons contact inner hair cells (Kiang et al., Science, 217:175-177, 1982). Thus there is segregation of the afferent pathways for the two hair cell systems. Although much is known about the central projections of the type-I neurons, information concerning the type-II terminations is just emerging (Brown et al., preceding abstract; Ryugo et al., Soc. Neurosci. Abstr., 12:779, 1986). In the present study, we have analyzed central terminals of presumed type-II axons in the cochlear nucleus of the cat.

Data were derived from 2 cats where extracellular injections of horseradish peroxidase (HRP) were made into the auditory nerves and the tissue processed using diaminobenzidine. Thin fibers (0.5 µm diameter) were anterogradely labeled, and were identified as type-II axons using light microscopic criteria (ibid.). Serial section electron microscopy was then used to analyze 3 such fibers in the interstitial region of the cochlear nucleus, concentrating on varicosities, terminal swellings and postsynaptic targets. Throughout the lengths examined, these thin fibers were unmyelinated. Some varicosities and short collaterals contained mitochondria and vesicles but had no associated synapses.

Synapses originated from en passant and terminal swellings. All were of the asymmetric variety as judged by their postsynaptic density and contained vesicles having the same degree of roundness as those of HRP-labeled type-I terminals. Terminals of type-II axons, however, had vesicles of smaller diameter (46.3 $\pm$ 0.4 nm) compared to those of type-I axons (54.6 $\pm$ 0.4 nm) and a characteristically large proportion (41-77%) of their apposition with target structures was synaptic.

Dendritic shafts and spine-like profiles were the postsynaptic targets of type-II axons. One postsynaptic dendrite was observed to originate from a granule cell. In fact, the ultrastructure of all targets thus far examined is consistent with their origin from granule cells of the cochlear nucleus (Mugnaini et al., J. Neurocytol., 9:537-570, 1980). Granule cells may therefore represent a central locus for the convergence of type-II afferents and collaterals from the medial olivocochlear efferents. Such efferents contact OHCs (Liberman and Brown, Hearing Res., 24:17-36, 1986). A previously undescribed system for processing acoustic information involving OHCs and granule cells may be unfurling. [Supported by NIH grants NS13126, NS20156 and NSF grant BNS-20833]

350.4 MODULATION TRANSFER CHARACTERISTICS OF NEURONS IN THE DORSAL COCHLEAR NUCLEUS OF THE CAT. C.E. Schreiner* and R.L. Snyder* (SPON: J. Mendelson). Coleman Laboratory, 971 HSE, University of California at San Francisco, San Francisco CA 94143-0526.

In the face of growing evidence that neurons in the dorsal cochlear nucleus (DCN) might specifically encode behaviorally relevant aspects of temporally varying signals, amplitude modulated tones and noises were used to characterize temporal response properties of neurons within the DCN of chloralose-anesthetized cats. Single units were classified following the scheme of Young and Brownell (*J. Neurophysiol.* 39:282-300, 1976). After a basic characterization of148 single units, discharge rate and synchronization (vector strength = VS) modulation transfer functions (MTFs) were constructed. Best-frequency tonal stimuli and white or bandpass-filtered noises were used as carrier signals.

For signal levels 30 dB or more above the response threshold, synchronization MTFs had a low-pass, band-pass or double-peaked form. Double-peaked (W-MTF) curves consisted of a low-pass portion separated from a band-pass portion by at least a 30% deep notch in vector strength (VS). Two additional types of MTFs were frequently seen for rate MTFs: high-pass MTFs and all-pass MTFs. The majority of type II units (approximately 55%) exhibited low-pass synchronization MTFs. By contrast, relatively few type III and type IV units exhibited low-pass characteristics (26% and 11%, respectively). Band-pass synchronization MTFs constituted the largest group for type IV (67%) and type III units (62%), compared to 27% of type II units. The striking W-MTFs for synchronization were seen in approximately 22% of type IV units, 18% of type III units and 12% of type II units. Insufficient numbers of type I units were found to allow for any significant assessment of their MTF response characteristics.

For the populations of units with band-pass synchronization MTFs, a range of "best modulation frequencies" (BMF = the modulation frequency that produced the highest VS) was recorded. For units with CFs between 4 and 8 kHz, the encountered BMFs ranged from about 90 Hz to 630 Hz, with a mean of 290 Hz. The upper boundary of the BMF range appeared to be a function of CF. BMF ranges for different unit types largely overlapped ; no clear distinction of their BMF ranges was apparent.

The phase function of band-pass synchronization MTFs showed a smooth progression as a function of modulation frequency, compatible with a constant-delay hypothesis. In contrast, phase function of W-MTFs often showed a discontinuity at modulation frequencies corresponding to the notch in the VS curve; the MTF portion below and above the notch frequency had different phase characteristics. These results indicate that the two different portions of W-MTFs probably reflect different sources of input and/or modes of operation.

Additional evidence for the interaction of various inputs in the definition of temporal response characteristics of single unit was obtained by the use of amplitude modulated noises. Using noise of various bandwidth, the synchronization MTF type could commonly be converted, for example, from a low-pass MTF for a carrier tone, to a band-pass MTF for a narrow-band noise, to a W-MTF for a broad band noise.

The possible significance of these phenomena for periodicity coding is complicated by observed population differences, and by the apparent mutability of MTF response characteristics as a function of signal complexity.

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350.5 SYNAPTIC DEVELOPMENT IN THE MONGOLIAN GERBIL DORSAL COCHLEAR NUCLEUS G. DiCarlantonio* & I.R. Schwartz, Head & Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024

As the first step in an ongoing investigation of synaptogenesis in the gerbil cochlear nucleus, synaptic connections within the neuropil of the DCN were assessed. As the major synaptic element of the DCN molecular layer (ML), the development of the parallel fiber (PF) synapse is emphasized. Animals at 6,7,10,12,14,16,18, 20,22,28,76,88 & 120 days after birth (DAB) were perfused with mixed aldehyde fixatives and prepared for electron microscopy.

At 7 DAB, granule cell PFs are of small caliber with a few neurotubules and mitochondria and frequently contain vesicles. Although vesicle-containing terminals are rare, they increase in number at successively deeper levels of the ML. Most terminals are only slightly enlarged with a few round synaptic vesicles loosely associated with the presynaptic density. In the deeper DCN, PFs are sparse and large unmyelinated axons are present. Most dendrites are smooth-contoured with infrequent spines; these rarely receive synapses.

Three striking changes are present at 14 DAB. 1) There is a great proliferation of dendritic profiles; most have a swollen appearance, sparse matrix, and a few spines. 2) There is a great increase in number of PF boutons which tend to be more mature at deeper levels. The more developed terminals are expanded in volume with a greater density of synaptic vesicles, many of which are associated with the presynaptic density. The postsynaptic density is markedly thickened. Boutons which contact dendritic spines are frequently invaginated by the spine. 3) Number of synapses per unit area has increased. The majority of synapses are formed by PF boutons, but both the number of classes and density of terminals increase at successively deeper levels. Several types of boutons with a denser matrix occur. The most frequently encountered is large, irregular, contains 3-6 mitochondria, numerous large, round synaptic vesicles and forms moderately long, postsynaptically thickened synapses on large caliber dendrites.

By 21 DAB most PF terminals contact dendritic spines and are invaginated by the spines to form cup-shaped profiles. There is further proliferation of dendritic profiles; most are the profusely branching trunks studded distally with spines typical of fusiform and cartwheel neuron dendrites. Although present at 14 DAB, large caliber myelinated axons become more prominent in the deeper areas at this time.

Older animals showed few additional changes in synaptic structure, indicating that synaptic maturation, as manifested by morphologic features and density of synaptic connections, is virtually complete as early as 21 DAB.

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350.6 LOSS OF CARTWHEEL NEURONS IN THE DORSAL COCHLEAR NUCLEUS OF THE MOUSE MUTANTS LURCHER, PURKINJE CELL DEGENERATION AND STAGGERER. A.S. Berrebi, J.I. Morgan, and E. Mugnaini, Laboratory of Neuromorphology, Biobehavioral Sciences Graduate Degree Program, Univ. of Connecticut, Storrs, CT 06268 and Dept. of Neuroscience, Roche Institute of Molecular Biology, Nutley, NJ 07110.

Several lines of research have suggested that within the mammalian dorsal cochlear nucleus (DCoN) there exists a cerebellar-like network of neurons. Like the cerebellar cortex, the DCoN contains mossy fibers, granule and Golgi cells, and parallel fibers. In the superficial layers of DCoN, two distinctive neuron families, the spiny cartwheel cells and the nearly aspiny stellate cells, bear some striking similarities to cerebellar Purkinje and stellate cells. In particular, cartwheel cells and Purkinje cells share numerous cytological and synaptic features and are glutamate decarboxylase and cerebellin-positive.

We have previously shown in "nervous" mice, a mutation which produces a characteristic mitochondrial alteration in Purkinje cells, that cartwheel cells are the only neurons in the cochlear nuclear complex that express the same anomaly as Purkinje cells. Moreover, the respective mitochondrial alterations appear within a comparable time frame (Berrebi and Mugnaini, Anat. Rec., 1987). We report here a cartwheel cell loss in lurcher, pcd, and staggerer, all murine mutants that suffer Purkinje cell death. On the contrary, in the weaver mutation cerebellar Purkinje cells and, likewise, DCoN cartwheel neurons are spared. The data are derived mainly from immunocytochemical analysis of serial brain sections treated with the PAP procedure of Sternberger after incubation with a rabbit primary serum raised against the recently discovered peptide, PEP-19 (Ziai et al., P.N.A.S., 1987). This neuropeptide, which contains a calcium-binding sequence, is an excellent marker for both Purkinje and cartwheel cells and the immunoreaction reveals not only their cell bodies, but also their dendritic and axonal processes (Mugnaini et al., Arch. Ital. Biol., 1987, in press). These findings support the hypothesis that cartwheel cells in the cochlear nuclei are homologous to Purkinje cells in the cerebellar cortex. These neuronal populations may arise from the same embryonic precursors, although their mature phenotypes are not identical (Wouterlood and Mugnaini, JCN, 1984). Studies of additional murine mutations affecting the cerebellum are in progress.

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350.7 IMMUNOSTAINING OF GABA-ERGIC AND GLYCINERGIC INPUTS TO THE ANTEROVENTRAL COCHLEAR NUCLEUS. J. C. Adams, and R. J. Wenthold. Department of Otolaryngology and Communicative Sciences, Medical University of South Carolina, Charleston, SC 29425 and Lab of Neuro-otolaryngology, NIH, Bethesda, MD 20205.

There are a variety of inputs to the rostral pole of the cochlear nucleus but few details concerning the nature of these inputs are known. In this study HRP was injected into the anteroventral cochlear nucleus of cats to identify individual inputs. Sections were incubated to visualize the transported HRP and then incubated to immunostain either GABA or glycine. In the deep portions of the dorsal cochlear nucleus (DCN) there are many medium sized pleomorphic neurons that project to the rostral portion of the ventral nucleus. Large numbers of similar appearing cells in the deep DCN immunostain for glycine. A much smaller number of similar appearing cells in the deep DCN immunostain for GABA. HRP-labeled cells in the deep DCN were found to be immunoreactive for glycine and others for GABA. The possibility that both antigens are in the same neurons was not tested. In the lateral nucleus of the trapezoid body, HRP-labeled cells were also found to be immunostained for glycine and others for GABA. In the ventral nucleus of the trapezoid body, there are large numbers of GABA-ergic cells and few, if any, glycinergic cells. Accordingly, in this region HRP-labeled cells were found that immunostained for GABA, but none for glycine. These results suggest that a large proportion of the inputs to the anteroventral cochlear nucleus from the olivary complex and perhaps all intrinsic inputs from the DCN are inhibitory. The bulk of the DCN inputs appear to be glycinergic; all the inputs from the ventral nucleus of the trapezoid body appear to be GABA-ergic; and numbers of inputs from lateral portions of the olive appear to be equally glycinergic and GABA-ergic.

There is considerable heterogeneity between the various inputs to the anteroventral nucleus with regard to immunostaining with antisera to peptides. The glycinergic cells in the deep DCN appear to also immunostain for enkephalin and for neuropeptide Y. The GABA-ergic cells in the ventral nucleus of the trapezoid body immunostain for enkephalin and a subpopulation of these that project to low frequency portions of other nuclei immunostain for neurotensin. The heterogeneity of staining of these various inputs may be helpful in indentifying specific terminals within the anteroventral cochlear nucleus. Elucidation of these inputs should promote understanding of the organization of the nucleus.

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350.8 CHANGES WITH ADVANCED AGE IN THE MORPHOLOGY OF THE RAT AUDITORY NERVE. V. Hoeffding* and M.L. Feldman. Dept. of Anatomy, Boston Univ. School of Medicine, Boston, MA 02118.

While cell loss in the spiral ganglion and degeneration of terminals in the cochlear nucleus have both been observed in aging rats (J. Comp. Neurol., 188, 429, 1979; Neurosci., 7, S67, 1982; Gerontologist, 22, 213, 1982), the effect of advancing age on the auditory nerve has not been investigated in this species. The present study is investigating auditory nerve structure in a series of 24 rats ranging from young adulthood (2 mo) to extreme advanced age (36 mo). The animals were perfused via the aorta with mixed aldehydes; blocks including the auditory nerve and part of the ventral cochlear nucleus were removed, embedded in Araldite, and sectioned in a plane transverse to the axis of the nerve. The analysis of the material includes counts of normal and degenerating auditory nerve fibers and of glial cells, mapping of fiber packing densities, and measurements of vascularity and of the cross-sectional areas of the nerves.

Results obtained thus far suggest that the number of normal auditory nerve fibers declines from a mean of about 21,500 in young adult rats to a mean of about 17,000 in the oldest animals studied. While there is substantial variability around these numbers, this represents an approximate fiber loss of 21% at 36 mo. Despite this loss of fibers, there is an age-related increase of about 50% in the cross-sectional area of the nerve. This increase in area accompanies a decrease in the packing densities of auditory nerve fibers and an increase in the thickness of myelin sheaths in older animals. Glial lipofuscin and cytoplasmic debris are occasionally encountered in young adult animals, but these deposits clearly increase in frequency and size with advancing age.

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350.9 SPIRAL GANGLION CELL DENSITY IN YOUNG AND OLD GERBILS. E.M. Keithley, A.F. Ryan and N.K. Woolf. Division of Otolaryngology, Dept. of Surgery, UCSD Medical School and Veterans Administration Medical Center, La Jolla, CA 92161.

Recently it was shown that the cochlear nucleus (CN) of adult gerbils (Meriones unguiculatus) contains a microcystic neuropathy (Ostapoff et al., ARO, 10:209, 1987). In order to determine if there is an associated loss of spiral ganglion cells (SGC) which project to the CN, the number of SGC was counted in 2 month old and 29 month old animals. The maximum reported lifespan of gerbils in 48 months (average = 36 month old).

Subjects chosen for this study had normal external and middle ears and physiological data was gathered prior to perfusion. (see Neuroscience Abs. Woolf et al, 1987). Animals were perfused with mixed aldehydes. Cochleas were dissected and embedded in araldite. 2 um sections were stained with azure II. Nuclei of SGC were marked on paper with the aid of a drawing tube attachment on the microscope (500x) and counted. The density of SGC within each profile of the ganglion (#/mm^3) was computed by dividing the number of cells in a given profile by the volume of that profile. The profile volume was determined by measuring the area of Rosenthal's canal (mm^2) with a computer planimetry program and multiplying by the section thickness (mm) plus a factor to correct for the fact that the sections are much thinner than the cell nucleus.

The average cell density of the gerbil SGC is 1 x 10^5 cells/mm^3 and is relatively constant throughout the ganglion and the older animals had similar cell densities. The SGC appeared normal in size and did not contain lipofuscin granules in the older animals.

Young adult gerbils have a similar cell density to that seen in the rat and the cat. The lack of cell loss and lipofuscin accumulation in the older animals suggests that these animals may be just below the onset time for degenerative changes seen in the aged animals (60% of maximum life span). Cell loss and lipofuscin were reported for the rat to occur at 70% of the maximum life span. (Keithley and Feldman, J.C.N. 188:4292, 1979). It seems then that the microcystic inclusions in the CN are not the result of SGC degeneration.

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350.10 PHYSIOLOGY AND ANATOMY IN THE AGING MONGOLIAN GERBIL AUDITORY SYSTEM. N. K. Woolf[1], A.F. Ryan[1], E.J. Silva*[1], E.M. Keithley[1] and I.R. Schwartz.[2] [1]Division of Otolaryngology, Department of Surgery, UCSD Medical School and Veterans Administration Medical Center, La Jolla, CA 92161 and [2]Division of Head and Neck Surgery, UCLA School of Medicine, Los Angeles, CA 90024.

Cochlear microphonic (CM), auditory nerve N1 compound action potential (AP) and auditory brainstem evoked potentials (ABR) were recorded from mongolian gerbils at either 30 DAB (days after birth) [N=7], 90 DAB [N=10] or 2-3 years of age (Mean=2.4 years; S.D=0.275) [N=9]. The average life span of the gerbil is 3.03 (±0.64) years.

Only cochleas for which external canals and middle ears were normal were included. All of the 30 DAB and 90 DAB candidates met these criteria. In contrast, 2/3's of the 25 older candidates presented impacted ears: 8 had two normal ears and 9 had only one.

Relative to 90 DAB subjects, the mean CM audiogram for the older gerbils exhibited a flat loss of approximately 10 dB for frequencies at or below 16 kHz, with a more pronounced loss of 22 dB at 32 kHz ($p < .02$, ANOVA). AP and ABR thresholds for the older gerbils were also increased approximately 20 dB above the 90 DAB values ($p < .02$, t-test). At 20, 40 and 60 dB SL, ABR morphology, peak latencies and inter-peak latencies for the three groups were similar.

Light microscopic examination of the cochleas of the older gerbils revealed good preservation of the organ of Corti, spiral ganglion cells, and stria vascularis.

The spongiform encephalopathy first noted by Ostapoff et al. (ARO 10:209,1987) was observed in all of the older gerbils. However, the older gerbils with clean external and middle ears used in the present study showed less pathology than a group of unselected animals 4-6 months old (which showed similar pathology to that reported by Ostapoff). Preliminary examination of the PVCN and other affected regions of the older subjects indicate no marked decrease in cell size or number, or loss of specific nuronal populations, associated with the pathology.

The physiologic and anatomic data suggest that the hearing loss in the older animals is of peripheral origin. They further indicate that when the condition of the external and middle ears is normal, brainstem cytopathology, though present, is not extensive. The data also suggests that the encephalopathy may not be strictly progessive with age.

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350.11 ENDOCOCHLEAR POTENTIALS DECREASE WITH AGE IN GERBILS. R.A. Schmiedt, J.H. Mills*, and J.C. Adams. Department of Otolaryngology and Communicative Sciences, Medical Univ. of S.C., Charleston, SC 29425.

Endocochlear potentials (EPs) were recorded at four places along the cochlear duct in young and old gerbils. Measurements were obtained with micropipettes in scala media at 0.5, 4.0, 8.0 and 11.0 mm from the oval window. These distances correspond respectively to best frequencies of about 30, 8, 2 and 0.5 kHz. EP values measured in eight controls with ages less than 12 months showed little scatter between animals and followed known trends. EP values measured in similar fashion in 10 gerbils with ages between 29 and 31 months were significantly less than that of the controls in the very basal and apical regions of the cochlea. The table below compares the average values of the EP and the standard error of the mean (SEM) at each cochlear location for young and aged gerbils.

Location (mm):	0.5	4.0	8.0	11.0
Frequency (kHz):	30.0	8.0	2.0	0.5
Young EP (mV):	91.2	78.8	72.3	72.7
Young (± SEM):	1.9	2.9	4.0	3.7
Aged EP (mV):	76.7	74.2	73.1	45.9
Aged (± SEM):	2.7	4.3	4.7	9.8

Whole nerve action potentials (APs) recorded at the round window to tone pips showed that the older gerbils had threshold shifts relative to the controls of about 12 dB at 20 kHz, 13 dB at 8 kHz, 10 dB at 2 kHz, and 14 dB at 0.5 kHz. These shifts were not expected given the previously reported dependence of neural thresholds on acute changes of the EP brought about with furosemide (Sewell, W.F., J. Physiol., 347:685, 1984). In particular, the 30 mV decrease in the EP of the apical turn would be expected to be associated with a greater threshold shift than 14 dB. On the other hand, the AP shifts associated with the middle turns are not correlated with any EP shift. However, AP amplitudes were greatly reduced at suprathreshold levels at all frequencies in the old gerbils. Thus, the relation between EP and neural characteristics in aging animals is complex. Further, there is evidence that the decreased EP in at least the apical turn is due to changes in the functional anatomy of the lateral wall of the cochlea.

350.12 ULTRASTRUCTURE OF THE END BULB OF HELD IN CONGENITALLY DEAF CATS. S.A.Larsen and T.M.Kirchhoff*. Depts. of Anat. and Otolaryn., Univ. of Louisville Med. Sch., Louisville, Ky. 40292.

The cochlear nuclear complex is the first synaptic relay in the central auditory pathway. Within this complex, all information conveyed from the hair cells of the cochlea through the auditory nerve is received and recoded to be conveyed to higher levels in the pathway. Bock et al (Brain Res, 239:608, 1982) concluded that at least some central connections remain functional in the central auditory pathway in the absence of peripheral stimulation and that function may be restored by direct nerve stimulation. On the other hand, Gulley et al (Brain Res, 158:279, 1978) suggested that peripheral stimulation is important for maintaining synapses.

Using computer-assisted morphometric analysis of electron micrographs, we have studied the end bulb of Held (EB) synaptic termination of the auditory nerve on the soma of large spherical cells in the rostral anteroventral cochlear nucleus of normal-hearing (NH) and white-deaf adult cats (WD). WD cats are profoundly deaf from birth as a result of genetically determined cochlear degeneration and were selected from the investigator's breeding colony. Auditory brainstem responses were used to determine if cats were unilaterally or bilaterally deaf. Scanning electron microscopy revealed massive degeneration of the hair cells in the cochleas of the WD cats compared to that of the NH cats. There was considerable variability to the degree of auditory nerve fiber degeneration found in the WD cats but they all had extensive degeneration of the neurons in the anteroventral cochlear nucleus. In WD cats, large spherical cells and synaptic terminations of the EB were smaller compared to that found in NH cats. In addition, the EB had fewer membrane specializations associated with active zones and intact active zones were characterized by extremely dense presynaptic specialization and few synaptic vesicles.

Electrical stimulation of remaining auditory nerve fibers of profoundly deaf patients assumes that transneuronal effects of hair cell loss does not impair the function of remaining neurons or that the function can be reversed by electrical stimulation. We have reported degenerative changes in both the termination of the first-order fiber and the second-order neuron of the central auditory pathway. Further work to determine the reversibility of these changes with electrical stimulation is in progress.

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350.13 AGE-RELATED CHANGES IN THE VESTIBULAR GANGLION OF C57BL/6 MICE. J. C. PARK and V. A. FEDOR*. Dept. of Biological Sciences, Florida Institute of Technology, Melbourne, FL 32901.

We have been examining age-related changes in the mouse vestibular system in order to understand normal vestibular aging. This preliminary report concerns the vestibular (Scarpa's) ganglion of C57BL/6NNia mice. Ganglia from six young (5-9 wk) and six old (26-29 mo) mice were dissected and immersed in buffered glutaraldehyde. The superior and inferior divisions were separated and embedded in epoxy after routine osmium post-fixation and dehydration in acetone. The right ganglion from each mouse was serially sectioned into 5 um thick sections for cell counts. Nucleoli in every eighth section were counted, multiplied by the intervening sections and the total corrected for split nucleoli. The left ganglia were sectioned for TEM examination.

The most prominent ultrastructural change in the old neurons was an accumulation of membrane bound pigment, lipid droplets and foamy appearing inclusions, especially at the axon hillock. In older neurons, the myelin sheath appeared less well organized and contained myelin whorls.

Contrary to the spiral ganglion serving the cochlea, old mice maintained a constant number of vestibular neurons well past the mean of their expected life span. Young mice had approximately 2231±171 neurons and old mice had 2198±403. About 65% of the cells comprised the superior division supplying the horizontal and anterior ampullae and the utricle. The remainder were in the inferior division supplying the posterior ampulla and the saccule. The majority (94-97%) of neurons in both young and old ganglia were large, oval to round cells with a mean size of 31X24 um. These large cells were sheathed by many layers of compact myelin. The remainder were unmyelinated, smaller cells with an average size of 18X15 um.

Compared to the spiral ganglion of this mouse strain, where cell degeneration and loss is severe by 26 mo (G. M. Cohen and J. S. Grasso, Assoc. Res. Otolaryngol. Abstr., p. 120, 1987), aging changes in Scarpa's ganglion develop more slowly.

(Supported by grant AGO-4889, National Institute of Aging.)

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CEREBELLUM III

351.1 AN ANALYSIS OF THE RECURRENT COLLATERALS OF PURKINJE CELLS IN ZONE X OF THE CAT'S CEREBELLUM. Georgia A. Bishop Department of Anatomy and Neuroscience Program. The Ohio State University, Columbus, Ohio 43210

In the present study, the axonal collaterals of 17 Purkinje cells in zone x have been analyzed subsequent to intracellular recording and staining of these cells with horseradish peroxidase. Nine cells responded to activation of the upper limb and 5 to stimulation of both the upper and lower limbs. The axonal collaterals of zone x Purkinje cells extend an average of 294.2 μm in the transverse plane and 520.8 μm in the sagittal plane. The collaterals of 10 of the 17 Purkinje cells remain confined to zone x. Five Purkinje cells have collaterals that extend into zone a and two project to zone b. Fifty-three percent of the varicosities derived from the recurrent collaterals (average number of varicosities/plexus = 98) are located within the Purkinje cell layer, 27% extend into the molecular layer, some near the pial surface, and 20% distribute to the granule cell layer. A comparison of collaterals derived from zone x Purkinje cells with those from zones a, b, and c (Bishop et al., New Concepts in Cerebellar Neurobiology, 1987) indicate that the primary lamina of distribution for the varicosities in all four zones is the Purkinje cell layer. However, two features distinguish the collaterals of zones x Purkinje cells. First, the average transverse extent of zone x collaterals is greater than that seen in other zones. Second the presence of beaded branches that extend to superficial aspects of the molecular layer are not present in other zones. This distribution pattern suggests that the sphere of influence of zone x Purkinje cell collaterals is greater in the transverse plane. In addition, the postsynaptic targets of zone x collaterals may be more varied than the collaterals derived from Purkinje cells in zones a, b, and c; potential targets in the molecular layer include stellate cells, the dendrites of Purkinje, basket and/or Golgi cells. However, the primary synaptic interactions are likely comparable to those described for zones b and c; these include the proximal dendrites of Purkinje cells and basket cells. The data obtained in the present study, combined with those derived from previous studies, indicate that there are features of the pattern of distribution of the recurrent collaterals that are uniform for Purkinje cells in zones, a, x, b, and c. However, the data also indicate that there are aspects of the collateral distribution, with respect to lamina and extent, that are specific to each zone. Therefore, afferent information may not be integrated via these local circuits in the same manner in each zone. (Supported by NSF BNS 8505971)

351.2 THE SYNAPTIC RELATIONSHIP BETWEEN PURKINJE CELL AXON COLLATERALS AND BASKET CELLS IN THE LATERAL VERMIS OF THE CAT. D.L. O'Donoghue, G.A. Bishop and J.S. King. Dept. of Anatomy and Neuroscience Program, Ohio State University, Columbus, OH 43210.

Intracellular recordings were obtained from basket cells (identified by intracellular injections of horseradish peroxidase, HRP) located in lobule V. Stimulation of the inferior cerebellar peduncle or peripheral nerves elicited a slow-rising, low amplitude, excitatory post synaptic potential that was interrupted by a fast-rising, large amplitude, inhibitory post synaptic potential (IPSP). Hyperpolarizing currents of low intensities, passed through the recording electrode, reversed the IPSP. The amplitude of the evoked IPSP was not graded with changes in stimulation intensity. At threshold intensities, the response was "all or none" in nature. These findings indicated this inhibitory input was derived from a single neuron. The IPSP's fast rise-time, and its reversal with low levels of hyperpolarizing currents, indicated that the inhibitory synapses derived from the single presynaptic cell were located on or close to the soma.

In order to determine the anatomical substrate for the unitary IPSP, the perimeters of two basket cells were examined in serial thin sections. The majority of synaptic profiles on the cells were small (<1 μm) and contained round vesicles. Two and four large boutons (1.5-2.5 μm) synapsed on each cell, respectively; they contained pleomorphic vesicles, numerous mitochondria, and a darkened cytoplasmic matrix. Two of the boutons originated from myelinated fibers of the ganglionic plexus. The cytological characteristics of the large boutons were comparable to the terminals of Purkinje cell collateral described previously by Mugnaini (in: The Comparative Anatomy and Histology of the Cerebellum, '72). However, this serial section analysis could not address the unitary nature of the relationship between Purkinje cells and basket cells. To determine this, Purkinje cells and their recurrent collaterals were stained intracellularly with HRP. Serial thin sections were cut through a portion of one collateral plexus. One to three HRP labeled varicosities from this plexus synapsed on at least three different basket cells. These labeled varicosities were 1.5-2.0 μm in diameter, contained pleomorphic vesicles, and numerous mitochondria. Although over 1/2 and 3/4 of the surfaces of two basket cells were examined, no unlabeled boutons were found with cytological features indicative of a recurrent collateral.

These physiological and anatomical data indicate that individual basket cells receive axosomatic terminals of recurrent collaterals derived from a single Purkinje cell. In addition, a single Purkinje cell may modify the activity of more than one basket cell. (Supported by NSF 8505971 and NIH 07814)

351.3 THE DEVELOPMENT OF ENKEPHALIN IMMUNOREACTIVE ELEMENTS IN THE OPOSSUM'S CEREBELLUM. J.J. Walker, R.H. Ho, and J.S. King. Department of Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

Enkephalin (ENK) immunoreactive climbing fibers, mossy fibers and a beaded plexus of axons have been described in the adult opossum's cerebellar cortex (King et al., New Concepts in Cerebellar Neurobiology, 1987). In the present study we have used this peptide as a marker to study the ontogeny of these three forms of axon terminals. The indirect antibody peroxidase anti-peroxidase technique was used to localize ENK immunoreactivity in the cerebellum of pouch young opossums from postnatal day (PD) 1 to PD 83. By PD 5 ENK fibers with growth cone-like terminal expansions are present in the intermediate layer of the cerebellar anlage. Subsequently, the number of ENK fibers increase in the intermediate, and ventricular layers and by PD 18 discrete regions of dense punctate immunoreactivity are present within the nascent Purkinje cell layer and the undifferentiated cellular area deep to this layer. These immunoreactive elements are likely composed of immature mossy fibers as well as climbing fibers. Pericellular nests (nids) around Purkinje cell somata can be visualized by PD 35 and follow a sequence of development through PD 83 paralleling that described in Golgi preparations of human climbing fibers (Marin-Padilla, M., J. Comp. Neurol., 235:82-96, 1985). Immature mossy fiber rosettes can be identified in the internal granule cell layer on PD 40 and subsequently follow a sequence of maturation comparable to that described in Golgi impregnations of the opossum's cerebellum (O'Donoghue et al., Anat. Embryol., 175:341-354, 1987). Beaded ENK axons can be identified on PD 68 where they form a diffuse system of fibers primarily along the granule cell-Purkinje cell border. Between PD 40 and PD 68 there is generally an overlap in the distribution of developing ENK climbing and mossy fibers in discrete regions of vermal lobules II-VIII and X, whereas in the hemispheres climbing fibers predominate. However, in the adult ENK positive climbing fibers are no longer seen in the lateral folia (King et al., New Concepts in Cerebellar Neurobiology, 1987). The present results indicate that the terminal expansions of early arriving ENK axons are not present in cellular layers undergoing mitotic division. In addition, the transient appearance of ENK in discrete populations of developing climbing fibers in the lateral hemispheres suggests that: 1) cell death in the inferior olive, 2) the retraction of collaterals, or 3) a transient expression of this peptide, may be developmental events characteristic of this chemically defined system of axons. (We thank Dr. R. Elde for ENK antiserum 156C. Supported by NS-08798)

351.4 CORTICOTROPIN RELEASING FACTOR DISTRIBUTION IN THE CEREBELLUM AND PRECEREBELLAR NUCLEI OF THE CAT. Sharon Cummings, Georgia A. Bishop and James S. King. Dept. of Anatomy and Neuroscience Program, Ohio State University, Columbus, Ohio 43210

Corticotropin releasing factor (CRF) has been reported in some cerebellar afferent systems (Cummings et al., J. Neurosci., in press). As part of a continuing analysis of CRF in cerebellar circuitry, we have employed the indirect peroxidase, anti-peroxidase technique to map the distribution of CRF-immunoreactive (CRF-IR) fibers within the cerebellum and the precerebellar nuclei of the cat. Serial 60um transverse and sagittal sections of brains from adult cats perfused transcardially with picric-acid/phosphate (0.1M) buffered 2% paraformaldehyde were incubated with a primary antiserum to the rat CRF sequence.

CRF-IR climbing fibers were present throughout all cerebellar lobules, though the density of the fibers and the intensity of immunostaining was not uniform. CRF-IR mossy fibers were also widely distributed in the cerebellar cortex, but also were not uniform in density or in staining intensity. A plexus of CRF-IR fibers was present in the Purkinje cell layer; collaterals of CRF-IR climbing and mossy fibers may contribute to this plexus. Within the overall distribution of CRF-containing cerebellar fibers, several prominent patterns were apparent. The vermis contained five bands of intensely immunoreactive mossy fibers; these were most easily defined at the base of the lobules and in the anterior lobe. Climbing fibers were continuous across the width of the vermis in lobules I-X, though more darkly stained median and parasagittal bands of climbing fibers accompanied the mossy fiber bands of the anterior lobe. A wide band of darkly stained climbing fibers also extended rostro-caudally through the intermediate cortex of the anterior lobe; caudally this band merged with a climbing fiber band in the lateral aspect of vermal lobules VI and VII. The paramedian lobule contained a prominent median band of immunostained climbing fibers; few mossy fibers were apparent in this lobule. CRF-IR climbing fibers were present throughout lobus simplex and crus I; mossy fibers within crus I were more evident rostrally than caudally. A dense band of climbing fibers extended through the midline of crus II; mossy fibers were few in number, but increased rostrally at the base of the lobule. CRF-IR climbing and mossy fiber distribution in the flocculus and paraflocculus was irregular and patchy in appearance, and confirmed observations of earlier studies. Both fiber types were more heavily represented in the flocculus than in the paraflocculus. Finely beaded CRF-IR fibers, present in all deep cerebellar nuclei, were more abundant laterally than medially.

In colchicine pretreated brains, CRF-IR cell bodies were localized in several brainstem nuclei known to project to the cerebellum. In addition to CRF-containing somata in the inferior olivary complex, previously reported to be a source of CRF-containing climbing fibers (Cummings et al., J. Neurosci., in press), immunostained perikarya were present in the lateral, subtrigeminal, magnocellular and paramedian reticular nuclei; medial and inferior vestibular nuclei; the perihypoglossal complex, locus ceruleus, parabrachial nucleus and several raphe nuclei.

The existence of CRF in regions of the brainstem known to project to the cerebellum, and the wide distribution of CRF-containing climbing fibers and mossy fibers in the cerebellum, suggest a major functional role for this neuropeptide in cerebellar circuitry. We thank Dr. Burt Sharp for the CRF antiserum. (NS 08798).

351.5 DORSAL COLUMN NUCLEI PROJECTIONS TO THE INFERIOR OLIVE IN THE CAT. M.C. Perreault*, J. Courville. Département d'anatomie, Université de Montréal, Montréal (Québec) CANADA H3C 3J7.

Injections of high specific activity ^{3}H-leucine in the dorsal column nuclei and in all adjacent structures were used to demonstrate the projections to the olive. Two major areas of distribution are demonstrated. Within the rostral half of the olive, a predominantly contralateral distribution to most regions of the dorsal accessory olive originates from the cuneate nucleus. Nucleus gracilis only provides bilateral projections to the lateral regions of dorsal accessory olive. Rostral parts of the nucleus gracilis were not investigated. In addition, the cuneate nucleus sends extensive and dense projections to the medial accessory olive in the caudal half of the olive. Those are mostly ipsilateral. The main source of the projections to the olive appears to arise from ventral cells of the caudal two thirds of the cuneate nucleus. However, cells of all regions of the cuneate nucleus participate to the projection without any clear topographical arrangement. Several control injections in surrounding nuclei have been used to assess the contribution of the spinal trigeminal nucleus, dorsal reticular formation, nucleus of the solitary tract and the vestibular nuclei. There are overlaps between the territories of distribution of the dorsal column nuclei, and between those of the cuneate nucleus and the spinal trigeminal nucleus.

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351.6 CORTICOTROPIN-RELEASING FACTOR-LIKE IMMUNOREACTIVITY (CRFLI) IN INFERIOR OLIVE PERIKARYA AND IN CLIMBING FIBER-LIKE PROFILES IN CEREBELLAR CORTEX OF MONKEY. C. I. Cha* and S. L. Foote (SPON: J. H. Carlson). Dept. Psychiatry, Univ. Calif., San Diego, CA, 92093.

Immunohistochemical methods were utilized to characterize the distribution of CRFLI in brain and spinal cord of two monkey species (Saimiri sciureus, Macaca fascicularis). As previously reported for both rodent and primate, many neurons in the parvocellular component of the paraventricular nucleus were labeled, and dense fibers were evident, streaming toward the median eminence from this nucleus. In other brain areas, substantial differences were evident between the distributions of labeled elements in monkey and those previously reported for rat. For example, although colchicine pretreatment was not used, densely labeled cells were evident in the monkey inferior olive. The vast majority of olivary neurons were immunoreactive, and perikarya in the medial accessory olive (MAO) were especially densely stained. In the cerebellar cortex, intensely reactive fibers were present in the molecular and Purkinje cell layers. These fibers exhibited many of the morphological characteristics of climbing fibers. For example, in sagittal sections, highly divergent axonal arbors, originating from individual axons at the base of the molecular layer, were evident. In coronal sections, only thin, paired profiles were present. Labeled fibers were most dense in the uvula, dorsal paraflocculus, and pyramis, areas which receive their climbing fiber input mainly from the MAO. In the vermis and intermediate zone, labeled axons were often organized into parasagittal "stripes" similar to those formed by subpopulations of climbing fibers. Labeled fibers were less dense in the lateral hemispheres, where stripes were not often apparent. Labeled axons exhibited other characteristics of climbing fibers: they formed efflorescences in the cortical granular layer and were evident in cerebellar nuclei. The present observation of CRFLI in the monkey olivo-cerebellar pathway is compatible with the previous observation of CRF mRNA within olivary neurons of rat, baboon, and human (Young et al., Molec. Brain Res. 1: 189-192, '86) and previous immunohistochemical findings in cat (Cummings et al., Neurosci. Abstr. 11: 683, '85) and human (Powers et al., Neurosci. Abstr. 12: 568, '86). The present observations thus reinforce the suggestion that CRF is contained within the primate olivo-cerebellar pathway. Furthermore, they indicate that MAO neurons and their climbing fiber projections may contain higher levels of CRF than other olivary subdivisions. Antisera generously provided by W. Vale and J. Rivier, Salk Institute.

351.7 EVIDENCE FOR THE EXISTENCE OF GLUTAMINERGIC PONTINE AFFERENT SYSTEMS IN THE RAT. B.G. Border and G.A. Mihailoff, Dept. of Cell Biology and Anatomy, Univ. of Texas Health Science Center, Dallas TX 75235.

The amount of biochemical evidence suggesting that glutamate (GLU) is an excitatory neurotransmitter in the central nervous system (CNS) has increased tremendously in the past decade. Recently, immunocytochemical (ICC) investigations have demonstrated large populations of neurons in many regions of CNS to be glutaminergic, including cells of the basilar pontine nuclei (BPN). In the case of the pontine nuclei, the glutaminergic neurons have been demonstrated to be components of the pontocerebellar system; these neurons are known to form mossy fiber terminals in the cerebellar cortex and synapse with glutaminergic granule cell dendrites. Little attention has been given, however, to the presence of glutaminergic axon terminals in the pontine neuropil, although several of the regions demonstrated to contain glutaminergic neurons are sources of afferent fibers to the pontine gray. Therefore, through the combination of retrogradely transported WGA/HRP and GLU immunocytochemistry, we have demonstrated several pontine afferent systems to be glutaminergic.

Following a ventral injection of WGA/HRP into the BPN, adult Long-Evans rats were perfused intracardially, the brains sectioned and reacted with TMB to visualize the WGA/HRP reaction product, and the tissue sections incubated with either a mouse monoclonal antibody (provided by Dr. A. Beitz) or a rabbit polyclonal antibody (provided by Dr. P. Petrusz), both directed against glutamate. Immunoperoxidase methods (either biotin-avidin or PAP) were used to visualize GLU-containing elements in the BPN. Our principal findings in these studies are the following. (1) As reported previously (Beitz et al., Neurosci 17(3):741-753, 1986), neurons in many subdivisions of the basilar pons appear to be immunoreactive for glutamate. (2) GLU-immunoreactive axons and axon terminals are present throughout the rostro-caudal extent of the BPN. (3) Double-labeling studies reveal neurons in the cerebral cortex, the dorsal column nuclei, the deep cerebellar nuclei, and the zona incerta that are labeled with both the black reaction product of retrogradely transported WGA/HRP and the reddish-brown product of ICC. Therefore, it can be assumed that these systems provide glutaminergic axons that terminate within the BPN. (4) Preliminary EM studies demonstrate GLU-positive synaptic terminals, neuronal somata, and dendrites within the BPN. Immunoreactive boutons contain round vesicles and exhibit asymmetric membrane specializations. Postsynaptic loci include both GLU-immunoreactive and nonreactive dendrites. Immunoreactive neurons exhibit large multipolar somata with infolded nuclei.

The present observations suggest that the origin of some of the GLU-positive axon terminals in the BPN arise from extrinsic sources. This finding provides evidence for the presence of multi-synaptic glutaminergic pathways within the CNS that involve basilar pontine afferent systems, neurons of the BPN and their respective pontocerebellar axons, and the granule cells of the cerebellar cortex. Supported by USPHS grant NS-12644.

351.8 POLYCLONAL HUMAN AUTOANTIBODIES REACTIVE WITH CYTOPLASMIC ANTIGENS OF CEREBELLAR PURKINJE CELLS ARE NON-SPECIES SPECIFIC AND OF MULTIPLE IMMUNOGLOBULIN CLASSES. J. C. Finley*, J. L. Smith* and V. A. Lennon. (SPON: E. Benarroch). Neuroimmunology Laboratory, Depts. of Immunol. and Neurol., Mayo Clinic, Rochester, MN 55905.

Purkinje cell autoantibodies are found characteristically in sera of patients with paraneoplastic cerebellar degeneration (PCD). At the ultrastructural level they bind principally to the endoplasmic reticulum and trans face of the Golgi (Rodriguez et al, J Neuropathol Exp Neurol 45:322, 1986). Apart from their serological usefulness in aiding the diagnosis of an autoimmune form of cerebellar degeneration, these antibodies offer potential tools for neuroscientists investigating cerebellar cells.

In this study we have investigated 6 positive PCD sera for immunoglobulin isotype and species specificity. Three of the patients had gynecological cancer, one breast cancer, one lymphoma and one suspected, but not yet proven, lung carcinoma. Control human sera (n = 145) included neurologically normal cancer patients, cancer patients with paraneoplastic syndromes not involving the cerebellum, and patients with non-neoplastic neurological disorders. These were negative for Purkinje cell antibodies of all isotypes.

The 6 PCD sera had high titers of IgG anti-Purkinje cell antibodies (1/1,000 to 1/128,000), two additionally had IgM (1/100 and 1/500), and another had IgA anti-Purkinje cell antibodies (1/1,000). Antibodies of IgE class were not found. Antibodies of IgG, IgM and IgA classes reacted as well with rat and mouse cerebellar tissue as with human, and antibodies of each class bound to the peripheral cytoplasm of discrete molecular layer cells in addition to Purkinje cells in the cerebellum of all species.

Because of its ready availability and superior viability, rodent cerebellar tissue should be a useful substitute for human tissue in clinical serological testing for Purkinje cell antibodies. Its use also should facilitate biochemical characterization of the autoantigen(s) of PCD. The antibodies themselves presumably will recognize cerebellar components of additional species, and may prove to be valuable probes for studying cerebellar anatomy and development. **Supported by grant CA 37343 from the National Cancer Institute.**

351.9 SPECIES DIFFERENCES IN CEREBELLAR CORTICAL MUSCARINIC RECEPTOR DISTRIBUTION: AN AUTORADIOGRAPHIC STUDY. A. Neustadt, A. Frostholm and A. Rotter. Department of Pharmacology, University of California, Irvine, CA 92717.

[^{3}H]Quinuclidinyl benzilate (QNB) was used to study the muscarinic acetylcholine receptor distribution in cerebella of mice, rats, guinea pigs and rabbits. Slide-mounted tissue sections of cerebellum were incubated for 90 minutes at 25^{o}C in 0.01M phosphate buffered saline pH 7.4, containing 1nM [^{3}H]QNB, and prepared for autoradiography. Control slides were incubated under the same conditions in the presence of 10μM atropine to determine the degree of non-specific binding. In mice, [^{3}H]QNB labeling was highest over the granule and Purkinje cell layers; relatively low labeling was present over the molecular layer and negligible binding was evident over the white matter. This distribution was seen in all lobules of the cerebellar cortex. In the rat, a similar laminar distribution of binding sites was observed. In addition, a considerable increase in autoradiographic grain density was seen over the molecular and granule cell layers of lobules 9 and 10 (uvula and nodulus) when compared to the remainder of the cortex, a distinction not observed in mice. A further characteristic feature of rat cerebellum was discrete columns of very high grain density traversing the molecular layer of lobule 10. Again, this pattern was not observed in mice. In the guinea pig, the molecular layer of the neocerebellum was of almost equal grain density to that of the granule cell layer. In the nodulus, ventral uvula, flocculus, ventral paraflocculus and lingula (lobule 1), the relative grain density between the two layers was reversed when compared to the same regions in rat and mouse, the molecular layer being of considerably higher grain density than the granule cell layer. In the vermis of lobules 6 and 7, a columnar pattern of high grain density was observed, similar to that over lobule 10 of the rat cerebellum. In the rabbit neocerebellum, the grain density was high, with barely discernible differences between the molecular and granule cell layers. In the paleo- and archicerebellum, a discrete band of very high grain density was observed over the Purkinje cell layer; moderate and low grain densities were present over the granule and molecular layers, respectively. Columns of high grain density were again observable over the molecular layer of several vermal regions, including lobules 1, 6, 7 and 10. The observed species differences in muscarinic receptor distribution reflect variations in the evolutionary development of phylogenetically older regions of the cerebellum and may indicate important functional distinctions between these animals.

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351.10 THE RELATIONSHIP BETWEEN SYNAPTOGENESIS AND CYTOCHROME OXIDASE ACTIVITY IN PURKINJE CELLS OF THE DEVELOPING RAT CEREBELLUM. A.E. Mjaatvedt* and M.T.T. Wong-Riley (SPON: E. Godfrey). Dept. of Anat. & Cellular Biology, Med. Coll. of Wis., Milwaukee, WI 53226.

In the adult CNS, the level of oxidative metabolism, as indicated by cytochrome oxidase (C.O.) cytochemistry, can be correlated with the level of neuronal activity (Br.Res. 171:11,'79, Neurosci. 7:2337,'82). Specifically, heightened C.O. activity in postsynaptic neurons can often be correlated with a greater proportion of excitatory input, whereas the predominance of inhibitory inputs often results in a low level of C.O. activity postsynaptically. This relationship has not be explored in developing neurons. Purkinje cells (PC) of the neonatal rat cerebellum serve as a useful model since their somata go through a postnatal transition from receiving a predominantly excitatory input to that of an inhibitory one. We proposed that this transition of synaptic input would be paralleled by a change in C.O. activity within PC, peaking at the height of climbing fiber synaptogenesis onto the somata and decreasing to a low level in the adult when the PC receive mainly inhibitory basket cell input. Rat pups from birth to adult were anesthetized, perfused and the cerebella processed for light and EM C.O. cytochemistry. Counts of both asymmetrical (climbing fibers) and symmetrical (basket fibers) synapses on the somata indicated that excitatory synapses were first seen at P3, peaked at P10, and were absent in the adult. Mitochondria (mito.) of the PC somata were classified as dark (D), moderate (M) and lightly (L) reactive and the area of the mito. and PC cytoplasm were measured. At birth, D and M mito. occupied 1.7% of cytoplasmic (cyto.) area; L mito., 3.2%. This ratio reversed between P3 and P10, with D and M mito. occupying 5.8-7.2% of the cyto. area and L 2-3%. In the adult, D and M mito. decreased to 2.4%, and L mito. rose to 3.5%. The overall packing density of mito. increased from 6% of cyto. area at P3 to a peak of 14% at P10, then decreased to 6% in the adult. The cyto. area increased 10 fold during this time. Analysis of dendrites indicated a high degree of C.O. activity, particularly in the secondary branches, which receive predominantly excitatory inputs. This pattern persisted into the adult, in which somata were only lightly reactive. At all ages, we found a relationship between mito. size and C.O. activity. D mito. were larger than M which were larger than L. This suggests that mito. size may be an indicator of C.O. activity. Our data also indicate that the change in C.O. activity from high levels at P3-10 to low levels in the adult can be predicted from the change in synaptic input from a predominantly excitatory to an inhibitory one on the PC somata during maturation. This is supported by the observation that dendrites, which receive mainly excitatory input, consistently maintain a high level of C.O. activity throughout development to adulthood. (Supported by NIH NS18122).

351.11 THE CEREBELLUM AND INFERIOR OLIVARY COMPLEX OF A MONOTREME, TACHYGLOSSUS ACULEATUS. M-C. Holst* (SPON: D.T. Tracey) Sch. Anatomy, Univ.New South Wales, Kensington,N.S.W.,Australia 2033

The primitive mammals of Australasia, the platypus and spiny anteater or echidna, have an alleged mosaic of reptilian and mammalian characters. However, the monotreme cerebellum has previously been described as consisting basically of vermis and paraflocculus and hence being "avian" rather than "mammalian" or "reptilian" (Larsell, O., The Cerebellum from Monotremes through Apes, Univ. Minnesota Press, 1970); the inferior olivary complex (IOC) was also said to resemble that of birds (Kooy, 1917).

The morphology of the cerebellum of the echidna, Tachyglossus aculeatus was re-evaluated in intact and sectioned specimens. The pattern of fissures and medullary rays is mammalian. The vermis, anterior lobe hemisphere and paramedian lobule are relatively large, but the ansiform lobule is relatively small. The paramedian lobule comprises the prominent posterolateral area. Lobules 4 and 8 form large parts of the anterior and posterior lobe vermis, and the pyramis forms the posterior pole. The flocculonodular lobe is very small. The paraflocculus is made up of a series of small folia and does not form an obvious protrusion.

The cytoarchitecture of the echidna's IOC was ascertained from Nissl sections. The caudal IOC is directly comparable with that of other mammals, with homologues of subnuclei a, b and c of the medial olive (MO), nucleus beta and the dorsal cap of Kooy apparent. The rostral IOC is at first sight unlike that of other mammals. The homologues of the MO and dorsal olive form an arch around the homologue of the principal olive; the latter is relatively undifferentiated. The homologue of the rostral MO has both medial and lateral components. The dorsomedial cell column (dmc) is ventral to the medial cell group of the MO. The conformation of the rostral MO in Tachyglossus is in a more derived state than that found in the marsupial IOC; in the latter, the rostral MO is entirely lateral in position.

Olivary afferents to the vermis and hemispheres of lobules 5-8 were studied using the retrograde transport of HRP, bisbenzimide, or propidium iodide. The IOC projects to the contralateral cerebellum in a topographically organized pattern with caudal areas plus dmc supplying the vermis, and the rostral IOC projecting to the hemispheres. A longitudinal zonal organization is apparent. The projection is comparable to that in other mammals and validates the present subdivision of the echidna cerebellum.

In Tachyglossus, the total numbers of olivary and Purkinje cells, and the extent of climbing fiber divergence provide further evidence of "mammalian" character.

351.12 CERTAIN CEREBELLOPONTINE FIBERS PROJECT IN COLLATERAL FASHION TO THE THALAMUS, SUPERIOR COLLICULUS, OR INFERIOR OLIVE OF THE RAT. H.S.Lee, R.J.Kosinski, and G.A.Mihailoff. Dept. of Cell Biology, Univ. of Texas Health Science Center, Dallas, TX 75235.

Based on HRP and EM observations, Mihailoff et al. (J.C.N.195:221) provided indirect evidence that cerebellopontine fibers arise as collaterals of ascending and descending projections of the cerebellar efferent system. The objective of the present study was to directly visualize the collateralization of cerebellopontine fibers to the thalamus(Th), superior colliculus(SC), or inferior olive(IO), using a double fluorescence technique or a combined fluorescence-HRP method.

First, True Blue (TB) was injected into the ventrolateral nucleus of the thalamus and after three days, Nuclear Yellow (NY) deposited within the basilar pontine nuclei (BPN) of the rat, using a retropharyngeal(ventral) approach. With this combination, double-labelled (DL) cells were located in the interpositus (anterior and posterior) nuclei and the rostral part of the lateral cerebellar nucleus. The majority of DL neurons are medium to large in size and multipolar-shaped. In a second combination, TB was injected into the SC and after three days, NY was again deposited within the BPN. A much smaller number of DL cells relative to the first injection combination were present throughout the rostro-caudal extent of the lateral cerebellar nucleus and an even smaller number were located in the interpositus (anterior and posterior) nuclei. These cells are intermediate to large-sized and either bipolar or multipolar in shape. Finally in a third combination, NY was injected into the BPN and WGA-HRP deposited within the IO, again using a ventral surgical approach. In these cases, the majority of DL cells were located in the interpositus (anterior and posterior) nuclei and the medial portion of the lateral nucleus. The number of DL cells was similar to the BPN/SC combination and far less than that seen in the BPN/Th combination. These cells are relatively small in size and most are spindle-shaped. In each injection combination, single-labelled (SL) neurons of various sizes were also observed in the cerebellar nuclei. From all injection sites, SL but not DL cells were seen in the medial (fastigial) nucleus.

The morphology of DL cells in this study included a range from small to large multipolar somata as well as small bipolar or spindle-shaped neurons. Since the cerebellar input to BPN and IO is at least in part GABA-ergic, some DL cells in the BPN/IO cases might be GABA-ergic and probably inhibitory. Based on the relative number of DL cells observed in the interpositus and lateral cerebellar nuclei in these three injection combinations, a large proportion of cerebellopontine fibers originate as collaterals of axons projecting rostrally to the thalamus, while only a relatively small fraction arise as collaterals of cerebellar efferents to the SC or IO. Interestingly, ongoing studies of dorsal column nuclear efferents similarly reveal that while many of the cells projecting to the BPN also project in collateral fashion to the VPL thalamus, relatively few provide collaterals to the BPN and SC.

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CLINICAL CNS NEUROPHYSIOLOGY I

352.1 ATROPINE EFFECTS SPONTANEOUS ELECTROENCEPHALOGRAM (EEG), PERFORMANCE AND INSTRUMENTAL ESTIMATES OF VIGILANCE IN HUMANS. W.B. Pickworth, R.I. Herning*, S.T. Higgins*, J. deBorja* and B. White* NIDA, Addiction Research Center, Baltimore, MD 21224.

The use of atropine in clinical medicine is widespread and recent reports of illicit use have appeared (Bower, Am J Psychiat 144, 383, 1987). Atropine increases scores on scales which measure drug-induced sedation and some subjects report euphoria and drug liking (Penetar and Henningfield, Pharmacol Biochem Behav 29:1111, 1986). Atropine disrupts performance on complex tasks (Dellinger et al, Aviat Space Environ Med 57:1185, 1986) and impairs memory. The purpose of these experiments was to compare changes in the spontaneous EEG, behavior, performance and instrumental estimates of vigilance, that follow atropine administration.

Seven male volunteers with a mean aged of 25.8 yrs and a mean weight of 73.5 kg were given a dose of atropine (1.5, 3 and 6 mg/70 kg) or a placebo (saline) on two occasions in a randomly assigned order of a crossover double-blind experiment. Subjects performed the auditory oddball task and the continuous performace task (CPT); two-minute samples of EEG were obtained while the subjects relaxed with eyes closed (EC) and eyes open (EO). Data were collected prior to the IM administration of drug, and at 2 hrs and 8 hrs after the drug. The power and peak frequency were analyzed by analysis of variance techniques in: delta (0.25-3.75 Hz), theta (4.00-7.00 Hz), alpha (7.25-14.00 Hz) and beta (14.25-25 Hz) bands. Instrumental estimates of vigilance were derived from power spectra criteria, theta/alpha power and beta/alpha power, proposed by Matousek and Petersen (EEG Clin Neurophysiol 55: 108, 1983).

Atropine caused several significant changes in the frequency and power of the spontaneous EEG. There were significant increases in delta power (EC, EO); whereas, alpha power (EC) and theta power (EC) were reduced by dose. Beta power (EC) was increased by the small doses but not larger ones. Delta, theta and beta frequency decreased over dose and time while alpha frequency (EO) increased over dose and time. The EEG slowing in all frequencies and enhanced power in the lower frequency bands coincided with subject reports of drowsiness. Atropine caused dose-related increases in errors on the oddball task and the CPT task. The P300 amplitude in the oddball task was systematically reduced across the dosage range. The effects of the 3 and 6 mg doses on most EEG measures were evident at the 8 hr recording. Situational tolerance was not apparent because dose repetitions did not cause significant decrements of EEG effects.

352.2 **UNFOLDING THE HUMAN CEREBRAL CORTEX INTO TWO DIMESIONAL FLAT MAPS**

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Correlations between neocortical areas and cognitive function in humans have been difficult to draw in fine detail for many reasons that have included: 1) Theoretical problems inherent in relating lesions to dysfunction; 2) The need to develop over the years sufficiently sensitive methods capable of qualitatively and quantitatively measuring human cognitive or emotional dysfunction following organic brain trauma; 3) The inability to precisely describe, until the recent development of Magnetic Resonance Imaging (MRI), the anatomical location and extent of cortical lesions in the brain of living humans independently of signs and symptoms; 4) A total lack of statistical information on the variations in human gyral and sulcal pattern which may constitute individual cortical "fingerprints"; 5) An absence of methodology capable of systematically relating, despite individual variations, the finer details of cortical gyral patterns to functional areas in the cortex.

Although the recent advent of MRI has made possible the visualization of white and grey matter and the precise localization of lesions in the brains of living humans, it is still difficult to appreciate the dimensions of cortical lesions and their relations to surrounding cortical areas and landmarks when they are spread over several individual brain slice images. It is also difficult to appreciate how a lesion in one individual may be comparable to a roughly similar lesion in another individual.

Computer reconstruction techniques designed to open the cortical sucli and flatten the convoluted cerebral cortex into the shape of two dimensional maps, developed only in the last several years in the study of animal cortical anatomy, may now be profitably applied to re-examine in greater detail localization of function in the human cerebral cortex. The classical approach to localization of fuction has involved first describing sensory, motor, cognitive and behavioral changes and then patiently waiting sometimes years for the final pathology reports describing the location and extent of the lesion. MRI has made the cortex and the lesion immediately accessible; the two dimensinal reconstruction progams simplify neocortical geometry. These maps will permit more precise quantitiative relations between lesion distribution and extent to higher cognitive dysfunction than has been hitherto possible by classic neurological methods.

Single human hemispheres have been flattened in 2 dimensions with the aid of new programs designed and written in our laboratory for the Macintosh Plus computer. For purposes of standardization and replicability, the first two brains processed were those taken from An Atlas of the Human Brain for Computerized Tomography (Matsui and Hirano, 1978). A two dimensional cortical map was reconstructed from a coronally sectioned brain, and another from a horizontally sectioned brain. The programs process line drawings of brain slices on which sulcal and gyral landmarks are registered. The individual sections are then aligned with one another. Further studies utilizing 2 dimensional maps are now in progress in our laboratory on MRI slices of living patients.

352.3 DERIVATIZED Tc-99m DIAMINE DITHIOL COMPLEXES FOR IMAGING OF REGIONAL CEREBRAL BLOOD FLOW. R.C. Walovitch, R.A. Morgan*, A.D. Watson*, E.A. Cheesman*, S.T. Garrity*, S.J. Williams*, Diagnostic Imaging Research, E.I. DU PONT DE NEMOURS AND CO., INC., N. Billerica, MA 01862.

A technetium based radiopharmaceutical which localizes in the brain according to regional cerebral blood flow will allow the non-invasive assessment of rCBF alterations under a variety of pathological states. Greater than 60 derivatized of neutral, lipophilic Tc-99m diamine dithiol complexes have been synthesized and evaluated in rats for potential clinical utility. An appended amine functionality has been determined to be the primary factor in increasing retention of the complexes. Two of these complexes RP-37 (N-Ethylpiperidine diamine dithiol) and RP-120 (N-Ethylpyrrolidine diamine dithiol) have adequate pharmacokinetic characteristics for further evaluation. Both compounds are extracted efficiently by the rat brain (brain uptake index values above 100). Dynamic planar imaging in monkeys also indicates that both compounds are well extracted (RP-37 = 5.3 and RP-120 = 4.5 %ID in the brain). Time activity curves from the monkey planar imaging studies were used to calculate whole brain biological half-life of the complexes which for RP-37 and RP-120 were 33 and 56 min respectively. Dual-labeled autoradiography in rat brain using [^{14}C]-iodoantipyrine and ^{99m}Tc show both complexes to be distributed according to rCBF. By 30min post administration both compounds show selectively hippocampal localization. Dynamic tomographic imaging in monkeys (CLEON 710 dedicated head system) corroborate the initial rat autoradiographic studies. However, by approximately 30min post compound administration the concentration of activity in various cortical brain regions appear to change at rates not related to blood flow. Clinical results with RP-37 are in accordance with these monkey imaging studies and this agent appears to be useful as an indicator of rCBF at early times post-injection.

352.4 A COMPARISON OF ELECTRICAL AND MAGNETIC COIL STIMULATION OF PERIPHERAL NERVES IN MAN. P.J.Maccabee*, R.Q. Cracco and V.E. Amassian. Depts. of Neurology and Physiology, SUNY Health Sci. Ctr. at Brooklyn, New York 11203.

Magnetic coil stimulation was recently introduced by Barker et al (1987, Neurosurgery, 20:100-109) as a convenient method of stimulating peripheral nerves and the cerebrum. Conventionally, the plane of the coil has been applied tangentially, which ensures that a high fraction of the magnetic flux enters the tissue, but introduces uncertainty as to the exact site of stimulation. Using ourselves as subjects, we compared focal cathodal electrical stimulation of arm nerves with various orientations of the magnetic coil. The latency-amplitude relations of the hypothenar EMG was first measured to electrical stimulation of the ulnar nerve, the focal cathode being located just above the medial epicondyle. Selective ulnar nerve stimulation was obtained when the plane of the circular magnetic coil (9 cm diameter) paralleled the long axis of the extended arm and was orthogonal to the arm. With this orientation, the effective site of stimulation was displaced proximally from the distal edge of the coil by up to ½ of the coil diameter. Maximal amplitude of the hypothenar EMG was obtainable by appropriately tilting the coil. Transverse orthogonal orientation was less effective. Laying the coil flat on the arm (i.e., tangentially) activated additional structures locally.

The lateral extent of stimulation was determined by longitudinal, orthogonal stimulation of the median nerve at the wrist, with recording of the thenar EMG. Moving in 5 mm increments in the ulnar-radial axis, a maximum was found over the tendon of palmaris longus, i.e., over the median nerve; the EMG was markedly attenuated at 10-15 mm on either side of this point. Again, transverse orientation of the coil stimulated less effectively. (Tangentially applied at the wrist, magnetic coil stimulation was surprisingly uncomfortable).

Orthogonal stimulation at an angle to the long axis of the humerus permitted the radial nerve to be identified laterally in the upper arm, the EMG recording being taken from the forearm extensors.

Summarizing, our findings suggest the utility of magnetic coil stimulation in clinical peripheral nerve studies, provided that its orientation is appropriate for focal stimulation.

352.5 ELECTROPHYSIOLOGICAL CORRELATES OF DYSLEXIA IN ADULTS: A 20 YEAR FOLLOW-UP. C. Naylor, R. Harter, F. Wood*, and R. Felton*. Sect. of Neuropsychology, Bowman Gray Sch. of Med., Winston-Salem, NC 27103 and UNC-G, Greensboro, NC 27412.

The purpose of this study was to a) assess the comparability of childhood and adult ERP indicants of reading disability (RD), and b) use these indicants to understand improvement in reading in a physiological context. The subjects were 38 males, 32 of whom had been tested in childhood. Of those 32, 24 met the criteria of specific RD in childhood (achievement scores on the Gray Oral and/or WRAT-R reading tests two or more years below grade level). All childhood and adult IQ scores were within normal limits.

Many subjects with RD in childhood had improved their reading skills by adulthood, although as a group they remained impaired relative to normal readers. Oral reading speed and spelling skills proved to be most resistent to remediation.

Event-related potentials were recorded over O1, O2, C3, C4, F3 and F4 to letter and color stimuli using a single stimulus presentation paradigm. Subjects with RD showed a general reduction in positivity starting at 150 msec and extending beyond the latency of the behavioral response. Adult subjects with RD, compared to the children with RD in the Harter et al. study, showed more diffuse reductions in electrophysiological response to both color and letter stimuli. Unlike children who showed greater left hemisphere deficits in central regions when compared to normal readers, the deficit in adults with RD was more widely distributed and bilateral in nature. As for children, the effect of RD was more prominent over central regions.

Reduced positivity at 240 msec over central regions replicated the findings of Harter et al. with children. The amplitude of central P240 was significantly correlated with both childhood and adult reading deficits. Central P240 was found to be a promising marker of dyslexia. The amplitude of central P240 may serve as a predictor of potential for reading improvement as well as an indicant of the degree of deficit in childhood and adulthood.

352.6 A TECHNIQUE FOR VISUALIZING THE NEURAL SYSTEMS INVOLVED IN ACTIVITY RELATED BRAIN DAMAGE. G.O. Ivy and N.W. Milgram. Div. of Life Sciences, Univ. of Toronto, Scarborough, Ontario, M1C 1A4

Hypertrophy of astrocytes in the CNS is a well known marker of neural trauma. We demonstrate here that patterns of astrocyte hypertrophy (AH) can be used to trace neural systems that are involved in abnormally heightened levels of electrical activity. Using additional histological and immunocytochemical methods, the degree of specific neural damage or death in various parts of the system can then be determined.

Rats were given one of several treatments: systemic injection of kainic acid (KA), repetitive electrical brain stimuation, or localized electrolytic lesions or stab wounds to various brain regions. After various survival times, the rats were perfused and the brains processed to demonstrate AH (using antibodies to GFAP) or necrosis (using PAS or nissl stains).

Both localized electrolytic lesions and stab wounds produced discrete patterns of AH which reflected known anatomical connectivity. In contrast, both KA and electrical brain stimulation produced recurrent seizure activity and induced patterns of AH that labeled specific neural systems. Three weeks following seizure activity AH was focused in the ventrolateral forebrain, the medial thalamus and hippocampus. Within these regions specific structures were affected. In the forebrain, AH was consistently seen in olfactory cortex, external capsule, endopyriform nucleus, the deep layers of insular cortex, and lamina Va of lateral neocortex; within thalamus AH was pronounced in the intralaminar nuclei, the rhomboid nucleus and nucleus reuniens; within hippocampus AH was particularly notable in the hilus of dentate gyrus. The PAS stain revealed that these areas contained cells in various stages of necrosis, a result that could also be detected in nissl stains. The numbers of regions affected and the extent of cell damage in those regions varied with extent of seizure activity. While cell death was typically seen in the hilar region of hippocampus, it was not typical in dorsal areas CA1 and CA3. In contrast, the endopiriform nucleus, external capsule and lamina Va of temporal and parietal neocortex exhibited marked necrosis, possibly indicating much lower thresholds for damage.

Our results indicate that a variety of different treatments, all of which produce generalized seizure activity cause a common pattern of degenerative changes in the brain that is clearly reflected in patterns of AH. Futher, while fields CA1 and CA3 of hippocampus are most commonly emphasized as being vulnerable to the excitotoxic effects of seizure activity, we show that structures such as the endopiriform nucleus, specific nuclei of the medial thalamus and the hilus of fascia dentata have a far greater vulnerability. It seems likely that similar patterns of degenerative changes occur in individuals afflicted with recurrent seizure disorders.

352.7 **ELECTROENCEPHALOGRAPHIC SPIKING ELICITED DURING SLEEP FOLLOWING MELATONIN ADMINISTRATION IN CATS.** R.K. Harper*, R.M. Harper and H.L. Lesse (SPON: S. Eiduson). Depts. of Psychiatry and Anatomy, and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

Melatonin has been shown to reduce sensory-related paroxysmal EEG activity and has been suggested to have anticonvulsant properties (Anton-Tay et al., Life Sci. 10:841, 1971; Fariello and Bubenik, Neurosci. Lett. 3:151, 1976; Brailowsky, EEG Clin. Neurophysiol. 41:314, 1976). Melatonin secretion rises nocturnally and may be associated with specific sleep stages. Certain sleep states activate some types of seizure discharge, and melatonin may interact with these states to modulate the incidence of abnormal EEG activity. We examined the incidence of abnormal EEG discharge during different sleep states following melatonin administration in cats kindled to produce amygdaloid seizure discharge.

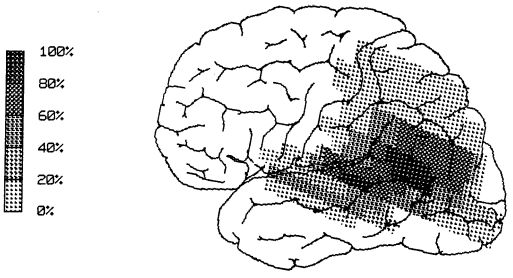
Five adult cats (4 female, 1 male) were surgically prepared with arrays of bipolar needle and concentric electrodes implanted bilaterally in the dorsal hippocampus and basolateral amygdala under stereotaxic guidance. Screw electrodes placed over the orbital plate and occipital bone provided indications of eye movement and cortical EEG respectively. The cats were kindled by daily stimulation of 3-Hz, 1/2-sec current delivered to the amygdala. Following kindling, daytime (0830-1630) control and test recordings of waking and sleeping EEG activity were obtained in a sound-attenuated chamber. Melatonin (40-45 mg/kg) was administered orally 24 hrs after onset of the control recording. Sixty-second samples of polygraphic activity were obtained during quiet and active (rapid eye movement) sleep periods, and instances of rapid-rise-time "spike" activity were tabulated. The occurrence of spike discharge in the cortex and amygdala was dramatically (as much as a factor of 17) increased during quiet sleep epochs following melatonin administration over control periods in all cats. Hippocampal spiking was increased to a lesser extent in two cats. EEG spiking during active sleep showed a smaller, but significant increase following melatonin.

The origin of this enhanced spike discharge following melatonin administration is unclear. Interictal spike discharge in kindled epilepsy has been associated with decreased probability of generalized seizure discharge, and a similar phenomenon may be operating here.

352.8 DEFINING FUNCTIONAL CORTICAL AREAS WITH "AVERAGED" CT SCANS R. T. Frey*, D. L. Woods, R. T. Knight, D. Scabini*, and C. C. Clayworth* Clinical Electrophysiology Laboratory, Dept. of Neurology, U.C. Davis, VA Medical Center, Martinez, CA, 94553 (SPON. I. Kwee).

Lesions of particular cortical areas may vary considerably in extent. We have developed computer software that permits the "signal averaging" of CT scans from patients with particular sensory, motor or cognitive symptoms. Lesions from individual patients are outlined on computer templates of CT sections. The computerized representations of the horizontal sections can then be added across subjects and averaged. In addition to lesion averaging, the lesions can be projected in lateral perspective and estimates are provided of (1) the variability in reconstructions from independent reviewers, (2) lesion volume, (3) the Brodmann areas and subcortical structures that have been damaged.

Averaging CT sections from selected patient groups reveals structure-function correlates more clearly than data from a single patient. Consider cortical lesions that produce left homonymous hemianopia: These may include parietal and temporal lobe structures, but an "averaged CT" from several cases would reveal the critical role of the right occipital cortex. The averaging of lesions can also clarify cortical areas implicated in less well understood functions. For example, shown below is a lateral perspective of left hemisphere lesions in 5 aphasic patients with bilateral reductions of the N_{100} component of the auditory evoked potential. This suggest that cortical areas near the parietal/temporal junction are critical for receptive speech perception and for the generation of the N_{100}.



352.9 **ENHANCEMENT OF CYCLIC AMP-STIMULATED Na^+ CURRENT BY THE CONVULSANT DRUG PENTYLENETETRAZOL IN IDENTIFIED NEURONES OF *Lymnaea stagnalis*.** Catherine R. McCrohan* and Rhanor Gillette (SPON Rebecca Prosser). Department of Physiological Sciences, Univ. of Manchester, Manchester M13 9PT, U. K. and Department of Physiology & Biophysics, University of Illinois, Urbana, IL 61801.

The convulsant drug pentylenetetrazol (PTZ) induces characteristic paroxysmal depolarizing shifts (PDS) in molluscan neurones resembling those recorded from mammalian neurones during epileptogenesis. Onozuka et al. (Brain Res. 269, 277) showed that PTZ elevates intracellular cAMP, while Argemi et al. (Bull. Math. Biol. 46, 903) suggested PDS generation required a persistent inward Na^+ current (I_{Na}). We have examined effects of PTZ on a cAMP-stimulated I_{Na} in neurones of the pond snail *Lymnaea*.

For injection of cAMP under voltage clamp, buccal neurones 1 and 2 were impaled with a voltage electrode and a 2-barreled current electrode, one barrel of which was used for iontophoretic injection of cAMP. PTZ was applied in saline at concentrations of 10-30 mM.

Injection of cAMP elicited a slow inward current in buccal neurones, which was voltage-independent, and which was abolished in 0-Na^+ saline. PTZ caused depolarization and intense bursting, sometimes leading to inactivation of the spike-generating mechanism. In the presence of PTZ, at a holding potential of -60 mV, both amplitude and duration of the cAMP-stimulated I_{Na} were significantly increased. Amplitude increased from 1.63 (0.6 s.e.m.) nA to 2.05 (0.64) nA ($P<0.001$) and duration from 27.79 (2.27) s to 36.71 (2.43) s ($p<0.001$; paired t-test, N=14). This effect was reversed by washing with normal saline. Similar enhancement occurred in the presence of the phosphodiesterase inhibitor IBMX (0.1 mM). PTZ inhibition of phosphodiesterase may contribute to PDS by augmenting endogenous cAMP, and thereby I_{Na}.

The cAMP-stimulated I_{Na} recorded at -70 mV was reduced following a 30 second depolarizing command step to 0 mV. This suggests that during PDS cAMP-stimulated I_{Na} may partially inactivate, thus contributing towards repolarization of the PDS. Supported by The Royal Society, The Nuffield Foundation and NSF BNS 8603816.

352.10 DIMINISHED P3 AMPLITUDE IN SCHIZOPHRENICS DURING CPT PERFORMANCE. R.J. Strandburg, J.T. Marsh, W.S. Brown, R.F. Asarnow,*D. Guthrie.* Dept. of Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024.

We previously observed that schizophrenic children produce significantly lower P3 amplitudes (compared with normals) during performance of the span of apprehension task. This task which is sensitive to schizophrenic psychopathology requires detection of a target letter within a briefly presented (50 msec) 12 letter array. Here we present a similar P3 reduction in schizophrenic children during a vigilance task, the Continuous Performance Task (CPT). Like the span, the CPT is sensitive to schizophrenia, however, it differs in its information processing requirements.

Single digits were presented at a fixation point every 1.25 sec. for 50 msec and were surrounded by up to nine distractor digits. Subjects were required to respond to the repetition of the same digit on successive trials (f=18%). Twelve schizophrenic and 13 mean age-matched normal children (S=11.5±1.2 yrs., N=11.2±1.2 yrs)each received 400 trials of the CPT. Informed consents were obtained for each subject.

ERPs were recorded from 19 International 10-20 System loci referenced to linked earlobes. A 2.5 sec. epoch including both the target and preceding stimulus was averaged offline. Only correct response trials (hits) were included in the averages. Baseline-to-peak and latency measurements were made for the P3 component elicited by both the target (T) and the preceding nontarget (NT) stimuli at the peak of the observed P3 field (PZ and CZ).

As expected, the hit rate for schizophrenics was significantly lower than for normals (69±14% vs 89±8%). The two groups did not differ in response latency or within and across subject response latency variability.

Schizophrenics produced significantly ($p<.05$) smaller P3 amplitudes at both leads for both the T (12.5 vs 16.8 μv) and NT (4.1 vs 8.7 μv) stimuli. There was no group by stimulus interaction suggesting that the two groups showed comparable increments in P3 to the rare, target stimulus. They also did not differ significantly in P3 latency (S=520±55 ms vs N=493±44 ms).

That P3 amplitude is diminished in schizophrenics across a wide variety of information processing tasks suggests that this component is measuring some aspect of performance evaluation such as confidence. The comparability and stability across groups of latency measures for both the P3 and the behavioral response suggests that this amplitude difference is not the result of latency jitter. These results are consistent with the ERP data from adult schizophrenics.

352.11 AUDITORY BRAIN STEM RESPONSES IN CHRONIC SCHIZOPHRENIC PATIENTS.

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Many studies have been made recently on evoked potentials in schizophrenic patients to assess their perceptual abnormalities. We studied auditory brain stem responses (ABSRs) in chronic schizophrenics and compared with those of controls. We also examined a relationship btween ABSRs and psychiatric symptoms in chronic schizophrenics.

[Methods]

Subjects were 17 chronic schizophrenics and 17 controls. ABSRs were elicited by click stimuli (2 kHZ, 80 dBHL, 5/sec), and were recorded between the vertex (CZ) and mastoids. The amplitudes and the latencies of ABSRs (Wave I - V) were analyzed. Brief Psychiatric Rating Scale was used to assess the psychiatric symptoms in each patient.

[Results]

(1) No differences in latencies of the ABSRs were observed between chronic schizophrenics and normal controls. (2) The amplitude of wave V in chronic schizophrenics was significantly lower than that in normal subjects. (3) There were significant positive correlations between the amplitude of wave V and florid symptoms such as hallucinatory behavior, excitement and suspiciousness in the chronic schizophrenics.

[Comments]

ABSRs are considered to arise from structures in the auditory pathways. Wave V is thought to originate from generators in the region of the inferior colliculus (midbrain).
These results suggest; (1) chronic schizophrenics have neuronal low activities or synchronous disturbances of neuronal events with click stimuli in the midbrain area. (2) As concerned with chronic schizophrenics with florid symptoms, the neuronal activites of midbrain area are not so much decreased from those in normal subjects.

352.12 "CAT P300" RECORDED FROM MEDIAL SEPTAL AREA. J. Harrison and J. Buchwald. Brain Res. Inst., Ment. Res. Ctr., Dept. Physiol., UCLA Med. Ctr., Los Angeles, CA 90024.

The human P300 potential is a scalp-recorded positivity of approximately 300 msec latency which appears to be a neural correlate of cognitive functions such as sequential information processing and short-term memory. We have been studying an animal model of the P300 in an attempt to elucidate the generator substrate. The "cat P300" is a positivity in the 200-500 msec range which is significantly larger for rare than for frequent stimuli. We previously showed that complete bilateral ablation of the primary auditory cortex did not affect the auditory "cat P300", nor did bilateral ablation of polysensory association cortex. In contrast, bilateral ablation of the medial septal area resulted in disappearance of the "cat P300" several days after the lesion, which suggested that the septum is involved in modulation but not actual generation of the response.

In the present study we mapped the medial septal area with depth recordings using a P300 stimulus protocol. Blocks of 250 click stimuli with a 1.5 sec interstimulus interval were presented with loud clicks occurring 80% of the time and soft clicks occurring 15% of the time in a random order. These probabilities were reversed in some blocks. A tone pulse, followed by eyelid shock, occurred 5% of the time and elicited a conditioned response which focussed the cat's attention on auditory stimuli. Field potentials were recorded from a stainless steel depth electrode, and EEG was recorded simultaneously from a stainless steel skull screw at the vertex. We recorded from five adult cats, awake and restrained. In each 250-stimulus block the EEG and depth potential were averaged separately for the loud and soft clicks and tone. Recordings were made bilaterally in tracks which passed through the medial septal area, lateral septum and vertical limb of the diagonal band of Broca. Responses to the rare loud click were recorded at 1 mm intervals, and if a positivity relative to baseline occurred in the 200-500 msec range, responses to frequent loud clicks were recorded at the same site.

In all five cats P300-like potentials, i.e., a positivity in the 200-500 msec range, larger to the rare than to the frequent stimuli, were recorded in the dorsal part of the medial septal region. These responses were localized to an active site and generally disappeared abruptly at sites 1 mm lateral or ventral. Polarity inversions, i.e., reversal of the positivity referenced to a prestimulus baseline, were recorded along a number of tracks, usually ventral to a nearby positivity. A P300 was not always present at the vertex when a P300-like potential was recorded in the depth. These results support and extend our prior conclusion from the septal lesion studies, that the septal area is involved in P300 generation. (Supported by USPHS grants AG04088 and HD04612).

352.13 P300 RESPONSES TO LINGUISTIC AND PROSODIC STIMULI. R. Erwin, J.S. Buchwald, D. Van Lancker, J. Schwafel and D. Guthrie. Departments of Physiology and Psychiatry, Brain Res. Inst. and Mental Retardation Res. Ctr., UCLA, Los Angeles, Ca 90024.

Prosody is the intonational component of language which conveys emotional content (e.g., anger, happiness)) as well as linguistic information (e.g., the rising terminal inflection of a question). Whereas linguistic comprehension and production become abnormal or absent in cases of left (dominant) hemisphere damage, the comprehension and production of emotional prosody become abnormal after right hemisphere damage. Such data suggest that linguistic and prosodic components of language may be processed by relatively independent brain subsystems. In order to investigate this problem further, and to develop a normative data base as a forerunner to projected clinical studies, we have recently completed a sequence of electrophysiological experiments.

Subjects were a group of 14 normal adults (7 males, 7 females, 20-30 years of age). Recordings of the P300 event related potential were carried out from electrodes placed centrally (Cz) and laterally at left and right parietal (P3,P4) and temporal (T5,T6) locations. In an "oddball" P300 protocol, the rare stimulus (p=.2), to which the subject responded with a button press, was randomly interspersed with the frequent stimulus (p=.8). Stimuli were presented at 1.5 sec intervals in 300-trial blocks. All blocks were counterbalanced so as to reverse the probability of the rare and frequent stimuli. Four stimulus pairs were utilized: 1) phoneme pairs: ba/pa, 2) word pairs: rip/lip, 3) prosodic (linguistic) pairs: Bob (statement)/Bob (question), and 4) prosodic (emotional) pairs: Bob (angry)/Bob (happy). The stimuli were digitized from tape recordings and were equalized with regard to duration (440 msec $\pm$ 20 msec) and peak intensity (60 dB SPL $\pm$ 5 dB). Amplitude and latency measurements were made for evoked potential components N1,P2,N2,P3 and principal components analysis were also carried out. All data were subjected to ANOVA statistics.

In all cases the rare target stimulus produced a significant P300 response while none of the shorter latency components was consistently affected by stimulus probability. An electrode by condition interaction consistently occurred which reflected a larger P300 at parietal than at the temporal sites. Response latency varied as a function of stimulus: longest latencies were produced by word pairs and prosodic (linguistic) pairs while the shortest latencies resulted from phoneme and prosodic (emotional) pairs.

These data suggest that the neural substrate of the P300 reflects auditory discrimination involving a processing continuum, with fewer stages engaged by phonemes and emotional prosodic contrasts than by linguistic prosodic contrasts and words. (Supported by USPHS grant HD 05958).

352.14 EFFECTS OF ASSOCIATION CORTEX ABLATION ON CAT MIDLATENCY AUDITORY EVOKED POTENTIALS. L. Dickerson and J. Buchwald, Brain Res. Inst., Ment. Ret. Res. Ctr., Dept. of Physiol., UCLA Med. Ctr., Los Angeles, CA 90024.

A sequence of auditory evoked potentialS can be recorded from the vertex of the awake cat in response to repeated click stimuli. The auditory brainstem responses, originating from the brainstem auditory relay nuclei, occur within the first 10 msec. Subsequent to these short-latency components is the response generated by primary auditory cortex (wave 7, 10-15 msec latency), followed by a positive potential at 20-25 msec (wave A), attributed to post-synaptic projections of the ascending reticular activating system to the intralaminar thalamus. Subsequent to wave A is a pronounced negativity at 30-35 msec, wave N_A, and a large amplitude positivity, wave C, at 50-75 msec.

In a continuation of efforts to determine the generator origins of scalp recorded auditory responses, the objective of this study was to investigate the source or sources of waves N_A and C. Previous lesion studies indicate that wave C is abolished by complete bilateral hemispherectomy but is unaffected, as is wave N_A, by ablation of primary auditory cortex bilaterally, or by frontal lobectomy anterior to the pericruciate cortex. Unit studies of auditory association cortex, (especially the pericruciate area, PCA, anterior lateral gyrus, ALA, and medial suprasylvian gyrus, MSA) indicate that response latencies fall in the 16-50 msec range (Irvine and Huebner, J. Neurophysiol., 42: 107, 1979). Because of these and other data and because waves N_A and C follow wave A, we hypothesized that they might evolve from projections from intralaminar thalamus (probable source of wave A) to the various cortical association areas. Also, the prolonged duration of wave C suggested dual or multiple generators.

In a series of 6 cats, 10-12 recording sessions were carried out before and after surgery. Histological confirmation was made of all lesions. Three cats were subjected to bilateral ablation of auditory association cortex, areas PCA, ALA, and MSA. Wave C amplitude was reduced by 50% or more for 2-3 weeks; in some cases it began to approximate normal amplitude and configuration by 3-6 weeks post-operatively. The most striking and persistent alteration, however, was the persistent reduction and near-elimination of wave N_A. Three other cats were subjected to bilateral ablation of association areas ALA and MSA (but not PCA). Again, the most striking change was the great reduction of the negative wave N_A, while wave C was reduced in amplitude to varying degrees across subjects. These data suggest that association cortex (areas PCA, ALA and MSA) is essential for the 30-35 msec negative wave, N_A, and, although not essential, importantly contributes to the subsequent 50-75 ms positivity, wave C. (Supported by USPHS grant HD 05958).

353.1 OPERANTLY CONDITIONING MEDIAN NERVE SEP COMPONENT P100 IN HUMANS: SPECIFICITY AND EFFECTS ON SOMATOSENSATION.
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Earlier work (Dowman and Rosenfeld, Brain Res. 333(1985)201) demonstrated rats could increase and decrease cortical SEP amplitude when reward was made contingent upon change, and that these changes were associated with alterations in nociceptive sensitivity. The present work is an attempt to demonstrate this phenomenon in humans.

SEPs, elicited by electrical stimulation of the median nerve at the wrist, were recorded from the scalp overlying the contralateral somatosensory cortex and frontal cortex, each referenced to the contralateral ear lobe, in 4 human subjects. Each SEP operant conditioning session consisted of 500 evoking stimuli given with a randon interstimulus interval (4.1 - 5.1 s). The SEP conditioning procedure consisted of 3 phases: Baseline, Uptraining (UTR) and Downtraining (DTR). During the UTR and DTR phases reward (money) was given for making P100 amplitude greater than (UTR) or smaller than (DTR) its Baseline mean. Correct responses were indicated by a 2000 Hz tone given 300ms following the evoking stimulus. Detection thresholds (DT), pain thresholds (PT), and pain tolerance levels (Ptol), determined by electrical stimulation of the index and middle fingers of the ipsilateral hand, were obtained immediately following each conditioning session. Subjects were given 10-30 sessions (1 session/day) in each phase.

Two subjects failed to demonstrate change in P100 amplitude. There were no changes in any SEP component amplitudes, DT, PT, or Ptol levels between UTR and DTR in these subjects. The other 2 subjects demonstrated significant change (40-60%) in P100 amplitude associated with conditioning. Both also showed comparable change in N60 amplitude. One of these subjects exhibited a smaller (15-20%) change in postcentral P25 and P40 component amplitudes in the direction of training, but no change in the precentral P22 and N30 component amplitudes. This subject demonstrated a 34% change (UTR> DTR) in DT, but no change in PT or Ptol. The other successful conditioner exhibited a 33% change in P40 amplitude that was inversely related to the change in P100 amplitude. This subject demonstrated change (UTR> DTR) in PT and Ptol, but not DT.

These results indicate that human subjects can operantly condition P100 amplitude, and that these changes may be associated with change in somatosensation. The different specificity of change in SEP amplitude exhibited by the successful conditioners may represent different strategies used to change P100 amplitude. These different strategies may be responsible for the different effects on somatosensation.

353.2 VISUAL EVOKED POTENTIALS (VEP) IN HYDROCEPHALIC CHILDREN.
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Visual evoked potentials are severely altered in children with hydrocephalus. During acutely increased intracranial pressure (ICP) an increase of latencies and amplitudes as well as disappearance of wave components can be observed. Although localization of the structures which may be encroached upon by the increased ICP is uncertain, involvement of the posterior thalamic radiation may be postulated, on account of its close spatial relationship to the lateral ventricle. We recorded VEP to diffuse light stimulation in a group of hydrocephalic, 2 to 11 year-old children treated with a shunt operation. Most of them were studied during periods of normal, stabilized ICP. In some cases, however, recordings were made before, during and after episodes of acute ICP increase, during which the following VEP changes could be frequently observed: a) increase of N70 latency, b) increase of P100 latency and amplitude, c) disappearance of N120 and P170 components, d) decrease of P100 and N200 amplitudes at increasing stimulation frequencies. These changes showed variable degrees of reversibility following normalization of ICP brought about by neurosurgical treatment. In hydrocephalic children with normal ICP similar VEP abnormalities could be observed, in spite of absence of evident neurological impairment, as well as of ventricular enlargement. These pathological VEP changes were often accompanied by deficits in cognitive visual performance. These results suggest that VEP analysis in hydrocephalic children a) may be used to monitor acute ICP increases; and b) may help detect, and allow correlation with chronic changes in visual information processing.

353.3 IMAGING OF EEG AND MEG (MAGNETOENCEPHALOGRAPHY) FOR THE ESTIMATE OF SOURCES IN THE HUMAN BRAIN RESPONSIVE TO ANTIDEPRESSANT DRUGS.
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The relative new MEG (magnetoencephalography) technology gives the possibility to further investigate drug effects in human brain. In our experiment, EEG (electroencephalography) and MEG midlatency responses to 40Hz auditory stimulation were combined to make estimates of sources responsive to drug treatments in depressed patients.

In the 40Hz response, EEG and MEG data were analysed in female patients (35-50 years old), suffering from major depression (diagnosed by standardized psychological tests), before, during and after drug treatment (imipramine or desipramine). EEG and MEG recordings were compared to those of control subjects matched for age and sex with respect to distribution, amplitudes, latencies and localization. The stady-state auditory midlatency response in humans is known to be maximal in amplitude and sinusoidal in form (in EEG and MEG) in response to 40Hz rates of stimulation. The MEG data reported suggest sources in the temporal lobe, which could be a part of a thalamo-cortical loop, becoming resonant at 40Hz. A number of different central brain systems, whose activities are changed during depression and following drug treatment, could be expected to influence thalamo-cortical systems.

Initial data suggests that MEG and EEG may be combined to estimate sources of the 40Hz response, and that these dipole estimates may be responsive to drug treatments. Preliminary EEG results in control subjects indicated that an equivalent dipole can be located in each hemisphere, which systematically changes in orientation through aproximately 360 degrees corresponding to different phases of the sinusoidal 40Hz response with respect to stimulus onset.

The data will be discussed in relation to a) the usefullness of the combination of EEG and MEG in dipole estimates, b) the use of source estimates of midlatency responses as indices of the locus and degree of drug action and c) the hypothesis that thalamo-cortical systems are responsible for the 40Hz response.

353.4 REDUCTION OF MORBIDITY, MORTALITY, AND LESION SIZE IN A RAT MODEL OF CEREBRAL INFARCTION WITH AMPHETAMINE.
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A homologous embolic model of cerebral infarction was developed in rat to study long-term behavioral deficits after stroke. The procedure required neither craniotomy nor anesthesia at the time of infarct, factors which can confound stroke studies and detract from the clinical relevance of an animal model. The technique involved insertion of an indwelling cannula into the right common carotid artery while the animal was fully anesthetized. After recovery from surgery and anesthesia at 48 h, a fragmented clot suspension was injected into the cannula. All of the animals subjected to this procedure demonstrated symptoms of stroke within seconds of clot injection. These included obtundation, circling behavior, piloerection, tonic deviation of the head, splaying of the contralateral limbs, and/or contralateral hemiparesis. Following stroke, the rats were evaluated for 30 days on a locomotor agility task. Continuous infusion of amphetamine (1 mg/kg/d x 7 days) or saline was begun 24 h postinfarct. Amphetamine-treated stroke animals showed significant, enduring improvements in locomotor function and level of morbidity which persisted beyond the period of drug infusion to the end of the experiment at 30 days poststroke. The survival rate for the amphetamine stroke group was significantly higher than that of the saline-treated stroke animals. In addition, lesion size in cortex was significantly less in the amphetamine animals than in the saline group. Supported in part by Sigma Xi Grants-in-Aid of Research and funds from DHHS Grant# 1 RO1 NS20220.

353.5 RELATIONSHIP AMONG CURRENT SOURCE DENSITY, INTRANEURONAL CURRENT AND MAGNETIC EVOKED FIELD IN TURTLE CEREBELLUM. Y.C. Okada and C. Nicholson. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

We have recently shown (Soc. Nsci. Abst. 12: 1132, 1986; Brain Res., 1987 in press) that a magnetic evoked field (MEF) can be measured at 2-5 cm from the turtle cerebellum in vitro during neural activity evoked by electrical stimulations of the dorsal midline. Here a study was made to determine whether the MEF in the same preparation is directly related to the internal longitudinal current (I_i) in active neurons. The cerebellum was removed from the brain and placed in 800 ml Ringer solution (100 mM NaCl; 5 mM KCl; 40 mM $NaHCO_3$; 3.5 mM Cal_2; 3.5 mM $MgCl_2$; and 20 mM glucose) gassed with 95% O_2 and 5% CO_2. It was suspended vertically with nylon netting, so that Purkinje cells were oriented horizontally and parallel fibers vertically. The peduncle was then stimulated with a 50 µs electrical pulse every 2 s. To test whether the I_i is directly related to the MEF, we first estimated I_i from the current source density (I_m). The I_m was determined from the laminar profile of the extracellular potential (V_e) measured in 50 µm steps. It showed strong current sources and sinks near the soma and dendrites of Purkinje cells. I_m was integrated along the depth of the cerebellum to estimate I_i/mm^2 of active tissue as a function of depth. Then, I_i was integrated once again along the depth to estimate the current dipole moment (Q) per mm^2 of active tissue. This second integration in effect ignores the higher order terms of a generalized description of the current generator, but is justified since we have determined the MEF to be dipolar. The Q over the entire active tissue was then estimated by multiplying Q/mm^2 by the surface area of the active tissue (6 mm^2) determined from a 2-D profile of the V_e over the entire cerebellum measured in 500 µm steps at a fixed depth of 350 µm from the ventral surface. The profile showed that the peduncular stimulation produced a rather simple pattern of activation confined in the ipsilateral half of the cerebellum. Although there were certain latency shifts in V_e at different positions, the waveforms were sufficiently uniform to justify the simple multiplication of Q/mm^2 by the active surface area. Based on Biot-Savart law, Q was used to calculate the normal component of the MEF at a field extremum at a distance of 16 mm from the cerebellar midline. The predicted MEF was similar in temporal waveform and magnitude to the MEF measured simultaneously with the V_e during the determination of the laminar profile. [Supported by NINCDS grant NS21149]

353.6 NOVEL FLASH EVOKED POTENTIAL (FEP) CHANGE PRODUCED BY DIAZEPAM IN RATS. R.S. Dyer G.C. Rigdon and L.M. Mayo*. Neurophysiology Branch, U.S. E. P.A. Research Triangle Park, NC 27711.

Benzodiazepines might affect the visual process, since benzodiazepine binding sites are found in the retina and cortex. The FEP is a measure of visual function which has been used successfully in identification of toxicological effects of compounds, partly because it reflects not only the integrity of the retinogeniculostriate system, but also because it reflects the general state of the subject. For example, the late negative peak of the FEP, peak N3, is generally maximal in amplitude when the subject is relaxed and awake; either increased or decreased arousal from this state should decrease the amplitude. In the present study we investigated the effects of i.p. diazepam on FEPs. We presumed that FEP peak N3 would be decreased by diazepam. Since some effects of drugs on FEPs are dependent upon background illumination, we investigated the effects of diazepam under both light (115 lux) and dark (<10 lux) background illumination conditions. Rats with chronic skull screw electrodes overlying visual cortex were administered either 0 (vehicle), 1.25, 2.50 or 5.0 mg/kg diazepam i.p., and tested either 10 or 40 min later. Amplitude and latency of the characteristic 3 positive and 3 negative peaks occurring within the first 200 msec after the flash were determined. As expected, diazepam decreased peak N3 amplitude. However this effect was only manifest when testing occurred with a light background, perhaps reflecting the generally larger peak N3 amplitudes recorded under these conditions. Diazepam produced two other background-dependent changes in the FEP waveform. Peak P1 amplitude was increased and peak N1 amplitude was decreased, but both effects occurred only when testing was done with a light background. The most novel effect of diazepam treatment, however, was a curious diazepam-dependent change in shape of the early negative peak. Whereas the typical FEP recorded from unanesthetized rats is initiated with a positive wave which peaks at about 22 msec (P1), followed by a negative wave which peaks at about 30 msec (N1), rats treated with diazepam demonstrated a novel negative peak (at about 22 msec) prior to P1 (which shifted to about 24 msec). The appearance of this peak was not dependent upon level of background illumination. Recordings obtained from the optic tract following diazepam treatment did not indicate that this activity reflected an augmentation of an early output from the retina. These findings have implications for the normal interpretation of FEPs, which holds that peak P1 reflects the earliest arriving input to the cortex from the lateral geniculate. It remains to be determined whether recordings obtained from the lateral geniculate nucleus are altered by diazepam, or if this effect reflects a heightened response to the click which accompanies the strobe flash.

353.7 MK801 PRODUCES CHANGES IN RAT FLASH-EVOKED POTENTIALS WHICH ARE MORE PCP-LIKE THAN KETAMINE-LIKE. L.M. Mayo*, G.C. Rigdon and R.S. Dyer. (SPON: M.I. Gage) Neurophysiology Branch, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

MK801, Ketamine and PCP all appear to block ion channels controlled by the NMDA receptor, as well as serving as agonists for the sigma opioid receptor. In rats, both ketamine and PCP change flash-evoked potentials (FEPs) by increasing amplitude of peak P1 and decreasing amplitude of peak N3. Ketamine also decreases peak N1 amplitude, but the effects of PCP on N1 amplitude are uncertain. A fundamental difference between PCP and ketamine appears to be with respect to peak P2. PCP decreases P2 amplitude, while ketamine has no consistent effect at dosages as high as 150 mg/kg (Mayo et al., Fed Proc '87,46:709; Dyer and Rigdon, Toxicologist '87,7:98). In this report we describe the effects of MK801 on FEPs recorded from unanesthetized unrestrained rats, and compare them to effects obtained using either ketamine or PCP. Three studies on Long-Evans hooded rats with chronic skull screw electrodes over visual cortex are reported here. In the first study, rats were tested immediately before receiving either saline (n=12) or 10 mg/kg PCP (n=12) i.p., and were retested 15, 30 and 60 min later. In the second study, rats were tested immediately before receiving i.p. injections of either saline (n=14), ketamine, 150 mg/kg (n=14), or MK801, 2 mg/kg (n=14), and were retested 15, 60 and 120 min later. In the third study, all rats (n=30) received saline, 0.1, 1.0, and 10 mg/kg MK801 immediately following a pretreatment recording session and were retested 30 min later. The intertreatment interval was at least 2 days, and order of treatments was randomized. In these studies, ketamine produced a profound decrease in amplitude of peaks N1 and N3, while increasing amplitude of peak P1. PCP also decreased amplitude of peak N1 and N3, while increasing amplitude of peak P1. PCP reduced P2 amplitude, but ketamine did not. MK801 increased P1 amplitude, and decreased N1, P2 and N3 amplitude. MK801, ketamine and PCP therefore have consistent effects upon peaks P1, N1 and N3. However PCP and MK801 also reduce the amplitude of P2, while ketamine does not do so at dosages up to 150 mg/kg. The data indicate that MK801 and PCP produce similar changes in FEPs recorded from unanesthetized rats, although MK801 appears to be more potent. The decreased P2 amplitude found following PCP and MK801 also occurs following administration of physostigmine, a cholinesterase inhibitor. Ketamine and PCP have been shown to block cholinergic receptors. It is curious that physostigmine, which should increase availability of ACh, and Ketamine and PCP, which should block the effects reduce availability of ACh, should have similar actions on P2 amplitude. This paradox, as well as the basis for the difference between MK801 and PCP on the one hand, and ketamine on the other, remains to be resolved.

353.8 A FURTHER ANALYSIS OF CORRELATION BETWEEN CNV AND REACTION TIME D.M.Jiang*, W.H.Yang*, B.Y.Wang* (SPON: B.Y.Yang). Division of Physiology, Dept. of Biology, Fudan Univ. Shanghai, China.

Relationships between contingent negative variation (CNV) and reaction time (RT) have been investigated in numerous literatures for years. However, the results were equivocal showing that different ways of analyzing CNV and RT suggested opposite conclusions (Rebert, C.S.: Electroenceph. Clin. Neurophysiol., suppl. 1973, 33: 172-178.).

In this experiment, a typical S1-S2 respond CNV paradigm and a selective average technique were employed to study the correlation between CNV and RT. 15 subjects were presented with foreperiod reaction-time trials: a warning flash was followed 1890ms later by a 1 kc tone and subject was instructed to respond to turn off the tone by pressing a button-switch as quickly as possible. This reaction at once stopped a digital meter which provided a measure of RT. The intervals of successive S1-S2 pairs ranged randomly from 30-60 sec. Each subject received a session of 30 S1-S2 pairing trials test. EEG was recorded from Cz and left mastoid using matched biopotential (Ag/AgCl) electrodes. An earth electrode was placed on left forearm. The slow potential shifts were amplified by a preamplifier with a time constant of 8 sec. Data involving EEG, eye movement and trigger pulse were stored on a FM tape recorder. The selective EEG average over a 5 sec epoch and area measurement of CNV were off-line worked out by an IBM-pc.

The results showed that there were no significant differences between the averaged CNV total area and RT ($P>0.05$). But significant differences were found by comparing the 5 fastest with the 5 slowest RTs in 30 trials session, the 5 CNV total areas with the fastest RTs were significantly larger than the 5 CNV total areas with the slowest RTs ($P<0.01$). However, further examining showed that the significant differences were only in the CNV late half area ($P<0.01$) not in the CNV early half area ($P>0.05$).

Our finding implies that the late component of CNV may be related to motor facilitation and reflect the cortical 'priming' process. (Supported by the Science Fund of the Chinese Academy of Sciences)

353.9 SCALP-RECORDED EVENT RELATED POTENTIALS FOR MOTOR SPEECH EVENTS AND LIP ELECTROMYOGRAPHY. M.E.Pratarelli*.(SPON:D. Strelioff), Salk Institute for Biol. Stud., Lab for Neuropsychol. P.O. BOX 85800, San Diego, CA., 92138.

Cortical evoked potentials that may reflect the planning of motor speech events were studied in an effort to assess the degree to which simple/basic differences in monosyllabic speech samples may be observable. Twelve scalp electrodes were positioned over visual and language centers and motor cortex to record the event related potentials. In addition to vertical and horizontal Electroocculograms, a single labial surface electrode was patched into two channels (one bipolar and the other monopolar in configuration;monopolar EMG and scalp electrodes and EOG were linked to both mastoids) to study the contractile latencies. The stimulus paradigm to elicit the speech samples consisted of 4 possible visually presented samples of the phonetic sounds /pa/, /pi/, /po/ and /pu/, presented 75 times each in random order for a total of 300 stimuli/utterances to which the subject was asked to utter the sounds as quickly as possible. In addition, 30 presentations of the word 'wait' were presented in a typical 'oddball' paradigm to which the subject was to refrain from speaking. 'Oddball' paradigms consist of presentations that are expected and a percentage (variable) that are not. 10 percent of the stimuli were thus oddballs. Stimulus ISI was 3500 milleseconds (msec) with 400 msec durations. Bandpass was set at .01 to 100 Hertz except for the EMG channels which were set at 10 to 100 Hertz. These variations were implemented to a) assess the optimum resolution of the muscle potentials for the purpose of subtracting muscle artifact from the EEG, and b) to isolate the mean latencies for the motor events.

A P300 component which is typically elicited in 'oddball' paradigms was observed with a latency of approximately 600 msec after the presentation of the word 'wait'. The subject, supported by the EEG records and the averages reported initiating an utterance they expected to be one of the four possible /p?/s but stopped short of vocalizing or releasing the burst seen in /p/ sounds. Assymetries over left/right hemispheres observed by other researchers were seen in certain conditions as was the enhancement of the N1 component (150 msec latency) over the left occipital region. The motor speech event may be reflected in a slight positivity following N1 and which differs from the averages of the identical stimulus set presented with instructions to read the verbal stimuli and not utter them.

Comparisons of read stimulus sets and read/uttered stimulus sets are presented as are comparisons of the reaction times extracted from the averages and by the technique of 'back-averaging'.

353.10 ELECTROPHYSIOLOGICAL INDICES OF NEURAL DEVELOPMENT IN HUMANS. J.H. Daruna and A. E. Rau. Department of Psychiatry and Neurology, Tulane University School of Medicine, New Orleans, LA 70112

The brain's spontaneous electrical activity (EEG) as well as its event-related activity (ERPs) undergo significant change from infancy to adulthood (Courchesne, E., Electroenceph. Clin. Neurophysiol., 45:468, 1978; John, E.R. et al., Science, 210:1255, 1980). The extent to which the various electrophysiological changes reflect independent aspects of neural development has not been sufficiently investigated. The present study addressed this issue by examining the developmental course of several electrophysiological measures as well as their intercorrelations within narrow age groups.

The subjects were healthy individuals (N=24) of above average intelligence selected from the following age groups: 4,5,6,7,12 and 26 years. Brain activity was recorded from F3, F4, P3, and P4 scalp locations referred to the linked mastoids, while the subjects either passively viewed a static colorful display of animals or performed an auditory vigilance task. The electrophysiological measures were: 1) the absolute power within the delta, theta, alpha and beta bands derived from the spontaneous EEG recorded during the passive viewing task; and 2) the amplitude and latency of the N100, N200, and P300 components from ERPs to target events recorded during the vigilance task.

Several of the measures were found to have non-parallel courses of development. Delta power progressively decreased with age, whereas theta, alpha, and beta power initially increased, peaking at 5 to 6 years of age, and then gradually declined into adulthood. The latencies of N100 and N200 remained relatively stable with increasing age. In contrast, the P300 latency decreased rapidly during middle childhood and reached adult values by the age of 12 years. Changes in the amplitude of N100 and N200 paralleled each other until the age of 7, subsequently N100 increased and reached adult values by the age of 12, whereas N200 began declining and continued this trend into adulthood. P300 amplitude remained relatively stable with increasing age. Intercorrelations between the various measures within age groups were not significant. It is concluded, therefore, that these EEG and ERP measures are sensitive to relatively independent aspects of neural development.

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353.11 VARIATIONS IN ABERRANT PURSUIT EYE TRACKING AS A FUNCTION OF CLINICAL STATE: IMPLICATIONS FOR STATE-TRAIT CONSIDERATIONS. R.T. Pivik, P.M. Cooper*, F.W. Bylsma*. Lab. of Psychophysiology, Univ. of Ottawa and Ottawa General Hospital, Ottawa, Ontario K1H 8L6.

Pursuit tracking dysfunction in psychotic patients has been repeatedly verified, but conflicting evidence exists regarding whether the incidence of these tracking interruptions varies with changes in clinical symptomatology. None of these investigations, however, has examined tracking performance in the same patients when actively ill and again when in remission. The present study provides such data.

Pursuit eye tracking performance was assessed in 10 psychiatric patients (9 schizophrenics; 1 manic depressive) and 10 age matched non-patient controls. Diagnoses were made independently by two psychiatrists (DSM-III criteria). All subjects were recorded twice: patients once when displaying active psychotic symptomatology and again when in remission ($\bar{X}$ interval = 9 months); control subjects were rerecorded after an average interval of 5.2 months. Horizontal and vertical eye movements were assessed electrooculographically (EOG: DC-300 Hz) while subjects tracked an oscillating target light (2.2 Hz) subtending a 20^{o} visual angle ($\pm 10^{o}$ from midline) under both light and darkened conditions ($\geq$10 min in each condition prior to testing). Data were tape recorded for off-line electronic processing and computer analyses. Artifact-free HEOG data were processed for incidence of velocity arrests (VAs: eye velocity $\leq 2^{o}$/sec) and root mean square error deviations (RMS: global target-eye movement discrepancy) according to standard criteria [Pivik, Biol. Psychiat.,14:859-879, 1979]. Data were statistically analyzed using repeated measures ANOVA procedures with post hoc tests when appropriate.

Control-patient tracking performance (VA & RMS) differed significantly only in the lighted condition when patients were actively ill ($p<.01$). Clinical improvement was associated with a significant decrease in VA scores for patients ($p<.01$) and the elimination of significant differences between groups. Dark adaptation reduced patient tracking errors to control levels regardless of clinical status. RMS scores were reduced by dark adaptation, but not as dramatically as VA scores.

The results, which for the first time provide eye tracking data from the same subjects while actively ill and again in remission, support a state-based interpretation of eye tracking dysfunction. Marked decreases in aberrant eye tracking during dark adaptation corroborate previous reports of this phenomenon [Pivik et al., Soc. Neurosci. Abst., 12:1092, 1986; Pivik et al., Prog. NeuroPsychopharmacol. Biol. Psychiat., in press] which have attributed this effect to subclinical cerebellar dysfunction in these patients.

Supported by the Ontario Mental Health Foundation.

354.1 INTERACTION OF THE INHIBITORY NEUROPEPTIDE GALANIN WITH EXCITATORY AMINERGIC AND PEPTIDERGIC MESSENGER SUBSTANCES IN MYENTERIC NEURONS OF GUINEA-PIG SMALL INTESTINE. C. Winkelmann, J.M. Palmer*, K. Tamura* and J.D. Wood. Dept. of Physiol., Coll. of Med., Ohio State Univ., Columbus, OH 43210.

Colocalization of peptides and amines in neurons of the enteric nervous system suggests that their opposing or orchestrated neuromodulatory actions on electrical behavior of nerve cells is important for appropriate signal transmission and control of gastrointestinal effector systems. Our aim was to investigate the interaction of the inhibitory neuropeptide galanin with other putative messenger substances on the electrical behavior of enteric ganglion cells and to elucidate its action on signal transduction in these cells. Conventional intracellular methods with 3 M KCl-filled microelectrodes were used to inject current and record electrical activity in AH/Type 2 myenteric neurons of guinea-pig small intestine in vitro. Application of galanin (0.1 nM to 1uM) to AH/Type 2 myenteric neurons mimics slow synaptic inhibition (slow IPSP). The inhibitory action of galanin consists of membrane hyperpolarization, decreased input resistance and decreased membrane excitability marked by suppression of action potential discharge. Membrane hyperpolarization by galanin was reversible near the estimated K^+ equilibrium potential suggesting that opening of K^+ channels was the ionic basis of its inhibitory action. Galanin, histamine, 5-hydroxytryptamine (5-HT), substance P, vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and forskolin were applied to neurons by addition to the superfusing Krebs solution or by ejection from fine-tipped pipettes with nitrogen pulses of controlled pressure and duration. Prior application of galanin (1 nM to 0.1 uM) abolished or strongly suppressed the excitatory actions of histamine, VIP, the slow depolarizing response to 5-HT, substance P and forskolin. The excitatory response to CGRP was weakly suppressed after much longer exposure to galanin. Galanin exerted its antagonism of the other messenger substances in the presence of tetrodotoxin (0.5 uM) indicating that the responses were due to a direct action upon the impaled neurons. Inhibition of excitatory responses to these substances by galanin differed from that of adenosine. Adenosine inhibits receptor-coupled increase of cyclic AMP and does not offset the excitatory responses to substance P and CGRP. Our results suggest that galanin is a potent inhibitory peptide in the enteric nervous system and modulates excitatory responses to other putative messenger substances possibly by multiple receptor interactions through a common or convergent signal transduction pathway in the same neuron. Galanin may not only suppress receptor-coupled cyclic AMP generation but also may modulate intracellular Ca^{2+} homeostasis. (Supported by NIH grants DK07308 to J.M.P. and DK26742 to J.D.W., and Tokai Univ. Overseas Res. Grant to K.T.)

354.2 EFFECT OF GALANIN ON ELECTRICAL AND SYNAPTIC PROPERTIES OF MYENTERIC NEURONS OF GUINEA-PIG SMALL INTESTINE. K. Tamura*, J.M. Palmer*, C. Winkelmann and J.D. Wood (SPON: A. Humbertson). Dept. of Physiol., Coll. of Med., Ohio State Univ., Columbus, OH 43210.

Galanin, a 29 amino acid peptide localized in neurons and neural fiber tracts of the central and enteric nervous systems, is a putative brain-gut messenger. In myenteric neurons of the guinea-pig small intestine, galanin mimics slow inhibitory postsynaptic potentials (slow IPSPs). This action of galanin consists of membrane hyperpolarization, decreased input resistance and decreased membrane excitability marked by suppression of spontaneous and stimulus-evoked action potential discharge. Our aim was to elucidate the mechanism of galanin's inhibitory actions on neuronal electrical membrane properties and on synaptic transmission between myenteric ganglion cells. Conventional intracellular methods with 3 M KCl-filled microelectrodes were used to record electrical and synaptic behavior of myenteric neurons in vitro. Somal action potentials were evoked in AH/Type 2 neurons either by intracellular injection of depolarizing current pulses or by antidromic invasion of action potentials generated by focal extracellular shocks applied to neurites in interganglionic connective fibers. Single or repetitive electrical focal stimuli applied to interganglionic fibers also evoked fast or slow excitatory postsynaptic potentials (EPSPs). Galanin was applied by addition to the Krebs superfusion solution (0.1 nM to 10 uM) or it was microejected from fine-tipped pipettes (20 uM) with pulses of nitrogen at a controlled pressure and duration. Galanin suppressed somal membrane excitability marked by reduced frequency of spike discharge, increased threshold for spike discharge by depolarizing current, and blockade of evoked antidromic invasion of initial segment spikes. Galanin significantly reduced the Ca^{2+} component of tetraethylammonium (5 mM) enhanced somal action potentials, decreased the amplitude and duration of post-spike hyperpolarizing potentials (AH), and hyperpolarized the cell membrane (range 5-25 mV) which was reversible near the estimated K^+ equilibrium potential. The amplitudes of evoked fast EPSPs were reversibly reduced in the presence of galanin (ED_{50} = 20 nM), and slow EPSPs were aborted or blocked by microejected galanin. The fast nicotinic excitatory response to microejected acetylcholine was also suppressed in the presence of galanin suggesting a postsynaptic inhibitory action at cholinergic synapses in the myenteric plexus. Our results suggest that galanin exerts its potent inhibitory action on myenteric neurons through multiple mechanisms. The mechanism of suppression of synaptic behavior by galanin remains unclear; however, its inhibitory mechanism on somal membrane excitability appears to involve opening of K^+ channels and a reduction of Ca^{2+} current. (Supported by Tokai Univ. Overseas Research Grant to K.T., and NIH grants DK07308 to J.M.P. and DK26742 to J.D.W.)

354.3 THE CO-LOCALIZATION OF SUBSTANCE P AND PRODYNORPHIN IMMUNOREACTIVITY IN THE MEDIAL PREOPTIC AREA, BED NUCLEUS OF THE STRIA TERMINALIS AND MEDIAL NUCLEUS OF THE AMYGDALA OF THE GOLDEN HAMSTER (Mesocricetus auratus). S.W.Newman, C. Neal, Jr.* and J.Swann (SPON P.Coyle). Department of Anatomy & Cell Biology, University of Michigan, Ann Arbor, MI 48109.

Substance P and opiate neurons have been found in similar locations throughout the central nervous system in various species. The co-localization in the same neuron of substance P-like immunoreactivity with dynorphin-like immunoreactivity has been reported in the basal ganglia of birds, reptiles and mammals, and a comparison of substance P and enkephalin distribution in the rat brain has also been described. Although substance P and opiate immunoreactivity has been demonstrated in similar locations, with areas of co-occurrance, to date the co-localization of substance P and prodynorphin (the precursor for dynorphin A (1-8 & 1-17) and dynorphin B (1-13)) has not been demonstrated specifically for individual neurons in the medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST) and medial nucleus of the amygdala (AMe). These three areas in the Golden hamster have been demonstrated to be critical for male sexual behavior and show similar patterns of immunocytochemical staining for substance P and prodynorphin marker antibodies.

We have incubated colchicine-treated hamster brain tissue with primary antisera to substance P generated in rat and to the prodynorphin molecule generated in rabbit, in conjunction with FITC-labelled anti-rabbit and TRITC-labelled anti-rat secondary antibody. Using this technique we have demonstrated the co-localization of substance P and prodynorphin immunoreactivity in MPOA, BNST and AMe. Immunoreactivity for both peptides is strongest in mid to caudal MPOA with the most double labelling in the lateral part of caudal MPOA and almost no co-localization found in the medial part of caudal MPOA. Strongest immunocytochemical staining for both peptides in BNST is found in the medial BNST at its more dorsal-caudal extent, with extensive co-localization observed at this level. Co-localization in medial BNST is also observed as the nucleus extends ventrally at this caudal level. Though prodynorphin and substance P immunoreactivity is found in scattered locations from mid to mid-caudal AMe the strongest labelling for both peptides is found in the most caudal extent where extensive co-localization is observed. Since connections between BNST, MPOA and AMe are essential for normal mating behavior in the hamster, and both opiates and substance P have been shown to modulate this behavior in rodents, our findings suggest that these peptides may interact in the regulation of sexual behavior. (Supported by NIH:NS20269 to SWN)

354.4 STUDIES ON INTERACTIONS BETWEEN NEUROPEPTIDE Y (NPY) AND NORADRENALINE IN RAT CEREBRAL CORTEX. C. Wahlestedt*, R. Ekman and R. Håkanson*. Departments of Pharmacology, and Psychiatry and Neurochemistry, University of Lund, Lund, Sweden.

Neuropeptide Y (NPY) is a 36 amino acid peptide with a C-terminal α-amide group. NPY occurs throughout the mammalian cerebral cortex, mostly in local circuit neurones but also in nerve fibres deriving from (noradrenergic) brain stem neurones (de Quidt, M.E. and Emson, P.C., Neuroscience, 18:545, 1986). In various other locations NPY interacts with noradrenergic transmission (Wahlestedt C. et al., Regul. Pept., 13:307, 1986).

This study examined synaptosomal preparations from the rat cortex with regard to the effect of NPY on the following parameters: i) Binding of α-1- (3H-prazosin), α-2- (3H-rauwolscine), and β-adrenoceptor (3H-CGP-12177) ligands, ii) uptake of 3H-NA, and iii) potassium-induced release of 3H-NA. Also, the effect of NA on the binding of 3H-NPY was studied.

NPY (0.1 μM) approximately doubled the number of α-1-binding sites (Bmax) without affecting the affinity (Kd). The NPY-induced up-regulation of α-1-sites was attenuated by GTP. Peptide YY (PYY) 13-36, which is believed to have certain properties in common with NPY, did not increase the Bmax for prazosin. NPY (0.1 μM) did not affect the binding of the α-2- or the β-adrenergic ligands.

Conversely, NA up-regulated NPY-binding sites. This effect is probably mediated by β-adrenoceptors, since isoprenaline mimicked the effect of NA.

Finally, the present study failed to detect any effects of NPY on the NA uptake by and release from the cortical synaptosomes.

354.5 **NEURONAL AND VASCULAR ASSOCIATIONS OF NEUROPEPTIDE Y IN RAT STRIATUM: SYNAPTIC INTERACTIONS AND CO-LOCALIZATION WITH GABAERGIC NEURONS, BUT NOT CATECHOLAMINERGIC TERMINALS.** *C. Aoki and V.M. Pickel,* Div. of Neurobiology, Cornell Univ. Med. Coll., NY, NY, 10021

Neuropeptide Y (NPY) co-exists with catecholamines (CA) in certain central and peripheral neurons and, like norepinephrine, exerts potent vasoconstriction of peripheral vessels. We examined the ultrastructural localization and neuronal and vascular associations of NPY in the rat dorsal striatum, a region enriched with CA (principally dopamine) terminals and GABAergic neurons. Antisera against NPY, the CA-synthesizing enzyme, tyrosine hydroxylase (TH), and GABA were supplied by the laboratories of Drs. T.L. O'Donohue, T.H. Joh and A.C. Towle, respectively. These were produced in goats, rabbits and rats and were simultaneously visualized in pairs by peroxidase-antiperoxidase and immunoautoradiographic labeling methods. NPY-like immunoreactivity (-LI) was detected in a few perikarya and proximal dendrites of neurons having indented nuclei and other characteristics of aspiny neurons. The dendrites received sparse (1 per 4 µm dendritic shaft) synaptic inputs from terminals forming either asymmetric or symmetric junctions. In dual labeling studies, 7% (1 per 13) of the terminals forming symmetric junctions exhibited GABA-LI; whereas none of the afferents were labeled for TH. Axon terminals were the most frequently encountered profiles exhibiting NPY-LI (168 terminals vs. 21 NPY dendrites/8500 μm^2). Only 83 out of the 163 terminals which exhibited NPY-LI formed recognizable junctions. The remaining processes were in contact either with other neurons, glia or the basement membranes of blood vessels. The obvious junctions were exclusively symmetric and included unlabeled (33 of 36) and GABA-labeled (3 of 36) dendrites and dendritic spines. The NPY terminals also frequently were seen in apposition with other unlabeled (13) or GABA-IR (1) terminals, but not to terminals immunolabeled for TH (n=135). However, in 3 cases so far examined, convergent inputs of NPY and TH terminals onto common unlabeled dendrites were identified. Co-localization of TH and NPY also was never detected, although a few cells exhibited both NPY and GABA-LI. We conclude that in the dorsal striatum of rat, 1) NPY is localized to aspiny interneurons which receive GABAergic afferents and terminate on GABAergic neurons and may have additional intracellular interactions with GABA; 2) NPY and CA may jointly modulate the output of common target neurons, but are not detected in direct synaptic contact or within the same terminal; 3) NPY - exclusive of NE - may modulate vascular diameter or themselves depend on circulating factors. (This work was supported by NSF grant BNS 8320120 to VMP and NIH grant NS07782-01 to CA.)

354.6 NEUROPEPTIDE Y/SEROTONIN INTERACTIONS IN THE RAT HYPOTHALAMUS. J. Guy*,[1], O. Bosler*,[2], G. Pelletier[1], (SPON: A. Dupont), [1]MRC Group in Molecular Endocrinology, Le Centre Hospitalier de l'Université Laval, Quebec G1V 4G2, Canada.[2] Laboratoire de Neurobiologie, CNRS, 13402 Marseille-Cedex, France.

Numerous studies have shown that neuropeptide Y (NPY) is largely distributed in the mammalian brain. In the hypothalamus, dense plexus of NPY immunoreactive fibers are present in the ventral part of the suprachiasmatic nucleus. In the arcuate nucleus, NPY staining is found in both cell bodies and fibers. These two hypothalamic nuclei are also known to receive a dense serotoninergic (5-HT) innervation from the mesencephalic raphe nucleus. Using a combined radioautographic and immunocytochemical technique in rats subjected to intraventricular injection of [^{3}H]-5-HT, we observed a striking overlap between the distribution of NPY immunoreactive fibers and [^{3}H]-5-HT uptake sites in these nuclei. By contrast, in the dorsal raphe nucleus of colchicine pretreated rats, NPY immunoreactive perikarya did not show any selective radiolabelling. Intraventricular injection of 5-7-DHT was used in order to destroy the serotoninergic afferents. Two weeks after this treatment, the NPY immunoreactivity was not affected while [^{3}H]-5-HT uptake sites were not detectable. These results suggest that NPY and 5-HT are present in two different neuronal systems. Accordingly, electron microscope observations confirmed that NPY immunoreactive and [^{3}H]5-HT labelled profiles belonged to separate neuronal systems with distinct morphological features. In the suprachiasmatic nucleus, direct axo-axonic appositions between the two types of differentially labeled terminals were ocasionally seen. More commonly, a radiolabeled and an immunoreactive afferent contacted the same perikaryon or dendrite. In the arcuate nucleus, direct appositions between ^{3}H-5-HT labeled terminals and immunoreactive cell bodies or dendrite could be observed. These direct appositions in both the suprachiasmatic and arcuate nucleus were seen without synaptic membrane differentiation. These data should be helpful in determining the possible importance of NPY/5-HT interactions in some physiological processes such as circadian rythmicity and pituitary hormone secretion.

354.7 INTERACTIONS AMONG SEROTONIN, SUBSTANCE P AND THYROTROPIN RELEASING HORMONE IN RAT SPINAL CORD VENTRAL HORN. S.R. White and G. Crane*. Dept. Vet. Compt. Anat., Pharmacol. and Physiol., Washington State Univ., Pullman, WA 99164-6520

The neuropeptides, substance P (SP) and thyrotropin-releasing hormone (TRH), appear to be primarily, if not exclusively, colocalized in terminals with serotonin (5HT) in the rat spinal cord ventral horn (Gilbert et al., Neuroscience, 7, 1982, 69; Wessendorf and Elde, J. Histochem. Cytochem. 33, 1985, 984). Microiontophoretic application of each of these three substances enhances rat spinal motoneuron excitability in situ (White, Brain Res. 335, 1985, 63). However, it is not known how these substances, which may be co-released from terminals in the ventral horn, interact to affect motoneurons.

Seven barrel micropipettes were used to apply TRH, 5HT, SP, glutamate (GLU) and control solutions while recording from antidromically identified lumbar motoneurons in urethane-anesthetized rats. 5HT (10-30 nA, 30-60 sec) and SP (10-40 nA, 1-3 min) each facilitated GLU-evoked activity of motoneurons and the facilitation was additive when these substances were applied simultaneously. Although the initial application of TRH (40-100 nA, 1-3 min) also enhanced GLU-evoked activity of motoneurons, rapid desensitization developed to repeated TRH administration. TRH applied simultaneously with 5HT decreased the facilitatory effect of 5HT applied alone. Consequently, peptides colocalized with 5HT in the spinal cord ventral horn appear to be capable of enhancing or diminishing the facilitatory effect of 5HT on spinal motoneuron excitability. (Supported by NIH, NS24388).

354.8 5-HYDROXYTRYPTAMINE DEPRESSES RETICULOSPINAL EXCITATORY POSTSYNAPTIC POTENTIALS IN MOTONEURONS OF THE LAMPREY. J.T. Buchanan* and S. Grillner. The Nobel Institute for Neurophysiology, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden.

The spinal cord of the lamprey, a lower vertebrate, is richly innervated by 5-hydroxytryptamine (5-HT) fibers, most of which originate from an intrinsic spinal system of cells that forms a dense ventromedial fiber plexus. The dendrites of motoneurons and interneurons extend into this plexus (Van Dongen, P.A.M. et al., J. Comp. Neurol. 234:523-535, 1985) where they make synaptic contact with the large axons of reticulospinal Müller cells. The histological association of 5-HT fibers with Müller axons and neuronal dendrites suggested that 5-HT might be modulating the excitatory postsynaptic potentials (EPSPs) from Müller axons to spinal neurons. We have tested that possibility, using paired intracellular recordings of motoneurons and single Müller axons in the in vitro preparation.

We found that 5-HT always reversibly depressed the chemical component of the Müller EPSPs in motoneurons (50 to 80% reduction). The effect was observed with either local application of a small volume of 10 mM 5-HT to the surface of the spinal cord or by bath-application of 1 µM 5-HT. In addition, 5-HT always slightly hyperpolarized the cells and either left unchanged or increased the amplitude of the electrotonic component of these dual-component EPSPs (<10% increase) indicating an increase in input resistance.

To investigate 5-HT's site of action, the sensitivity of the motoneurons to L- or D-glutamate was tested in tetrodotoxin, before and after 5-HT application. No significant change was observed. Glutamate activates the same postsynaptic receptors as the endogenous Müller axon transmitter (Buchanan, J.T. et al., Brain Res. 408: 321-325, 1987). In contrast to the findings in normal physiological solution, 5-HT application in tetrodotoxin only rarely hyperpolarized the motoneurons and produced no change in their input resistance.

The intracellularly recorded resting potential and action potential of the presynaptic Müller axons were not affected by 5-HT. A small calcium component of the action potential was revealed by application of tetraethylammonium, but 5-HT had no effect upon it.

We conclude that 5-HT depresses the EPSPs in motoneurons from Müller axons by a mechanism that is most likely to be presynaptic. The effect is not due to a decrease in postsynaptic receptor sensitivity, nor to a shunting effect in the motoneuronal dendrites. The functional significance of 5-HT's depression of Müller EPSPs may be that it is a mechanism for segmentally modulating the amplitude of descending brainstem input without altering the responsiveness of the segmental neurons to other inputs.

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354.9 BLOCKADE OF DOPAMINERGIC OR SEROTONERGIC RECEPTORS DOES NOT PREVENT THE METHAMPHETAMINE- OR 3,4-METHYLENDIOXYMETHAMPHETAMINE-INDUCED DECREASE OF TRYPTOPHAN HYDROXYLASE ACTIVITY. G.R. Hanson, D.M. Stone*, L.A. Matsuda*, J.W. Gibb and M. Johnson*. (SPON: J.W. Woodbury). Dept. Pharmacol. and Toxicol., University of Utah, Salt Lake City, UT 84112

A single administration of methamphetamine (METH) or 3,4-methylenedioxymethamphetamine (MDMA) decreases brain tryptophan hydroxylase (TPH) activity in several brain areas. METH differs from MDMA in its greater ability to release DA, while MDMA is a better releaser of 5-HT. The decrease of neostriatal TPH activity induced by METH can be prevented by depleting DA with α-methyl-p-tyrosine (Schmidt et al., 1985) or by 6-hydroxydopamine lesions of the substantia nigra (Johnson et al., 1987). This study attempts to determine 1) if METH- or MDMA-released DA affects TPH activity by interacting with its receptors, or 2) if 5-HT released by either of the amphetamine-like drugs, or induced by released DA, affects TPH activity by interacting with its receptor or auto-receptor. Sprague-Dawley rats were injected i.p. with haloperidol (3mg/kg), methiothepin (20 mg/kg) or ritanserin (1 mg/kg) 15 min prior to treatment with either METH (10 mg/kg, s.c.) or MDMA (5 mg/kg, s.c.). Concentrations of DA, DOPAC, HVA, 5-HT and 5-HIAA were measured with HPLC-EC. TPH activity was determined according to Ichiyama et al. (1970) and Sitaram and Lees (1978). Neostriatal TPH activity was decreased to 65% of control 1 h after the administration of METH; enzyme activity reached 45% of control 3 and 6 h after injection. Haloperidol did not attenuate the effect of METH observed 1 h and 3 h after the administration. This treatment with haloperidol increased DA release as suggested by increased concentrations of neostriatal DOPAC and HVA 75 min later. Coadministration with METH further increased the concentration of these metabolites. Methiothepin had a similar effect to haloperidol on the neostriatal dopaminergic parameters but increased concentrations of 5-HIAA in the frontal cortex and hippocampus, indicating that methiothepin interacted with 5-HT autoreceptors. This treatment with methiothepin had no effect on the decrease of neostriatal TPH activity induced by METH as well as the pretreatment with the 5-HT_2 receptor antagonist, ritanserin. MDMA decreased neostriatal TPH activity to 70% of control after 1 and 6 h, while the enzymatic activity reached 50% of control 3 h after treatment. Haloperidol did not prevent this decrease at 3 h after the MDMA injection, nor did methiothepin at any of the 3 observation times. These results do not support the involvement of DA receptors, 5-HT receptors nor autoreceptors in the decrease of central TPH activity induced by a single dose of METH or MDMA. (Supported by USPHS grants DA 00869, DA 04222 and by Janssen Pharmaceutica Inc.)

354.10 EFFECTS OF COCAINE ON NEUROTENSIN SYSTEMS OF THE RAT BRAIN. P.L. Smiley*, J.W. Gibb, and G.R. Hanson (Spon: L.M. Partlow). Dept. of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112

Many of the stimulant effects of cocaine are similar to those produced by methamphetamine (METH). Our laboratory has previously demonstrated that single and multiple high doses of METH increase concentrations of neurotensin-like immunoreactivity (NTLI) in the striatum and substantia nigra of the basal ganglia as well as in the nucleus accumbens of the mesolimbic complex of the rat brain. In order to make comparisons with METH, we have evaluated the response to cocaine of neurotensin systems in these brain regions by measuring tissue concentrations of this peptide. We used for our radioimmunoassay a highly selective antiserum which could detect 2 pg of NTLI per sample. Six hours after single injections of cocaine (20 mg/Kg or 40 mg/Kg; i.p.), we observed significant increases of NTLI in the substantia nigra but found no apparent effect on neurotensin concentrations in the neostriatum, nucleus accumbens, and hippocampus. One hour following multiple doses of cocaine (30 mg/Kg, i.p.; q6h/5 doses), neurotensin levels in the neostriatum and substantia nigra were significantly elevated, but no effects were seen in the nucleus accumbens and hippocampus. The neurotensin concentration remained significantly elevated three hours after multiple dosing, but had substantially recovered in the neostriatum. These data indicate there are similarities in the response of striatal and nigral neurotensin systems to cocaine and METH. In contrast, the effects of cocaine on mesolimbic neurotensin systems are markedly different than those of METH. Possible mechanisms of action of cocaine on neurotensin systems will be addressed. (Supported by USPHS Grants DA 00869 and DA 04222)

354.11 EFFECTS OF METHAMPHETAMINE ON MESOLIMBIC NEUROTENSIN SYSTEMS OF THE RAT BRAIN. K.M. Merchant*, A.A. Letter*, J.W. Gibb and G.R. Hanson. Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112

We have previously reported that high doses of methamphetamine (METH) selectively alter the concentrations of neurotensin-like-immunoreactivity (NTLI) in the striatal-nigral regions of the rat brain. We hypothesize that these METH-induced changes are a result of the activation of the nigral-striatal dopamine pathway by this stimulant. Another dopamine system which is responsive to METH treatment projects from the ventral tegmental area to other CNS regions such as the nucleus accumbens and frontal cortex. For comparison with our previous findings, we have now examined the effects of METH administration on neurotensin systems associated with these mesolimbic structures. Eighteen hours following multiple doses of METH (15mg/kg, s.c.; q6h/total 5 doses), the concentration of NTLI increased by 100% to 150% in the nucleus accumbens and declined by 30% to 35% in the frontal cortex but was unchanged in the ventral tegmental area. A single dose of METH caused a similar change only in the nucleus accumbens suggesting that this structure is more sensitive than the frontal cortex to this drug treatment. Blockade of dopamine D_2 receptors with haloperidol (2 mg/kg, i.p.) or sulpiride (80 mg/kg, i.p.) altered the NTLI levels in both the nucleus accumbens and the frontal cortex in a manner similar to that of METH. This effect was additive to the alterations caused by METH when the drugs were administered concurrently. In contrast, D_1 receptor blockade by SCH 23390 (0.5 mg/kg, i.p.) had no effect alone, but attenuated the METH effect in both structures. These data suggest that the mesolimbic neurotensin pathways are closely associated with the dopaminergic systems and are differentially regulated by D_1 and D_2 receptors in much the same manner as the striatum. (Supported by USPHS Grants DA 00869 and DA 04222)

354.12 OPPOSING EFFECTS OF ROSTRAL AND CAUDAL ACCUMBENS CCK MICROINJECTIONS ON AMPHETAMINE-INDUCED LOCOMOTION F. J. Vaccarino and J. Rankin, Departments of Psychology and Psychiatry, University of Toronto, Ontario. M5S 1A1.

Current evidence indicates that the mesolimbic dopamine (DA) system is important for the expression of amphetamine (AMP)-induced behavioral activation. Studies indicating that a subset of mesolimbic neurons contain both DA and cholecystokinin (CCK) and that CCK terminals are present in the nucleus accumbens (N.Acc) raise the possibility that CCK may play a modulatory role in the expression of AMP-induced behavioral activation. Studies investigating the effects of intra-N.Acc CCK microinjections have found that CCK either potentiates (Crawley et al. J.Neurosci., 5: 1972, 1985) or attenuates (Van Ree et al. Eur. J.Pharmacol., 93:63, 1983; Weiss et al. Neurosci. Abs 12:1491, 1986) stimulant-induced behavioral activation derived from the N.Acc. The present study addresses the possibility that the effects of intra-N.Acc CCK microinjections on behavioral activation produced by systemic AMP are dependent on the rostral-caudal location of N.Acc CCK microinjections. To this end, rats were tested for the locomotor activating effects of systemic AMP following CCK microinjections into either the rostral or caudal N.Acc.

Male Wistar rats with cannulae implants aimed at either the rostral or caudal N.Acc were habituated to locomotor test cages. Following habituation, rats were tested for their locomotor response to intra-N.Acc microinjections of CCK in combination with systemic AMP (0.5 mg/kg S.C.) CCK was administered in the following doses: 0.0 (vehicle), 0.5 ng, 1.0 ng and 2.0 ng. Drugs were dissolved in saline. CCK was administered bilaterally in a 0.25 ul volume over 1 minute. CCK doses were administered in random order with a minimum of 2 no-pretreatment days separating drug tests.

The results demonstrate that rostral and caudal N.Acc CCK microinjections produce opposite effects on AMP-induced locomotion. Rostral N.Acc CCK microinjections dose-dependently attenuated, while caudal N.Acc CCK microinjections dose-dependently potentiated AMP-induced locomotion. CCK microinjections in the central N.Acc had no consistent effect. The present findings indicate that the effects of intra-N.Acc CCK on AMP locomotion depend on the rostral/caudal location of CCK suggesting that the N.Acc is functionally heterogenous with regard to CCK/AMP interactions. This research was supported by NSERC grant U0443 to FJV. CCK was generously donated by Mr. S. J. Lucania of E. R. Squibbs and Sons, Inc.

354.13 FAILURE OF CHOLECYSTOKININ TO ANTAGONIZE THE EFFECTS OF MORPHINE SULFATE WITHIN A CONDITIONED TASTE AVERSION PARADIGM. A.L. Riley, J.P. Mastropaolo, S. Pournaghash* and K. Moskowitz*. Psychopharmacology Lab, The American University, Washington, D.C. 20016.

Although conditioned taste aversions were initially examined in relation to their importance for traditional learning theory, they have recently been utilized as a pharmacological tool. One specific application of the taste aversion design is its use in assessing drug antagonism. For example, naloxone can block the induction of taste aversions by morphine in morphine-naive rats, as well as induce aversions in morhine-maintained subjects, presumably because of precipitated withdrawal.

The present experiments utilized the taste aversion design to assess the putative antagonistic interaction of cholecystokinin (CCK) and morphine. In Experiment 1, rats were given 20-min access to saccharin followed by an intraperitoneal injection of morphine sulfate (6 mg/kg). Subjects in Groups C10 and C30 also received an injection of CCK at 10 and 30 u/kg, respectively, while subjects in Groups N and W were given an injection of naloxone hydrochloride (10 mg/kg) or distilled water. This conditioning procedure was repeated every fourth day until three conditioning trials had been given. Although naloxone clearly attenuated the aversions induced by morphine sulfate, there was no attenuation of aversions in subjects receiving the combination of morphine and CCK, independent of the dose of CCK administered.

In Experiment 2, rats were injected for 21 consecutive days with either morphine sulfate (80 mg/kg) or distilled water. On Day 22, subjects in each of these two conditions were given saccharin followed by an injection of either CCK (10, 20 or 40 u/kg), naloxone hydrochloride (10 mg/kg) or distilled water. This conditioning procedure was repeated every fourth day until three conditioning trials had been given. Although morphine-maintained subjects acquired a robust aversion to the naloxone-associated taste (and displayed dramatic decreases in body weight following naloxone administration), CCK did not condition an aversion or affect body weight at any dose administered.

Although CCK has been reported to antagonize the effects of morphine within a range of behavioral paradigms, it was without effect within the taste aversion procedure, either as an antagonist in blocking taste aversions to morphine or as a precipitator of aversions in morphine-dependent animals. The basis for these differences are unclear although it is possible that the reported antagonism necessitates a procedure in which morphine and CCK produce opposite effects when given alone.

354.14 MODULATION OF HIPPOCAMPAL AND HYPOTHALAMIC [^{3}H]OXOTREMORINE-M DENSITY BY OPIATE AGONISTS AND ANTAGONISTS. Allan E. Johnson, Hector Coirini* and Bruce S. McEwen. Laboratory of Neuroendocrinology, Rockefeller University, N.Y., N.Y. 10021

Muscarinic cholinergic transmission mediates certain types of opioid stress analgesia (Terman et al., Brain Res.,372 (1986) 167). The administration of centrally active muscarinic anatagonists or lesions of the septohippocampal tract reduce this form of analgesia (Kelsey and Baker, Behav.Neurosci.,97 (1983) 945). To begin to specify the brain regions involved in the interaction of central muscarinic cholinergic and opioid transmission in the regulation of opioid stress analgesia, manipulations of opioid transmission on muscarinic receptor binding were studied with quantitative receptor autoradiographic techniques.

Female rats (200-250 grams) were ovariectomized and one week later implanted with Alzet minipumps containing either morphine sulfate (70mg/ml saline), naltrexone (70mg/ml saline) or saline. Forty-eight hours later, animals were decapitated, brains were removed, rapidly frozen in 2-methylbutane (-30°C) and stored at -70°C. Brains were sliced (14µm) with a cryostat at -12°C, thaw-mounted onto subbed slides, vacuum desiccated (2hrs, -5°C) and stored at -70°C. Muscarinic receptors were labeled with [^{3}H]Oxotremorine-M (OXO, 1.0nM; Spencer et al., Brain Res.,380 (1986) 59) with 1µM atropine sulfate used to define nonspecific binding. Labeled slices and plastic tritium standards (Amersham ^{3}H-microscales) were exposed to tritium-sensitive film (Amersham Hyperfilm-^{3}H) for 4 weeks. Binding was analyzed with a computer assisted densitometer that converted gray levels to femtomoles/mg tissue wet weight using the standard curve derived from tritium standards.

Autoradiographic analysis revealed that morphine treatment enhanced OXO binding in some hippocampal regions that contain high densities of µ-opioid receptors (Tempel et al., in press) such as the pyramidal cell layer of CA3, CA4, CA2 region of the radiatum, the molecular lacunosum layer and in the lateral habenula. Naltrexone treatment decreased OXO binding in areas containing high levels of κ-receptors such as the CA2 region of oriens layer and in the dorsomedial and ventromedial hypothalamic nuclei. Differences were not detected in other brain regions. Scatchard analysis of OXO binding in hippocampal and hypothalamic homogenates of morphine sulfate (100mg/ml) or naltrexone (100mg/ml) treated animals showed that differences in binding were due to differences in receptor density rather than affinity. These results support previous studies that implicate the muscarinic cholinergic system in the mediation of opioid stress analgesia. Supported by grant numbers NS07911 (AJ), TW03617 (HC) and MH41256 (BMc).

354.15 NALOXONE AND MORTALITY IN THE GERBIL STROKE MODEL, E.C. Benzel, C. Musgrove*. Division of Neurosurgery, Louisiana State University Medical Center, 1501 Kings Highway, Shreveport LA. 71130.

Endorphins and narcotics have been implicated in the exacerbation of neurologic deficits following stroke. In order to test the theory that narcotic antagonists might offer an improvement in neurologic sequellae following stroke, a gerbil stroke model was used to test varying doses of naloxone administered intraperitoneally, 45 minutes following carotid artery transsection, in 50 adult gerbils.

Lower dose naloxone (1.0-2.5 mg/kg) was more effective in preventing mortality than either control (sterile saline) or high dose (10 mg/kg) naloxone (1.0 and 2.5 mg/kg doses combined, offered a significant improvement in mortality over both the control and the high dose groups P=0.026).

The failure of high dose naloxone (10 mg/kg) in the face of low dose (1.0-2.5 mg/kg) efficacy illustrates a unique dose-response relationship. The etiology of this phenomena is uncertain. It could be that the narcotic agonist activity of naloxone dominates at a higher dose.

Other factors, perhaps, skewed the results presented here. This, however, is unlikely. A confounding effect of the anesthetic agent is unlikely since sodium pentobarbital was used in both the control and the naloxone treated animals. A systemic effect of naloxone may even play a role.

It is interesting that 10 mg/kg of naloxone is no more effective than the administration of saline. In this study, the dosage of naloxone demonstrated a clear correlation with survival and neurologic injury. It is apparent that a 1 to 2.5 mg/kg dosage of naloxone and the administration of the drug within 45 minutes of injury results in a substantial improvement in survival.

It appears that an appropriate dose of naloxone, administered early enough to alter outcome, may offer an improved survival in the gerbil stroke model. This obviously has significant implications with regard to the use of narcotic antagonists in humans with stroke.

355.1 ROLES OF THE DOPAMINERGIC SYSTEM ON THE REGULATION OF THE METABOLISM OF OPIOID PEPTIDES AND TACHYKININ IN THE STRIATONIGRAL PATHWAY. S. Li, P.M. Hudson, K. Nanry, M. Stachowiak, H.A. Tilson, and J.-S. Hong (spon: E. McNeill). LBNT, NIEHS, NIH, P.O. Box 12233, RTP, NC 27709.

It has been reported that the dopaminergic (DA) system exerts potent influence on the metabolism of [Met^5]-enkephalin (ME) and Substance P (SP) in the basal ganglia. Recent reports indicate that dynorphin-like immunoreactivity (DN-LI) is present in the striatonigral pathway and is found in high concentration in substantia nigra. However, the regulation of dynorphin (DN) in this pathway is not known. We have recently reported that repeated injections of a DA agonist, apomorphine (APO), caused dose-(0.5-5.0 mg/kg, s.c., twice daily) and time-related (1-7 days) increases in striatonigral DN-LI and SP-like immunoreactivity (SP-LI) but not ME-like immunoreactivity (ME-LI). These results, plus our previous finding that repeated injections of haloperidol increase the striatal level of ME-LI, but decrease the nigral level of SP-LI and do not alter the striatonigral levels of DN-LI, suggest differential regulation of these peptides by the DA system. The purpose of this study was to determine if APO-induced increase in the striatonigral levels of DN-LI and SP-LI is due to an increase in the biosynthesis by measuring the abundance of messenger RNA (mRNA) coding for these two peptides. Northern blot analysis, using cDNA or cRNA probes coding for precursor of dynorphin and tachykinin derived from rat brain, was used to quantitate the amount of mRNA. Seven daily injections of APO (5 mg/kg, s.c., twice daily) produced a 30-40% increase in mRNA coding for preprotachykinin and a 80-100% increase in preprodynorphin in the striatum. These increases in the abundance of mRNA were proportional to the increases in striatal levels of DN-LI and SP-LI, suggesting that APO treatment accelerates the rate of biosynthesis for these two peptides. To ascertain that the effects of APO are mediated through the nigrostriatal DA system, APO (5 mg/kg, s.c., twice daily for 7 days) and amphetamine (AMP, 5 mg/kg, s.c., twice daily for 7 days) were repeatedly injected to rats which had received prior unilateral nigral injection of 6-hydroxydopamine (6-OHDA, 10 ug in 0.5 ul). 6-OHDA did not alter the levels of DN-LI and SP-LI in striatum 3 weeks after lesion. APO caused significant increases in striatal DN-LI and SP-LI in both the nonlesioned and 6-OHDA lesioned sides. The increases of DN-LI and SP-LI in the 6-OHDA lesioned side were much greater than those in the nonlesioned side,presumably due to a hypersensitivity of DA receptors resulting from DA denervation. Seven daily injections of AMP increased striatal DN-LI and SP-LI in the nonlesioned side, but not in the 6-OHDA lesioned side. The striatal level of ME-LI was increased by 6-OHDA and this increase was attenuated by APO treatment. These results strongly suggest that the effects of APO and AMP on these neuropeptides are mediated through the nigrostriatal DA system.

355.2 SUBCELLULAR DISTRIBUTION OF MAMMALIAN TACHYKININS IN RAT BASAL GANGLIA. P.C.Emson, F.J.Diez-Guerra* and P.J.Richardson*. MRC Group, Dept. of Neuroendocrinology, Institute of Animal Physiology & Genetics Research, Babraham, Cambridge, CB2 4AT, UK.

A combined differential and density gradient centrifugation procedure was used to study the subcellular localization of the mammalian tachykinins in rat caudate-putamen and substantia nigra. Substance P, Neurokinin A, Neuropeptide K and Neurokinin B were found to be concentrated in the synaptosomal fractions, and in fractions containing heavy synaptic vesicles in both regions studied. Neuropeptide K showed a gradient distribution indicating its localization in a lighter vesicle population, compatible with its proposed role as Neurokinin A precursor. In contrast, the catecholamines dopamine and noradrenaline had a more wide spread distribution throughout the gradient. HPLC analysis of the immunoreactivity recovered showed that the tachykinin immunoreactivity coeluted with the relevant synthetic tachykinins, except in the soluble gradient fraction where neurokinin A immunoreactivity eluted in position consistent with neurokinin A3-10. These results show that, in the basal ganglia, the mammalian tachykinins are localized in fractions containing large dense cored synaptic vesicles. This vesicular localization would be consistent with the proposed role of the tachykinins as neurotransmitters and neuromodulators. The differences observed in the gradient distribution of neurokinin A and neuropeptide K in caudate-putamen and substantia nigra are consistent with peptide processing occurring during the migration of the tachykinin containing vesicles from the former region to the latter.

355.3 OPTIMIZATION OF HPLC-RIA ANALYTICAL PROTOCOLS FOR THE DETERMINATION OF SUBSTANCE P AND ITS FRAGMENTS IN THE SPINAL CORD. O.J. Igwe*, L.J. Felice*, V.S. Seybold and A.A. Larson. Depts. of Veterinary Biology, Veterinary Diagnostic Investigations and Cell Biology and Neuroanatomy, University of Minnesota, St. Paul, MN 55108.

A simple and sensitive reversed phase high pressure liquid chromatographic procedure combined with radioimmuno- assay (HPLC-RIA) was developed and optimized to quantify substance P_{1-11}(SP) and its amino and carboxy terminal fragments, SP_{1-4}, SP_{1-7}, SP_{1-9}, SP_{2-11}, and SP_{5-11}, some of which are natural metabolites in the spinal cords of mice. An extremely selective and efficient solid-phase extraction protocol was used for the preparative purification of tissue homogenates prior to subsequent analyses. The actual concentrations in spinal tissues were quantified from the calibration curves of the ratios of the peak areas of sample peptides and the internal standard versus the concentration of SP and its fragments. The sensitivity of the HPLC assay was 0.25 ug/ml for SP and all peptide fragments analyzed. The recovery from spiked acidified aqueous standards carried through the analytical protocol ranged from 60 to 92%. The precision, expressed as the coefficient of variation, ranged from 4.3% at the upper concentration limit to 25.8% at the lowest concentration limit. The sensitivity for the RIA was approximately 4 pg/ml for SP. The percentage cross reactivity of the SP antiserum to its amino terminal fragments was essentially zero in all cases while the cross reacticity with the carboxy terminal fragment (SP_{2-11}) was 50% with a sensitivity of approximately 16 pg/ml. This work was supported by USPHS grants #DA04190 and DA04090.

355.4 **ELEVATIONS IN UNAMIDATED SUBSTANCE P LEVELS FOLLOWING DISULFIRAM ADMINISTRATION TO RATS.** R. M. Kream*, M. S. A. Kumar*, W. El-Bermani*, M. L. Thompson*. (Sponsor: L. Shuster) Depts. of Anesthesiology, Anatomy and Pharmacology, Tufts University Schools of Medicine, Boston, MA. 02111.

Recent work has demonstrated that the relatively selective copper chelator N,N-diethyldithiocarbamate and its disulfide dimer, disulfiram (Antabuse) are effective inhibitors of peptide amidation in the pituitary. We have developed a highly sensitive and highly selective radioimmunoassay (RIA) for detection of the unamidated form of substance P (SP), SP-glycine (SP-G). We are thus able to quantify levels of SP-G without prior HPLC fractionation of tissue samples. In the present study, 10 rats were distributed into two groups, one receiving vehicle, the other 40 mg/kg bw disulfiram (suspended in 0.5% Tween-80 normal saline) subcutaneously daily for 10 days. At the end of the treatment period, the animals were sacrificed by decapitation and the following brain areas were rapidly dissected out: olfactory bulbs (OB), forebrain (FB), preoptic area (POA), medial basal hypothalamus (MBH), pons (P), medulla oblongata (MO). In addition, lungs and dorsal and ventral aspects of the spinal cord were also dissected out. Tissues were extracted in 2N acetic acid, freeze-dried and reconstituted for RIA and assayed for levels of SP and SP-G. Levels of SP-G were found to be increased over 200-fold (0.1-20 pg/mg tissue in brain and spinal areas receiving sensory afferents (medulla and dorsal spinal cord). In addition, similar increases in SP-G immunoreactivity were found in diencephalic areas. In contrast, SP levels were found to decline only 15% in the above areas, suggesting relatively slow turnover of the peptide in central nervous tissues. The most dramatic changes in SP/SP-G levels were found in the lung extracts where SP-G increased 300-fold with a corresponding decrease in SP levels of 25-30%. We are presently examining whether these changes in SP processing are physiologically relevant to pain responsiveness, as well as inflammatory processes in the respiratory system. (Supported by NIDA DA04128, RMK, and DA03797, MLT)

355.5 ONTOGENY OF SUBSTANCE P WITHIN MICRODISSECTED RABBIT BRAINSTEM NUCLEI. J.L. Gingras*. (SPON: MC McNamara). Dept. of Peds, Duke Univ. Med. Ctr., Durham, NC 27710.

Substance P has been demonstrated by immunohistochemical techniques within many discrete locations throughout the central nervous system (CNS), in some cases coexisting with other neuropeptides and/or classical transmitters (Ljungdahl,A., Hokfelt,T., Nilsson,G., Neurosci, 3:861, 1978; Chan-Palay,V., Jonsson,G., Palay,S., Proc. Natl. Acad. Sci., 75:1582, 1978). More specifically, substance P is found in brainstem areas known to integrate cardiorespiratory function and acts as a neurotransmitter and/or modulator. Additionally, when injected intraventricularly, substance P stimulates breathing (Yamamoto,Y., Lagercranz,H., vonEuler,C., Acta Physiol Scand., 113:541, 1981). This study reports preliminary data on five brainstem nuclei and describes the developmental characteristics of substances P within these specific microdissected brainstem nuclei (Locus Coeruleus, LC; dorsal raphe, dr; N. Parabrachialismedialis, NPBM; N.Reticulogigantocellularis, RGI; N. Tractus Solitarius, NTS). Rabbits of known age (postnatal days 3, 7, 14, 21, 2 months, adult) were sacrificed, brains were immediately removed, sectioned and frozen at -70 degree C. Central nuclear groups were removed by micropunch technique and pooled. Substance P was measured by RIA in duplicate samples and protein was measured by Lowry. Data are reported as ng substance P/mg protein. + denotes significance within groups by ANOVA $p<.05$.

	Day 3	7	14	21	2 mo	Adult
LC+	3.19±.77	3.15±.47	2.86±.65	1.45±.22	1.44±.46	1.26±.31
dr+	1.14±.11	1.94±.25	2.25±.54	1.81±.26	.89±.15	.99±.20
NPBM	1.78±.21	1.97±.16	1.91±.11	1.97±.19	1.63±.27	1.30±.18
RGI+	1.64±.02	2.38±.18	1.48±.09	1.46±.17	1.06±.21	.83±.10
NTS	5.01±.47	8.15±.55	6.70±.66	6.18±48	7.32±1.9	6.92±1.4

These data show that a) substance P levels are highest in the NTS at all ages, b) the developmental profiles for substance P are nuclei-specific and c) overall developmental patterns emerge. In the RGI and LC, substance P levels are relatively high at birth and decrease to adult values after the first few weeks of life. In contrast, substance P is low at birth in the dr, peaks at 14 days thereafter decreases to adult range. In the NTS and NPBM, substance P levels do not significantly change after birth. These data support the concept that neurotransmitter development is not synchronous throughout the CNS nor within specific regions of the CNS and suggests that the physiologic influences of substance P may also reflect these maturational changes.

355.6 TACHYKININ PEPTIDES AND mRNAS IN CULTURED MOUSE MESENCEPHALIC NEURONS. M.J Bannon and G. Kapatos. Center for Cell Biology, Sinai Reasearch Institute,Detroit,MI 48235.

Two to 3 week old cultures derived from dissociated embryonic day 13 mouse mesencephalon and plated at 1.5 million cells/35 mm dish were found after colchicine treatment to contain substance P (SP)-immunoreactivity localized primarily to small fusiform neurons. Acid-extracted cultures contained ~25-100 fm SP/dish was in the reduced form in situ, since 2-mercaptoethanol treatment did not alter immunoreactivity. Analysis by high performance liquid chromatography coupled to radioimmunoassays using both C- and N- terminally directed antisera demonstrated approximately equimolar amounts of SP and substance K (SK,neurokinin A), a related peptide derived from the same preprotachykinin (PPT) gene, in the cultures. The same cultures also contained the structurally related peptide neuromedin K(NMK,neurokinin B) which is derived from a seperate gene. Since SP immunohistochemistry had revealed both intensely stained and lightly stained neurons, it is conceivable that these represent authentic SP-containing and NMK-containing cells,respectively. Northern blot analysis using PPT gene and NMK gene derived clones confirmed the presence of mRNAs encoding SP,SK and NMK in culture. The interaction between these tachykinin neurons and the dopamine neurons with which they are cocultured (SN abstr 12:167.4,1986) is presently being investigated.

355.7 SUBSTANCE P REGULATION BY SECOND MESSENGERS IN DISSOCIATED CULTURES OF NEONATAL RAT VAGAL SENSORY NEURONS. D.B. MacLean and F.B. Wheeler*, Department of Medicine, Section on Endocrinology, Bowman Gray Sch Med of Wake Forest Univ, Winston-Salem, NC 27103.

The factors regulating substance P (SP) synthesis in and release from an unmyelinated subpopulation of vagal sensory neurons are largely unknown. To study this, we have developed dissociated cultures of neonatal rat nodose ganglion neurons. Neurons are dissociated in neutral protease and layered over previously dissociated rat atriacytes. In previous studies, we have demonstrated that substance P within these cultures is present in approximately 20% of neurons and accumulates in amounts of 10-20pg per plated ganglion/equivalent per day. The addition of NGF, 100ng/ml, doubles SP content. Basal release of SP is 0.036%/min of cell content during 20-40min epochs and in response to 50mM K+ rises to 0.15%/min. The sensory neurotoxin, capsaicin (CAP), stimulates SP release in amounts similar to K+.

To study possible second messenger regulation of SP, cultures were maintained for 5-7 days in the presence of phorbol myristate acetate (PMA, 200nM), the phosphodiesterase inhibitor IBMX (.1uM) or DbcAMP (100uM). In the presence of NGF, none of the treatments altered SP content. DbcAMP ± IMBX in NGF-deprived cultures restored SP levels to NGF-positive sister cultures. Prior exposure to PMA raised basal SP release during 20min release epochs (PMA 2.6±0.5 vs control 1.4±0.7% $p<.01$), did not alter K+ stimulated release but significantly reduced net stimulated K+ release (PMA 3.2±1.4 vs control 5.4±0.8%, $p<.02$). Both basal and stimulated release were enhanced by prior IMBX exposure in NGF+ cultures, e.g., 20 min K+ stimulated release, control 15±2 pg/well, IBMX 30±14 pg/well $p<.04$). Prior 6 day or acute exposure to forskolin stimulated SP release in amounts similar to CAP (2 hour release, basal controls 6.6±2.7%, prior forskolin 10.7±2.3%, acute forskolin 10.0±2.0% and CAP 11.9±1.9%).

These studies demonstrate that PMA (or protein kinase C) may modulate SP release mechanisms. The effect of NGF on SP content/synthesis is probably mediated by an adenylate cyclase-dependent mechanism. In addition, cAMP may have effects independent of NGF on SP release and, inferentially, on turnover.

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355.8 EXPRESSION OF SUBSTANCE P IN THE CELL CYCLE OF THE TUMOR CELL LINE COLO 320. S.C. Feldman, S. Barnett*, S. Ramachandran*, S. Kolla* and G.P. Studzinski*. Depts. of Anatomy and Pathology, UMDNJ-New Jersey Medical School, Newark, N.J. 07103.

The occurrence of neuropeptides and other putative neurotransmitters in cells outside of the nervous system has been well-documented. We are interested in the role(s) of these molecules in cell growth, differentiation and in disease states including neoplasia. In this study we report the occurrence of the neuropeptide Substance P (SP) in cultures of Colo 320 cells, a cell line derived from a tumor of the endocrine cells of the gut.

Colo 320 cells were maintained in continuous cultures. Smears of cells were made on glass slides, fixed in buffered-formalin acetate and SP localized by immunocytochemistry. Antiserum to SP was purchased from ImmunoNuclear Corp.; intracellular staining was judged specific by the lack of immunoreactivity in cells in which the antiserum was preadsorbed with synthetic peptide. To determine the relationship of appearance of SP to stages of the cell cycle, immunocytochemistry was combined with autoradiography. Cultures were pulsed (1h) with ^{3}H-thymidine, processed for immunocytochemistry then dipped in emulsion, exposed for 2 days, developed and fixed. Cells were scored for the presence or absence of SP and/or silver grains in the nucleus.

SP was found in 30-50% of the cells in normally growing cultures. Combined autoradiography and immunocytochemistry indicated that although 25-60% of the cells were synthesizing DNA, only 10% of these contained the peptide. To further investigate the relationship between the appearance of SP in the cell and DNA synthesis cultures were treated with the polymerase inhibitor aphidicolin (10 or 20ug /ml, 1-6h). Inhibition of DNA synthesis for as little as 1 hour resulted in a marked increase in the number of cells expressing SP (90%). To determine if this was the result of new protein synthesis, cultures were treated with cycloheximide (70ug/ml). SP immunoreactivity was reduced 1h after exposure to the drug; by 6 hours less then 1% of the cells were immunoreactive. After removal of the drug, the proportion of cells exhibiting SP returned to control levels, demonstrating that loss of immunoreactivity was not due to damage of the cells.

These results are consistent with the hypothesis that expression of SP is cell-cycle related. Colo 320 cells may provide a good model for studies of the relationship of neuropeptides to cellular growth processes.

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355.9 MOLECULAR CLONING OF MONKEY PROOPIOMELANOCORTIN (POMC) AND PHYLOGENETIC COMPARISON. P. D. Patel, T. G. Sherman, and S. J. Watson. Depts. of Neuroscience and Psychiatry, University of Michigan, Ann Arbor, Michigan 48109.

Proopiomelanocortin has received widespread attention due to its role as a neuroendocrine prohormone subserving numerous physiological systems. Some of the peptides cleaved from this prohormone have been intensively studied and fairly well characterized with respect to biological activity (i.e., adrenocorticotrophic hormone (ACTH), B-endorphin (BE), a-melanocyte stimulating hormone (MSH) in lower animals). Other sequences are equally well conserved across species, both at the nucleotide and at the peptide level, but as yet have not been physiologically characterized (e.g. N-terminal peptide, corticotropin-like intermediate lobe peptide (CLIP)). As part of a larger investigation into the processing and function(s) of POMC peptides, we have cloned and sequenced a cDNA for POMC from the pituitary of the old world monkey, Macaca nemestrina. We present here the complete nucleotide sequence, deduced amino acid sequence, and comparison at both levels with previously published POMC sequences.

In general, the monkey POMC clone is highly homologous to the human sequence. Certain peptide domains, e.g., signal peptide, b-MSH, ACTH, and the amino terminal portion of the N-terminal peptide through g-MSH are identical in these two species, and highly conserved in all species, lending support to the contention that all of these peptides serve the system in a vital capacity. The region between g-MSH and a-MSH, and the sequences spanning B-lipotropin show the greatest diversity in all species studied. These dissimilarities include single base frameshifts as well as larger deletions and/or insertions. Perhaps a more interesting observation, especially from the phylogenetic viewpoint, is that the monkey BE peptide contains residue changes intermediate between that of lower mammals (bovine, murine, rat) and that of the higher mammal (human). Other features include the expected pattern of dibasic cleavage sites and, in particular, the Arg-Arg cleavage site at the end of γ_1-MSH (in contrast to the corresponding Pro-Arg non-cleavage site found in rat and mouse POMC).

The 1.6 kilobase clone presented here has an unusually large 5' untranslated region, consistent with previous northern analysis of monkey mRNA and confirmed by S_1 nuclease analysis. The significance of this feature is not yet known, but studies are under way to analyze what may be two different sizes of message. RNase mapping will be utilyzed to compare this clone to Macaca rhesus POMC, for which a larger message is also observed.

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355.10 HALOPERIDOL-MEDIATED CHANGES OF GAMMA-MELANOTROPIN AND β-ENDORPHIN PROCESSING IN THE RAT PITUITARY. L.P. Taylor*, J.H. Meador-Woodruff*, L. Corkins*, N. Ling and H. Akil (Spon: S. Watson). Mental Health Research Institute and Department of Psychiatry, University of Michigan Medical Center, Ann Arbor, MI, 48109-0720.

Gamma-melanotropin (γ_3MSH) is in the N-terminal domain of the β-endorphin (BE)/ACTH precursor, proopiomelanocortin (POMC), and is an important component of the pituitary-adrenal axis, potentiating ACTH-induced adrenocortical steroidogenesis. Gamma-MSH-IR exists in rat pituitary primarily as 5K and 11K forms (representing fully processed and glycosylated γ_3MSH, and γ_3MSH extended by the extreme N-terminal fragment of POMC, respectively), although a small percentage exists as precursor-sized material. Although mechanisms controlling processing and release of BE from the pituitary have begun to be elucidated, little attention has been focused on the control of processing of the N-terminal region of POMC; for example, BE in the intermediate lobe of the rat pituitary is felt to be under partial dopaminergic control. To further address this issue, the effect of chronic haloperidol on the processing of both BE and γ_3MSH in the anterior (AL) and intermediate (IL) lobes of rat pituitary was explored. Male Sprague-Dawley rats received daily IP injections of haloperidol (dose range 0 to 2 mg/kg) for 14 days; 24 hrs following the last injections, the rats were sacrificed by decapitation, the pituitaries immediately dissected into AL and IL, and quickly frozen on dry ice. Tissue was extracted in acetone/HCl, lyophilized, and resuspended in 1% formic acid. Aliquots were assayed for γ_3MSH-IR and BE-IR by specific RIAs that we have developed. Additional aliquots were subjected to gel filtration and HPLC, and fractions assayed for both peptides. In IL, chronic haloperidol induced a near-doubling of both total BE-IR and γ_3MSH-IR. This increase was attributable to a dose-dependent induction of fully-processed BE-sized and γ_3MSH-sized (5K) material. In AL, total content of both BE-IR and γ_3MSH-IR was unchanged. Chromatography revealed, however, that high doses of haloperidol caused a selective increase of 22K γ_3MSH-IR, with a corresponding reduction of more processed forms of γ_3MSH in AL. Higher doses of haloperidol promoted a selective increase of 31K BE-IR (POMC), with a corresponding reduction of β-lipotropin and BE. These results suggest that haloperidol alters the processing of POMC in rat pituitary at multiple sites. Chronic haloperidol induces the IL to store increased amounts of fully-processed peptides (BE and γ_3MSH). In AL, however, it appears that chronic haloperidol causes a relative inhibition of the conversion of POMC to 22K-ACTH and β-lipotropin, and of 22K-ACTH to the 16K N-terminal fragment and ACTH, resulting in the accumulation of these larger molecules.

355.11 IMMUNOCYTOCHEMICAL STUDIES OF PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE (PAM). V. May, K. M. Braas, and B. A. Eipper. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD, 21205

The presence of a carboxyl terminal α-amide moiety confers biological activity to a number of neuroendocrine peptides. An α-amidating activity capable of producing α-amidated peptides from glycine-extended precursors has been described in plasma, CSF and several neuronal and endocrine tissues. The enzyme, peptidylglycine α-amidating monooxygenase (PAM), has been purified from bovine neurointermediate pituitary and occurs in multiple forms differing in apparent molecular weight and charge. To begin studies on PAM localization and regulation, and to enable screening of cDNA expression libraries for clones producing PAM, two rabbits were injected with purified enzyme; purified PAM preparations were labeled with ^{125}I to test for antibody production. To verify that the major labeled protein was the enzyme, the ability of the antisera to interact with ^{125}I-protein and active enzyme was compared. Incubation of bovine pituitary extracts to which ^{125}I-labeled purified PAM has been added with increasing amounts of antiserum covalently conjugated to Protein A Sepharose resulted in a dose-dependent parallel depletion of PAM activity and ^{125}I-protein from the supernatant. Bovine pituitary tissue extracts were subjected to SDS-PAGE and the resolved proteins transferred to nitrocellulose for Western analysis. When the blots were incubated with PAM antiserum and processed using either the avidin-biotin-peroxidase complex (ABC) or ^{125}I-Protein A technique, two major bands corresponding to PAM-A and PAM-B were observed. Although the anterior pituitary contains high levels of PAM activity, few amidated peptides are known to be synthesized there in sizeable amounts. Immunocytochemical studies with both antisera were performed to identify PAM producing cells in the pituitary. Bovine pituitaries were immersion fixed and embedded in paraffin. Tissue sections (8 μm) were incubated with PAM antisera diluted 1:2000-1:4000 in 0.1% gelatin and processed using a modification of the ABC immunocytochemical technique. Fixation with 4% paraformaldehyde preserved PAM antigenicity optimally without compromising tissue morphology. PAM immunoreactivity was localized to the cytoplasm of a distinct population of anterior pituitary cells and in specific regions of the neurointermediate pituitary lobe. Staining was absent when diluted antisera were preincubated with 1 μg/ml purified PAM. No staining was observed in rat or guinea pig pituitary suggesting that the antisera are species specific. Current studies utilizing serial sections and double labeling techniques are aimed at determining the identity of the PAM immunoreactive cells in the anterior pituitary.

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355.12 IN SITU HYBRIDIZATION ANALYSIS OF INCREASED PREPROENKEPHALIN mRNA FOLLOWING LIMBIC SEIZURES. C.M. Gall[1], J.C. Lauterborn*[1], and J.D. White[2], [1]Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717 and [2]Division of Endocrinology, S.U.N.Y., Stony Brook, NY 11794.

Previous work by ourselves and others has demonstrated that recurrent limbic seizures induce an increase in enkephalin synthesis within hippocampus and entorhinal cortex as measured by increases in enkephalin content, preproenkephalin mRNA, and incorporation of ^{35}S methionine into chromatographically identified enkephalin (White et al., J. Neurosci. 7: 753-759). In the present study, in situ hybridization techniques were used to identify the cellular localization of elevated preproenkephalin mRNA in limbic structures following an episode of recurrent seizures.

A small electrolytic lesion was placed in the dentate gyrus hilus of adult male Sprague-Dawley rats under ketamine/xylazine anesthesia. This treatment has been demonstrated to induce recurrent limbic and behavioral seizures which begin 1-2 hrs postlesion and continue for 6-8 hrs thereafter. Hilus-lesioned rats included in this study exhibited a minimum of two stage 4 behavioral seizures. At 24 hrs postlesion, experimental and paired control rats were sacrificed by perfusion with 4% paraformaldehyde. Free-floating vibratome sections through hippocampus, entorhinal cortex, and amygdala contralateral to the lesion were processed for the localization of preproenkephalin mRNA using an ^{35}S-labeled cRNA probe and both film and emulsion autoradiographic techniques.

In control animals, hybridization was low in the limbic structures evaluated. A few cells were lightly labeled in the posterior cortical amygdala, layers II and III of entorhinal cortex, and layer II of piriform cortex. Within hippocampus, lightly labeled cells were occasionally observed within the pyramidal dendritic fields, and only very rarely within the dentate gyrus granule cell layer. In contrast, labeling was uniformly dense over the granule cell layer of experimental animals. Autoradiographic grain density was also very high over cells in layers II and III of ventral lateral entorhinal cortex, layer II of piriform cortex, the amygdalohippocampal area, and the posterior cortical amygdala. Moderate grain densities were observed over cells in the more dorsal medial and lateral entorhinal cortices and the lateral amygdala.

These data demonstrate that seizure activity can induce increased enkephalin synthesis in a number of brain areas and cell types and thereby support the conclusion that physiological activity has a large influence over the regulation of enkephalin synthesis in C.N.S. neurons. Moreover, these results indicate that the full population of neurons capable of enkephalin synthesis is not evident in the resting state. (Supported by BNS 8417098 and RCDA NS 00915 to C.M.G., and RR05736 and MH42074 to J.D.W.).

355.13 PLASMA OPIOID PEPTIDES: CHARACTERIZATION OF MET-ENKEPHALIN-IMMUNOREACTIVE PEPTIDES GENERATED BY PEPTIC DIGESTION. F. S. Shen* and I. Lindberg (SPON: K. Kratz), Department of Biochemistry and Molecular Biology, L.S.U. Medical Center, New Orleans, LA 70112

Singer et al have shown that plasma from various species can yield micromolar concentrations of met-enkephalin-immunoreactive peptides (MEIP) after digestion with pepsin (Endocrinol. 119, 1527, 1986). In an effort to understand the biochemical basis for this observation, we have attempted to characterize MEIP generated from plasma.

Rat plasma was digested with pepsin and analysed by radioimmunoassay, gel filtration, isoelectric focusing (IEF), and HPLC. Results obtained using gel filtration on Bio-Gel P-2 were similar to those obtained by Singer et al. Two peaks of MEIP were observed. The first peak of MEIP, which accounted for 84% of the total, eluted several fractions after the void volume, while the second peak eluted several fractions before the salt volume. When the first peak of MEIP was further analyzed by HPLC using a slow acetonitrile gradient, it was found to be heterogeneous in nature, with six immunoreactive peaks. In contrast to results obtained by Singer et al, none of these HPLC peaks exhibited a retention time similar to met-enkephalin or met-enkephalin sulfoxide.

Isoelectric focusing (pH 3-10 gradient) of MEIP generated by peptic digestion of plasma showed five peaks of immunoreactivity with isoelectric points of 3.6, 5.9, 7.3, 8.1, and 9.6. The peak at pI 9.6 contained 40% of the immunoreactive peptides. In view of the results of Singer et al as to the molecular weight of the precursor protein which gives rise to MEIP (65,000), we digested rat serum albumin (RSA) with pepsin in order to examine the possibility that RSA might be the precusor of plasma MEIP. Gel filtration of digestion products of RSA yielded a peak of MEIP with the same elution volume as that of the second peak of digested whole plasma. This result suggests that MEIP present in this second peak (which represents only 15% of total plasma MEIP) may be due to plasma RSA. Analysis by IEF of digestion products of RSA showed a completely different profile of MEIP; the main peak exhibited a pI of 3.4 (75% of the total) and smaller peaks were apparent at pIs 4.3, and 4.9

From these results, it appears that at least two proteins (RSA and an as yet unidentified protein) can give rise to MEIP upon peptic digestion. In addition, MEIP produced by peptic digestion of whole plasma appear to be heterogenous in nature. (Supported by DK35159).

355.14 MONOCLONAL ANTIBODIES TO AN ENDOGENOUS NEUROPEPTIDE WITH PUTATIVE MORPHINE-MODULATING ACTIVITY C.-H. Lee*, R. Brown*, E.A. Majane* and H.-Y. T. Yang Naval Medical Research Institute, Bethesda, MD 20814 and National Institute of Mental Health, St. Elizabeth's Hospital, Washington, D.C. 20032.

Two neuropeptides, FLFQPQRF-NH_2 (F-8-F-NH_2) and AGEGLSSPFTSLAAPQRF-NH_2 (A-18-F-NH_2), originally detected by antiserum raised against Phe-Met-Arg-Phe-NH_2 (FMRF-NH_2), were recently isolated from bovine brain and found to modulate morphine analgesia. (Yang et al., Proc. Natl. Acad. Sci. USA 82:7757, 1985) Using the antisera raised in rabbits to these two peptides, they were found to distribute unevenly in bovine brain and spinal cord. In addition to F-8-F-NH_2 and A-18-F-NH_2, there are still other FMRF-NH_2-like immunoreactive materials in mammalian brains. Some immunohistochemical studies indicated that the FMRF-NH_2 and neuropeptide Y (NPY) immunoreactivities are both stored in the same neurons. However, it is not clear whether it is possible to differentiate NPY from FMRF-NH_2-like peptides by immunohistochemical means using polyclonal antisera. Therefore, in this study, monoclonal antibodies were generated in an attempt to select an antibody capable of differentiating NPY-like from FMRF-NH_2-like peptides. Three murine monoclonal antibodies of IgG isotype to F-8-F-NH_2 were obtained through cell fusion and cloning. In an RIA using ^{125}I-YLFQPQRF-NH_2 as a tracer, these antibodies cross-reacted with other structurally related peptides. The relative immunoreactivities of these peptides expressed in $(B/B_0)_{50}$ (pmol/tube) are: F-8-F-NH_2, 0.9; A-18-F-NH_2, 7; PQRF-NH_2, 60; FMRF-NH_2, 200; γ_1MSH, 500; and RF-NH_2, 1,000. NPY, PYY or Met^5-enk-R^6-F^7 showed no cross-reaction even at 10,000 pmol/tube. This result suggests that these monoclonal antibodies recognize the C-terminal portion of F-8-F-NH_2 and that RF-NH_2 is required for the immunoreactivity. It also indicates that these monoclonal antibodies are capable of differentiating -RF-NH_2, the C-terminal dipeptide amide of FMRF-NH_2-like peptides, from -RY-NH_2, the C-terminal dipeptide amide of NPY. An extract from bovine spinal cord was analyzed by HPLC coupled with RIA using the monoclonal antibodies. The major immunoreactivity was identified as F-8-F-NH_2 and the minor one as A-18-F-NH_2, although there are some very small amounts of unidentified immunoreactive species. The specificity of these monoclonal antibodies is thus further confirmed and will be useful in differentiating the distribution of F-8-F-NH_2 or mammalian FMRF-NH_2-like peptides from that of NPY. (Supported in part by NMRDC 62758/MM58.527.01.0003).

PEPTIDES: PHYSIOLOGICAL EFFECTS I

356.1 RELEASE OF ATRIAL NATRIURETIC FACTOR (ANF) FROM PERFUSED RAT HYPOTHALAMIC EXPLANTS. R. Nissen*, J. Gutkowska* and L.P. Renaud (SPON: E. Cooper), Neurosciences Unit, Montreal General Hospital and McGill Univ. Montreal, Quebec, Canada H3G 1A4.

Several observations indicate that ANF, a circulating hormone released from cardiac myocytes, may function as a neuropeptide. Selective ANF binding sites and ANF-like immunoreactive neurons and fibers are described in basal forebrain and diencephalic structures known to regulate fluid balance and blood pressure. ANF is released from blocks of rat hypothalamus by calcium-dependent potassium depolarization. The recent detection of an ANF-like peptide in the rat neurohypophysis (Gutkowska et al., Peptides, 1987) and report of a possible co-existence of ANF within hypothalamic magnocellular oxytocin-secreting neurosecretory neurons (Jirikowski et al., Neuropeptides 8: 243, 1986) prompted us to examine for in vitro release of ANF-like peptides from the neurohypophysis.

Experiments utilized intra-arterial perfusion of oxygenated, warmed artificial cerebrospinal fluid to maintain acutely prepared explants of rat hypothalamus and basal forebrain (Randle et al., Neurosci. Lett. 65: 219, 1986). Following removal of the anterior lobe of the pituitary, a suction pipette was positioned over the neurointermediate lobe to collect samples of superfusate. Under basal conditions, samples assayed for ANF by radioimmunoassay revealed baseline values of 5-20 pg/ml. ANF levels were increased 6-10 fold when the perfusion media contained 60 μM norepinephrine or elevated sodium chloride (from 124 mM to 144 mM). ANF appears to be released from both a median eminence and a neurohypophysial site, possibly by different stimuli at each site. Supported by MRC.

356.2 EFFECTS OF VAGOTOMY AND HEMORRHAGE ON ATRIAL NATRIURETIC PEPTIDE. M.I. Phillips, B. Kimura* and W.E. Hoffman. Department of Physiology, University of Florida, Gainesville, FL 32610 and Department of Anesthesiology, Michael Reese Hospital, Chicago, Il 60616.

Atrial natriuretic polypeptides (ANP) have potent natriuretic, diuretic and vasorelaxant activities. ANP is released from atria during volume loading and sodium intake. ANP has also been found in the brain. To study the effects of hemorrhage on the brain and plasma ANP levels, we used normotensive rats subjected to 33% hemorrhage.

Sprague Dawley rats were anesthetized with chloral hydrate and catheterized in the femoral artery. One group was hemorrhaged, the other was control. A 2 ml sample was collected 30 mins. later. Atria and hypothalamus blocks were boiled, purified and ANP extracted. ANP from all sources was characterized by HPLC. Levels of ANP were measured by radioimmunoassay after Sep-Pak C-18 extraction. The antisera was anti-Rat 28 amino acid ANP. Hemorrhage significantly decreased plasma ANP (244 ± 81 vs. 41 ± 7 pg/ml, $P < 0.05$), elevated ANP levels in the atria (126 ± 12 vs. 169 ± 24 μg/g tissue, N.S.) and significantly increased levels in hypothalamus (19.7 ± 1.0 vs. 25.5 ± 0.6 ng/g tissue, $P < 0.001$).

The fall in plasma ANP could be due to a volume change acting on the atrial stretch receptors or to neural afferent input to the brain. To attempt to separate these possibilities, bilateral vagotomy was performed. Twelve rats were anesthetized with chloral hydrate as above. In 6 rats, bilateral vagotomy at the level of the carotids was performed. Two ml samples of blood were withdrawn. Both the control group and vagotomized group were hemorrhaged 33% and 2 ml blood taken 30 mins. later. The results showed that the effect of vagotomy was a huge rise in plasma ANP in 5 out of 7 rats (means: control, 244; vagotomy, 731 pg/ml). However, hemorrhage produced a significant decrease in plasma ANP in both groups. Considering the high baseline levels of the vagotomized rats, the fall was greater than in controls.

It is concluded that the vagus is important in controlling ANP levels and acts to inhibit release. During hemorrhage less ANP is released from the atria and this may be partly controlled by the vagus nerve in addition to less stretch on the stretch receptors. Opposite effects of hemorrhage on plasma ANP and hypothalamic ANP levels indicate brain involvement in the regulation of hemorrhaged-induced hypovolemia. The results point to a reflex brain ANP response to hemorrhage. (Supported by NIH grant 1-R01-HL27334 to MIP.)

356.3 HYPOTHALAMIC ACTION OF ATRIAL NATRIURETIC FACTOR TO INHIBIT PROLACTIN SECRETION. W.K. Samson*, E. Ramos* and R. Bianchi* (SPON: R.L. Moss). Dept. of Physiology, UTHSCD, Dallas, TX 75235-9040.

Atrial natriuretic factor (ANF) is produced in cardiac myocytes and in brain neurons. High levels of the peptides have been detected in the hypothalamus (Brain Res 365:105), the ANF gene is transcribed there (PNAS 83:6697), and ANF receptors have been identified in the diencephalon (PNAS 83:174). Its presence in a variety of hypothalamic nuclei and in the median eminence (Histochem 83:1) suggests a hypothalamic action of ANF. Indeed the peptide alters neuronal firing rate in the hypothalamus (Neuroendo 44:49) and we have demonstrated that it inhibits the release of AVP and LHRH in vivo and in vitro. Recently, the interaction of ANF with brain dopaminergic systems has been demonstrated (EJP 131:171). We examined, therefore, the possibility that ANF plays a role in the hypothalamic control of prolactin (PRL) secretion in the rat. ANF (atriopeptin III and ANF-28) in log doses ranging from one pico- to one micromolar had no significant effect on PRL release from dispersed, cultured anterior pituitary cells under either basal or stimulated conditions. Intravenous bolus injection (0.1, 1.0 or 10 micrograms) or constant infusion for 30 min (0.01 or 0.1 microgram/kg/min) of ANF also failed to significantly alter plasma PRL levels in conscious, castrated male rats. Third cerebroventricular injection of ANF (in 2 microliters saline) resulted, however, in a dose-related inhibiton of PRL secretion.

ng PRL/ml plasma (X±SEM)

	0'	15	30	60	90	120 min
Control (23)	30±4	29±3	23±4	26±3	30±4	32±4
0.1 nmole ANF (10)	30±6	22±3	23±5	19±4	23±3	26±4
1.0 nmole ANF (10)	27±7	14±3**	17±5	17±3*	13±3*	26±5
2.0 nmole	26±10	12±3**	7±2**	11±3*	13±3*	17±5

*p<.025,**p<.005 analysis of variance, multiple comparisons.

These results suggest the possibility that centrally produced ANF plays some role in the hypothalamic control of PRL secretion. Whether this possible action is exerted via dopaminergic mechanism, perhaps by inhibiting dopamine-beta-hydroxylase activity, as has been demonstrated in pheaochromocytoma cells in culture (NEJM 314:321), remains to be established. Alternatively, the possibility exists that ANF alters the release of some other PRF/PIF thus resulting in a reduced hypothalamic drive for PRL secretion. (Supported by NIH Grant HD09988, Project II to WKS.)

356.4 THE EFFECT OF OXYTOCIN AND PSYCHOLOGICAL STRESS ON NOREPINEPHRINE, DOPAMINE AND SEROTONIN IN DISTINCT BRAIN REGIONS. J.L. Muir* and H.P. Pfister*. (SPON: ENA)
Dept of Psychology, Univ. of Newcastle, NSW 2308, Australia.

Oxytocin (OXT) acts on catecholaminergic and serotonergic activity in the brain and appears to be a member of the class of neuromodulators, i.e. modulates the activity of the putative neurotransmitters in the brain. The effects of OXT have been examined to some extent, the main findings indicating that this peptide induces impairment of memory consolidation and retrieval processes (Bohus, B., Kovacs, G.L. & De Wied, D., Br. Res., 157:414, 1978). OXT also appears to be involved in the stress response, potentiating corticotropin-releasing factor (CRF)-induced release of adrenocorticotropic hormone (ACTH) (Gibbs, D.M., Vale, W., Rivier, J. & Yen, S.S.C., Life Sci., 34:2245, 1984). Exposure to a stressor also produces a number of changes in catecholaminergic and serotonergic systems in the central nervous system. These changes are reflected as decreased concentration of transmitter and/or increased concentration of metabolites. The purpose of this study was therefore to investigate the effect of OXT on brain catecholaminergic and serotonergic activity following predictable and unpredictable novelty stress.

Female rats were allocated to four groups: a group which received no injections, a group which received 1 ml/kg injections of the vehicle solution, a group which received injections of 5.8 IU OXT/kg and a group which received 11.6 IU OXT/kg. All injections were given via the i.p. route. Each of these four groups were further subdivided into three stress treatment groups: a control group, a predictable stress group (animals were subjected daily for 5 successive days to a single 30 min. exposure to a novel apparatus) and an unpredictable stress group (animals received one 30 min. exposure to the novel apparatus on each of 5 days at a randomly selected time within each 24 hr period). Following OXT and stress treatment, the brain of each animal was dissected into five brain regions: hypothalamus, hippocampus, striatum, midbrain and brainstem.

The results will be discussed in terms of the effects of oxytocin and stress treatment on the steady state level and "turnover" of norepinephrine, dopamine and serotonin in the five brain regions indicated.

356.5 OXYTOCIN INFUSIONS IN CAUDAL VENTROLATERAL MEDULLA ACTIVATES SUPRAOPTIC VASOPRESSIN-SECRETING NEURONS IN THE RAT. L.P. Renaud, J.H. Jhamandas, R.M. Buijs* and T.A. Day. (SPON: A.T. Tan) Neurosciences Unit, Montreal General Hospital and McGill Univ., Montreal, Quebec Canada, H3G 1A4

The magnocellular neurosecretory neurons (MNCs) of the hypothalamic supraoptic and paraventricular nuclei receive a prominent noradrenergic innervation from the A1 neurons of the caudal ventrolateral medulla (Sawchenko & Swanson, Science 214:685, 1981). Recent electrophysiological data indicate that this A1 innervation is excitatory and selective for vasopressin-secreting MNCs (Day et al., J. Physiol. 355: 237, 1984). The A1 region receives input from several CNS sites. Of particular note is a descending projection arising in the parvocellular paraventricular nucleus which may comprise oxytocin immunoreactive fibers (Buijs, Cell Tiss. Res. 192: 423, 1978). In order to evaluate a possible transmitter role for oxytocin at this site, we infused the hormone into the A1 area while recording the firing frequency of supraoptic neurosecretory neurons. Experiments utilized urethane or pentobarbital anesthetized male Sprague Dawley or Long-Evans rats. The hypothalamus and brainstem were exposed by a transpharyngeal approach. MNCs were identified by antidromic activation from the neurohypophysis and categorized as vasopressin- or oxytocin-secreting according to their firing pattern and sensitivity to baroreceptor input. Oxytocin infusions (100-250 pg) restricted to the A1 area were accompanied by a rise in the firing frequency of 16/19 vasopressin-secreting neurons. In contrast, 0/10 oxytocin-secreting neurons were similarly influenced. These data suggest that the peptidergic hormone oxytocin may participate in central neurotransmission within the caudal ventrolateral medulla to induce activation of supraoptic MNCs that secrete vasopressin into the systemic circulation.
(Supported by MRC and QHF)

356.6 PARALYTIC AND PRESSOR EFFECTS OF INTRATHECAL ARGININE-VASOPRESSIN IN THE RAT. A. Martinez-Arizala*, J.B. Long and J.W. Holaday. Neuropharmacology Branch, Department of Medical Neurosciences, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307.

Endogenous opioids have been implicated in the pathophysiology of spinal cord injury since opioid antagonists improve neurologic outcome following experimental spinal cord trauma. Among the endogenous opioids, Dynorphin A has been specifically implicated in injury mechanisms since it produces flaccid hindlimb paralysis after intrathecal (i.t.) administration in rats. Other peptides endogenous to the spinal cord also share this effect, specifically, we studied Arg^8-Vasopressin (AVP) at doses that cause hindlimb paralysis and an arterial pressor response after i.t. injection in rats. Male Sprague-Dawley rats (300-350 gm) under anesthesia (ketamine 50 mg/kg and xylazine 10 mg/kg) were implanted with catheters in the external jugular vein, the tail artery, and the lumbar subarachnoid enlargement. After allowing 24 hours for recovery, the cardiovascular and motor responses to i.t. and intravenous (i.v.) AVP were compared. Intravenous AVP (0.01 nmoles) did not alter motor function, but produced a moderate pressor response (mean maximum MAP change = 34 mm Hg) which was blocked by the i.v. injection of the V_1 receptor antagonist $d(CH_2)_5[Tyr(Me)^2]AVP$ (10 nmoles). Intrathecal injections of an identical dose of AVP produced a marked pressor response (mean maximum MAP change = 73 mm Hg) accompanied by hindlimb paralysis. Intrathecal (0.2 nmoles), but not intravenous (10 nmoles) injections of the V_1 receptor antagonist blocked both the paralytic and pressor effects of i.t. AVP (0.01 nmoles). Following recovery of motor function and normalization of blood pressure, repeat injections of i.v. AVP (0.01 nmoles) produced equivalent pressor responses whereas repeat injections of i.t. AVP (0.01 nmoles) revealed a marked refractoriness to both the paralytic and pressor responses. We conclude that 1) Dynorphin A is not unique among endogenous neuropeptides in producing spinal cord dysfunction in the rat, 2) responses to i.t. AVP exhibit a period of refractoriness, and 3) the pressor and paralytic effects of i.t. AVP are centrally mediated through the V_1 receptor.

356.7 INFLUENCE OF ACUTE AND CHRONIC CHANGES IN BLOOD VOLUME AND PLASMA OSMOLALITY ON ATRIAL NATRIURETIC FACTOR (ANF) AND ARGININE VASOPRESSIN (AVP) CONCENTRATIONS. P.A. Mason, K.M. Chu*, C.R. Freed, D. Bhaskaran, L.G. Ganousis*, A.S. Nies*, and J.A. Durr*. Depts. of Med. and Pharm., Univ. of Colo. Health Sciences Center, Denver, CO, 80262.

Atrial natriuretic factor (ANF) and arginine vasopressin (AVP) regulate extracellular fluid volume. Anatomically, the brain regions containing ANF and AVP are in proximity to one another along the AV3V region. We have investigated the effects of changes in blood volume (BV) and plasma osmolality (Posm) on the plasma concentrations of ANF and AVP. Male Sprague-Dawley rats (300-350 gm, n=22) had BV and Posm acutely altered with injections of 35% polyethylene glycol (8000 MW, 2 ml/100 gm body weight, i.p.) dissolved in 200, 600, or 1000 mOsm/kg of NaCl solution. Animals were killed 2 hr after the fluid challenges. Other animals (n=16) were water deprived for 0, 24, or 48 hr to study more chronic BV and Posm changes. All rats were decapitated and trunk blood was collected for determination of Posm, hematocrit, protein concentration, and plasma ANF and AVP concentrations. Normal hydrated rats showed a baseline plasma ANF value of 169 pg/ml and an AVP concentration of 6 pg/ml. In treated rats, the change in BV ranged from +12 to -24% and Posm ranged from 291 to 322 mOsm/kg. In the acutely-treated rats, a reduction in BV led to a fall in plasma ANF ($r=0.73$, $p<0.001$), while AVP increased ($r=-0.87$, $p<0.001$). When BV was held constant at -20% and osmolality was varied, plasma ANF fell as Posm increased ($r=-0.74$, $p<0.001$) while AVP increased ($r=0.53$, $p<0.05$). Similar results were seen in chronic water deprived rats. Decreased BV was associated with a fall in plasma ANF ($r=0.87$, $p<0.001$) and an increase in plasma AVP ($r=-0.67$, $p<0.005$). With increased osmolality, there was a reduction in ANF ($r=-0.45$, $p<0.05$) and an increase in AVP ($r=0.78$, $p<0.001$). Our results predict that a 24% decrease in BV would be required to suppress ANF completely. In conclusion, acute and chronic BV depletion results in a linear decrease in plasma ANF concentration, suggesting that ANF has a physiological role in normal BV control rather than being important only in volume expanded conditions. In acutely-treated rats, plasma ANF correlated inversely with Posm when both BV and plasma protein concentration were held constant at -20% and 4 g/dl, respectively. This result indicates that ANF varies with changes in Posm as well as BV. As expected, plasma AVP increased in response to reductions in BV and increases in Posm. Because ANF and AVP appear to be reciprocally related to alterations in BV and Posm, it is possible that the two neurohormones are mutual antagonists.

356.8 ELECTRICAL STIMULATION OF THE BED NUCLEUS OF THE STRIA TERMINALIS (BST) SUPPRESSES PGE_1 HYPERTHERMIA IN THE RAT BY RELEASING VASOPRESSIN IN THE VENTRAL SEPTUM. A.M. Naylor*[1], Q.J. Pittman[2] and W.L. Veale[2]. [1]MRC Reproductive Biology Unit, Edinburgh, Scotland EH3 9EW and [2]Dept. of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Perfusion of arginine vasopressin (AVP) through the ventral septal area (VSA) of the rat brain suppresses the fever evoked by the intracerebroventricular (icv) infusion of PGE. Evidence suggests that vasopressin may function in this area of the brain as an endogenous antipyretic via an action on a receptor resembling the peripheral V_1 subtype. One of the potential sources of AVP to the VSA is from a vasopressinergic pathway originating in the BST. These experiments were undertaken to determine what effect electrical stimulation of the BST would have on the fever evoked by icv PGE and how this effect could be modified by vasopressin antagonists injected into the VSA.

Male Sprague-Dawley rats were anesthetized (pentobarbital 70 mg/kg i.p.) and guide cannulae were implanted bilaterally above the VSA and unilaterally above a lateral cerebral ventricle. In addition, monopolar stimulating electrodes were implanted bilaterally in the BST. Following a 7 day recovery, body temperature was monitored continuously with transmitters (Minimitter Inc.) implanted in the peritoneal cavity. Infusion of PGE_1 (200 ng/10 µl) into a lateral ventricle evoked a rise in core temperature of 1.0°C. When this injection of PGE_1 was accompanied by electrical stimulation of the BST (monophasic, square wave pulses, 0.5 msec duration, 20 Hz) for 10 min prior to and 20 min following the icv infusion of PGE_1, the rise in core temperature was attenuated significantly ($n=6$). The suppression of PGE-hyperthermia was observed only when the electrodes were located in the BST. Stimulation of nearby areas such as the lateral septum did not alter the hyperthermic response. When electrical stimulation of the BST suppressed PGE_1 fever, similar stimulation in the absence of PGE_1 did not affect body temperature in the afebrile rat. When the vasopressin V_1 antagonist $d(CH_2)_5Tyr(Me)AVP$ (400 pmoles/side) was injected into the VSA electrical stimulation of the BST did not attenuate the PGE_1-hyperthermia when compared to controls with saline or V_2 antagonist $[d(CH_2)_5DValVAVP]$ ($n=6$).

These data indicate that electrical stimulation of a potential source of AVP to the VSA, the BST, suppresses PGE_1-fever. In addition, a vasopressin V_1 antagonist blocks the stimulation-induced suppression suggesting that endogenous AVP, released in the VSA in response to electrical stimulation of the BST, may be responsible.

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356.9 DOES INTRATHECALLY ADMINISTERED VASOPRESSIN PRODUCE ITS PERIPHERAL CARDIOVASCULAR EFFECTS BY COMPROMISING SPINAL BLOOD FLOW? James P. Porter and Mark A. Schroer*. Dept. of Physiology, University of Louisville, Louisville, KY 40292.

Intrathecal administration of vasopressin (AVP) into the subarachnoid space of the spinal cord produces a marked increase in arterial pressure in rats. AVP-containing neurons are present in the spinal cord and it has been hypothesized that exogenously administered AVP mimicks a neurotransmitter-like effect of this peptide. However, AVP is a potent vasoconstrictor and the effects of intrathecal administration of the agent could be due to widespread neuronal hyperactivity resulting from compromised blood flow to the spinal cord rather than to a neurotransmitter effect on sympathetic preganglionic neurons. In the present investigation we tested the hypothesis that the effects of intrathecal vasopressin are due its vasoactive properties. Specifically, we compared the effects of intrathecal vasopressin to the effects produced by intrathecal administration of other vasoactive agents, namely angiotensin II (AII), norepinephrine (NE), and phenylephrine (PE). We reasoned that these agents should also produce an increase in arterial pressure when administered into the spinal cord if the effects of vasopressin can be explained by its vasoconstrictor action. Mean arterial pressure and heart rate were monitored in conscious, freely moving rats before and after administration of AVP (5 to 15 ng), AII (30 ng to 3000 ng), NE (200 ng to 1200 ng), or PE (200 ng to 1200 ng) into an indwelling intrathecal catheter. AVP produced the expected increase in arterial pressure that was accompanied by bradycardia. On the other hand, the other agents produced no effects at any of the doses used. In a second series of experiments, we sought to determine if prior intrathecal administration of vasodilating agents would attenuate the expected increase in arterial pressure with subsequent AVP injections. Bradykinin (1 µg), papaverine (1-30 µg), or vasoactive intestinal peptide (1-3.3 µg) were first given into the intrathecal catheter. Two minutes later 5 ng of vasopressin was also added to the spinal subarachnoid space. Papaverine and VIP had no effect on the increase in arterial pressure with subsequent administration of vasopressin. However, pretreatment with bradykinin significantly attenuated the AVP-response by 50%. It is unlikely that a vasodilator action of bradykinin contributed to this attenuation since the two other potent vasodilators had no affect. It is more likely that some neuroactive action of bradykinin was responsible. Taken together, these data do not support the hypothesis that AVP increases sympathetic outflow to the peripheral vasculature by constricting local spinal vessels and compromising blood flow to the spinal cord. The hypothesis that AVP acts directly on sympathetic preganglionic neurons is still tenable.

356.10 COLD EXPOSURE ALTERS ENDOGENOUS TRH AND NEUROTENSIN CONCENTRATIONS IN MOUSE BRAIN REGIONS. G. Bissette. Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

The study of thermoregulation has identified a large number of endogenous substances that can alter body termperature when injected into the CNS or periphery of a wide variety of experimental animals. These substances include classical neurotransmitters such as norepinephrine and serotonin, amino acids and neuropeptides. Few studies, however, have attempted to show neurochemical alterations in brain that are correlated with challenges to normal thermoregulation. Previous work has shown that centrally injected neurotensin (NT), an endogenous tridecapeptide can lower body temperature in a wide variety of animals and that a paradigm of cold-adaptation involving 4 days previous exposure to 4°C ambient temperatures can antagonize the hypothermic actions of subsequent CNS administration of NT. Thyrotropin-releasing hormone (TRH) has been shown to cause hyperthermia after CNS injection in several species and is also able to attenuate NT-induced hypothermia. Thus we examined regional brain concentrations of TRH and NT in male Swiss-Webster mice exposed to 4°C ambient temperatures for 3 hours daily for four consecutive days compared to naive controls. On the fifth day, one group of cold-adapted and one group of naive controls were exposed to one hour of 4°C cold before all mice were sacrificed and brains were removed and frozen. Eleven brain regions were subsequently dissected and extracted for peptide radioimmunoassay of TRH and NT. Protein estimation was performed using the Folin-phenol method and results were expressed as pg of peptide per mg protein. TRH and NT concentrations were similar to those reported previously in both regional distribution and absolute concentrations. TRH concentration was increased in the preoptic hypothalamus-diagonal band area and olfactory tubercles of animals exposed to the 4 day regimen compared to naive controls. Cold-adapted animals receiving one hour of cold stimulus on day 5 exhibited increased TRH concentrations in the frontal cortex and anterior caudate relative to cold-adapted animals without this stimulus. NT was unchanged in the preoptic hypothalamus-diagonal band of cold-adapted animals compared to controls and was increased in the substantia nigra-ventral tegmental area and anterior caudate in cold-adapted animals with one hour cold exposure on day 5 compared to animals without this final exposure. These data show that endogenous TRH and NT concentrations are altered by a regimen of cold-adaptation in brain regions known to subserve a major role in mammalian thermoregulation.

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356.11 INTRACISTERNAL INJECTION OF TRH ANALOGUE, RX 77368, STIMULATED SEROTONIN RELEASE INTO THE GASTRIC LUMEN. R. L. Stephens*, Jr. and Y. Taché (SPON: H. Weiner). CURE, VA Wadsworth Medical Center, Dept. of Medicine, Brain Research Institute, UCLA, Los Angeles, CA 90073.

Serotonin (5HT) is present in mast cells of the gastric mucosa, and together with histamine is released into the gastric lumen after vagal stimulation. The release and functional role of serotonin in response to agents which act as vagal stimulants has not been studied. Central injection of TRH produces marked stimulation of gastric acid secretion, motility and lesion formation, and acts primarily by stimulating the vagus (Reg. Peptides 13: 21, 1985). In 2 hr pylorus-ligated rats, intracisternal (ic) injection of the stable TRH analogue, RX 77368, produced a marked, dose-dependent increase in the content of 5HT and its metabolite, 5HIAA, in the gastric juice as measured by HPLC with electrochemical detection.

RX 77368 (ng)	N	Acid output (μmol H^+/2h)	5HT (ng/2h)	5HIAA
1	5	187 ± 43	49 ± 12	113 ± 40
10	4	286 ± 77	64 ± 15	266 ± 109
100	7	406 ± 36	190 ± 58	381 ± 109

Atropine pretreatment (0.1 mg/kg; 20 min before ic injection) reduced RX 77368 (100ng) induced stimulation of gastric acid output by 95 % and 5HT and 5HIAA content by 79 % and 85 % respectively.

Treatment	N	Acid output (μmol H^+/2h)	5HT (ng/2h)	5HIAA
Saline + RX 77368	4	273 ± 79	208 ± 16	254 ± 102
Atropine + RX 77368	4	14 ± 9	45 ± 16	38 ± 19

However, para-chlorophenylalanine pretreatment (300 mg/kg; -72 and -48 h before ic injection) resulted in a 60 % reduction in RX 77368-stimulated 5HT release into the gastric juice, while gastric acid secretion was not significantly altered. It is concluded that 1) ic injection of RX 77368 produced a dose dependent stimulation in 5HT into the gastric lumen, 2) 5HT release is vagally mediated, as indicated by its atropine sensitivity, and 3) the stimulation of 5HT release can be dissociated from that of gastric acid output produced by central injection of RX 77368. 5HT release may mediate other changes in gastric functional parameters produced by central TRH, such as stimulation in gastric motility.

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356.12 INHIBITION OF TRH STIMULATED GASTRIC SECRETION BY BOMBESIN MICROINJECTED INTO THE DORSAL VAGAL COMPLEX. T. Ishikawa* and Y. Taché. CURE, VA Wadsworth Medical Center, Department of Medicine and Brain Research Institute, UCLA, Los Angeles, CA 90073.

Bombesin injected into the cerebrospinal fluid inhibits gastric secretion stimulated by various secretagogues (Life Sci.,37:115,1985). Mapping of hypothalamic responsive sites demonstrated that bombesin suppressed gastric secretion only when injected into the paraventricular nucleus (Gunion & Taché, Brain Res., in press). The facts that midcollicular transection and ventricular plug did not prevent intracisternal bombesin induced inhibition of gastric secretion in pylorus ligated rats suggest that responsive sites must be located in the hindbrain (Gunion & Taché, Am. J. Physiol., in press).

Rats, fasted for 24 h, were anesthetized with urethane (1.5 g/kg, ip) and a double cannula was placed into the lumen of the non glandular part of the stomach. Saline or peptides were microinjected unilaterally into the dorsal vagal complex (DVC) using glass micropipet and pressure ejection of 100 nl. Microinjection of the stable TRH analog, RX 77368, (30 ng) into the DVC induced a long lasting stimulation of gastric secretion with a peak response occurring within 50 min. The gastric secretory response to TRH was dose dependently inhibited when bombesin (B) was concomitantly microinjected respectively at 1, 3 and 10 ng dose into the DVC.

Treatment	Dose ng/rat	N	Gastric Acid Output μg/60 min
Saline		8	10 ± 3
RX 77368	30	8	130 ±12
RX 77368 + B	1	6	85 ± 21
RX 77368 + B	3	8	51 ± 11
RX 77368 + B	10	7	23 ± 3

Peak secretory response to TRH (25 ± 3 μmol/10min) was inhibited by 84 % following bombesin (10 ng) microinjected into the DVC whereas that of pentagastrin infusion (10 ± 2 μmol/10min)) was inhibited only by 25 %. Bombesin injected intracisternally at 0.3, 1, 3 and 10 ng elicited a significant dose dependent suppression of pentagastrin response respectively by 11, 39, 56, and 74 %. These results demonstrated that bombesin acts in the dorsal motor nucleus to inhibit vagally-stimulated acid secretion whereas other brain stem or spinal site must also been involved in mediating the inhibition of pentagastrin-stimulated gastric secretion.

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356.13 COMPARISON OF THE TRH ANALOG RGH 2202 WITH TRH: EFFECTS UPON THE RAT EMG. C.K. Haun, S.R. Beydoun, E.F. Hawkins, W.K. Engel and I. Tan* Departments of Anatomy & Cell Biology and of Neurology, USC School of Medicine, Los Angeles, CA 90033.

Thyrotropin-releasing hormone (TRH) has been used to enhance strength of patients with degenerative motor neuron diseases, e.g., ALS; however, high- or repeated-dosage can cause, instead, a temporary muscular weakening, termed "autorefractoriness" (AR) (Neurology 34, Suppl. 1, 147, 1984). We have studied this phenomenon in adult male Sprague Dawley (Holtzman) rats and compared the effects of a low-affinity TRH analog, RGH 2202 (RGH) (a gift from Gedeon Richter, Ltd., Budapest, Hungary.)

The rats' spinal cord was transected at T-8/9, and 3 weeks later, EMG recording electrodes were implanted in their left triceps surae muscles (both surgeries performed under methohexital). After 1 to several days the rats were suspended, awake, in a sling inside a shielded cage, and both direct and integrated EMG recordings were made. After a baseline recording period, s.c. injections of TRH or RGH (10 mg/kg) were given.

TRH produced an immediate increase in the spontaneous EMG activity, which reached a peak in 3-10 min., but declined rapidly, and by 90-120 min. post-injection the EMG activity averaged only 41% of the peak value. By comparison, RGH acted more slowly: the peak in enhanced EMG activity was attained at an average of 35 min., and by 90-120 min. the enhancement was still 75% of its peak. By 210-240 min. post-injection, the integrated EMG activity to TRH was down to 28% of its peak, whereas the response to RGH was still at 54% of its peak.

When a second 10 mg/kg dose of TRH was given 120 min. after the first, a second EMG peak response occurred, again at approximately 10 min. post-injection, but the second peak amplitude was only 61% of the first -- an apparent AR ("tachyphylactic") response. Contrastingly, the peak response to a second 10 mg/kg of RGH occurred with a somewhat greater latency than the first (ave. 45 min.) and the magnitude of this second peak, compared to the first, was undiminished (i.e., no AR). When the EMG was examined at 90-120 min. after the second injections, the integrated EMG activity for TRH was 46% of the value of the first peak. With RGH, however, the EMG activity was still 75% of that attained with the first injection.

We propose: 1) the refractoriness of peak responses with TRH, but not with RGH, may be further evidence for our postulated agonist-dependent TRH-receptor desensitization, which is lacking with low-affinity ligands (Biochem. Biophys. Res. Commun. 138, 1184, 1986), and 2) RGH, with its longer-lasting excitatory effects, should be tested further for use in treatment of motor neuron disorders. (Aided by a Biomedical Research Support Grant to the USC School of Medicine.)

356.14 THYROTROPIN-RELEASING HORMONE (TRH) AS AN EXCITATORY NEUROMODULATOR IN THE MAMMALIAN SPINAL CORD. I. PHYSIOLOGIC CHARACTERIZATION. Shripad B. Deshpande* and Jordan E. Warnick. Dept. of Pharmacol. & Exptl. Ther., University of Maryland School of Medicine, Baltimore, MD 21201.

TRH (1-pyroglutamyl-1-histidyl-1-prolinamide) is a putative neuromodulator/neurotransmitter which is found in greater amounts in the extrahypothalamic nervous system than in the hypothalamus itself. Significant receptor binding of TRH occurs in the spinal cord. Because of this and other evidence, TRH has been tried in the treatment of amyotrophic lateral sclerosis and has been implicated in the pathophysiology of the disease. Although TRH is known to potentiate the monosynaptic reflex (MSR) in amphibian and mammalian spinal cords, its action has been variable and the exact mechanism of this excitatory action (e.g., a direct effect on motoneurons or mediation via disinhibition) is not clear. Therefore, we have studied the actions of TRH on the MSR in spinal cord from neonatal rats, *in vitro*.

Since TRH was virtually ineffective in cords from female rats (Deshpande and Warnick, *Fed. Proc.* 46:1449, 1987), all experiments were done on cords from male rats. Spinal cords were removed from 7- to 10-day old rats, hemisected and placed in an experimental chamber. Suction electrodes were then attached to a pair of dorsal and ventral roots of the L_3-L_5 segments. Supramaximal stimulation of a dorsal root evoked a MSR from the corresponding ventral root. In some experiments a dorsal root reflex was simultaneously recorded from an adjacent dorsal root. Alternatively, conditioning stimuli applied to an adjacent dorsal root evoked both a strychnine- and picrotoxin-sensitive inhibition of the MSR.

TRH (1 nM-1 μM) produced a concentration-dependent potentiation of the MSR but did not influence the dorsal root reflex. At 1 nM, the effect of TRH was variable, half-maximal potentiation occurred at about 50 nM with maximal potentiation to 147% of control at 1 μM. The frequency-dependent depression of the MSR elicited at 0.1-1.0 Hz was reduced by TRH (30-100 nM). TRH did not depolarize the ventral root at concentrations up to 1 μM although raising the $[K^+]$ from 5 to 25 mM produced a concentration-dependent depolarization (0.25 mV/mM K^+). Raising or lowering the external $[Ca^{2+}]$ and raising the external $[Mg^{2+}]$ did not affect the potentiation induced by TRH. Neither strychnine-, picrotoxin-, nor bicuculline-sensitive inhibition were altered by facilitating concentrations of TRH. The effect of TRH seems to be specific for transmission between I_a afferents and α-motoneurons since multisynaptic pathways are not affected by the tripeptide. Both pre- and postsynaptic mechanisms appear to be involved in this effect. (Supported in part by USPHS grant NS21312.)

356.15 THYROTROPIN-RELEASING HORMONE (TRH) AS AN EXCITATORY NEUROMODULATOR IN MAMMALIAN SPINAL CORD. II. PHARMACOLOGIC CHARACTERIZATION. Jordan E. Warnick and Shripad B. Deshpande* (SPON: F.C. Kauffman). Dept. of Pharmacol. & Exptl. Ther., University of Maryland School of Medicine, Baltimore, MD 21201.

TRH is a tripeptide hormone which has received much attention for its role in the pathophysiology of amyotrophic lateral sclerosis and possible utility in treatment of the disease. We have shown that TRH potentiates the monosynaptic reflex (MSR) in cords from neonatal male but not female rats (Deshpande and Warnick, *Fed. Proc.* 46:1449, 1987). TRH did not potentiate the MSR in cords from castrated male rats but did in cords from ovariectomized female rats treated with testosterone (Warnick *et al., The Pharmacologist* 29: 1987). Thus, gender is a significant factor in the responsiveness to TRH. We now attempt to further define the pharmacology of TRH-induced potentiation of the MSR.

Spinal cords were isolated from 7- to 10-day old male rats, hemisected, placed in an experimental chamber and suction electrodes were attached to a pair of dorsal and ventral roots of the L_3-L_5 segments. Supramaximal stimulation of a dorsal root evoked a MSR from the corresponding ventral root.

The potentiation of the MSR produced by TRH (100 nM) was not prevented by atropine (1 μM), haloperidol (10 μM), methysergide (30 nM), cyproheptadine (1-30 μM) or ICS 205-930 [(3α-tropanyl)-1*H*-indole-3-carboxylic acid ester; 10 nM-3 μM], a potent and selective serotonin M-receptor antagonist (Richardson *et al., Nature* 316:126, 1985). On the other hand, diazepam (1-30 μM) depressed the potentiation produced by TRH without causing ventral root depolarization. TRH remained effective in spinal cords from rats treated previously on days 1-3 with 6-hydroxydopamine (i.p.). Methysergide completely blocked the MSR at 30 nM, an effect that was reversed by TRH. The MSR was restored to nearly 40, 60 and 90% of the control magnitude by the addition of 0.1, 0.3 and 1 μM TRH, respectively, in the presence of methysergide. On the other hand, 3,4-diaminopyridine (10 μM), in the presence of methysergide (30 nM), caused a nonspecific increase in both the MSR and polysynaptic activity. The potentiation induced by TRH does not appear to involve dopaminergic, muscarinic or serotonergic (type 2 and M) receptors or the blockade of rectifier K channels. Although TRH did not affect strychnine-, picrotoxin- or bicuculline-sensitive inhibition, high concentrations of diazepam antagonize TRH-induced potentiation. Thus, the effect of diazepam may be unrelated to the classical $GABA_A$/benzodiazepine receptors. The reversal of methysergide-induced blockade of the MSR by TRH indicates that the action of TRH may be specific for the monosynaptic pathway. (Supported in part by USPHS grant NS21312.)

356.16 LEUTEINIZING HORMONE-RELEASING HORMONE (LHRH) AND LEUMORPHIN (LEUM) HAVE DIFFERENT EFFECTS ON THE NEURONS OF MIDBRAIN CENTRAL GRAY IN FEMALE RATS. Y. Koyama*, Y. Oomura and H. Nishino*. (SPON: K. Yamaguchi) Natl. Inst. Physiol. Sci., Okazaki, 444 Japan

The midbrain central gray (MCG) participates in supraspinal control of the lordosis in female rats by receiving the descending input from the medial preoptic area (MPOA) and ventromedial hypothalamic nucleus (VMH) and by giving its own output to the gigant cellular nucleus of the medulla (GCN). Central applications of LHRH and LEUM have facilitatory effects on lordosis and these peptides are found in the hypothalamus and midbrain, areas known to be involved in lordosis. In this study, the effects of electrophoretic applications of LHRH and LEUM were examined in the MCG neurons identified by the response to electrical stimulation of the MPOA, VMH and GCN.

Forty-two ovariectomized, estrogen primed female rats were used under light urethane anesthesia. MPOA and VMH stimulation had mainly facilitatory effects on the MCG neurons; 31% (48/154) were excited and 16% (25/154) were inhibited by the MPOA stimulation, while 33% (51/153) were excited and 11% (16/153) were inhibited by VMH stimulation. Electrophoretic application of LHRH excited 25% (25/101) of MCG neurons and inhibited 13% (13/101) of them. Excitatory effects of LHRH could be obtained mainly in the MCG neurons which received excitatory input from the MPOA; 42% (12/31) of neurons which were excited by MPOA stimulation increased firing rate by LHRH, while in the neurons received excitatory input from the VMH, 28% (7/25) were excited by LHRH. LEUM caused excitation in 8% (7/93) of MCG neurons and inhibition in 25% (23/93) of them. Thirty-one percent (8/26) and 39% (11/28) of neurons which received neural input from the MPOA and VMH respectively, were sensitive to LEUM. MCG neurons identified antidromically by GCN stimulation were not sensitive to LHRH or LEUM. LHRH sensitive neurons and LEUM sensitive neurons seem to belong to discrete neural substrate in the MCG; LHRH neurons were found in the dorsal MCG, while LEUM neurons were in the ventro-caudal MCG, and of 41 neurons on which examined the effects of both LHRH and LEUM, only 7 % (3/41) were sensitive to both chemicals. These results indicate that the LHRH and LEUM have different roles in the control of lordosis by acting on the MCG neurons in a different manner.

356.17 [D-ALA^2,F5-PHE^4]-DYNORPHIN AMIDE, AN OPIOID ANALGESIC WITH LETHAL PROPERTIES. R.M. Kostrzewa, A.J. Kastin , D.H. Coy*, R. Brus*, H. Criswell and P.S. Keegan*. Quillen-Dishner College of Medicine, East Tennessee State Univ., Johnson City, TN 37614 and Veterans Administration Medical Center and Tulane University School of Medicine, New Orleans, LA 70146.

Analgesic non-lethal actions of the opioid peptide, dynorphin (1-13) have been characterized over the past several years. A novel analog, [D-Ala^2,F5-Phe^4]-dynorphin amide was prepared, and its pharmacological spectrum of activity has been investigated in the present study. In the hot plate analgesic test on albino and black mice, a 20 µg intraventricular (i.vtr.) dose of the analog produced an analgesic effect that was similar in potency and duration (ca. 3 hr) to that produced by a 20 µg i.vtr. dose of the parent dynorphin. This action of [D-Ala^2,F5-Phe^4]-dynorphin amide was effectively antagonized by naloxone (2 mg/kg i.p.), administered either before or after peptide treatment. In addition to the analgesic action, [D-Ala^2,F5-Phe^4]-dynorphin amide produced a Straub tail and a catatonic-like state in mice, that was also attenuated by naloxone. When studied in vitro on the electrically-stimulated mouse vas deferens preparation, [D-Ala^2,F5-Phe^4]-dynorphin amide inhibited contractile activity and had an IC_{50} of 108.2 ± 34.7 nM (S.E.), about 4-fold greater than that of dynorphin. This action was also attenuted by naloxone. An i.vtr. dose of 150 µg of [D-Ala^2,F5-Phe^4]-dynorphin in mice, and a cumulative series of i.vtr. doses up to 2600 µg in anesthetized rats, failed to produce a lethal effect. In the diallylbarbital (70 mg/kg i.p.) and urethane (200 mg/kg i.p.) anesthetized rats, [D-Ala^2,F5-Phe^4]-dynorphin amide did not modify blood pressure, heart rate and respiratory rate. However, when mice were treated with single intraperitoneal (i.p.) doses of [D-Ala^2,F5-Phe^4]-dynorphin amide, convulsive episodes were produced, and lethal effects were observed with a 30 mg/kg dose (LD_{50} = 60 mg/kg at 24 hr). No pathological changes were observed in mouse liver and kidney at 24 hr after a 50 mg/kg dose of the peptide analog. The analgesic and opioid-like behavioral effects (e.g. Straub tail and catatonic-like state) were probably produced by a direct action of [D-Ala^2,F5-Phe^4]-dynorphin amide at opioid receptors. The lethal action of [D-Ala^2,F5-Phe^4]-dynorphin amide may have been induced by a metabolite of the parent molecule, since i.p., but not i.vtr. doses produced that effect. The novel fluorinated dynorphin analog, [D-Ala^2,F5-Phe^4]-dynorphin amide, may be a useful chemical tool for studying opioid systems in the central nervous system.

356.18 Heterogeneous Effects of Opiate Drugs on Neurons Recorded from the Nucleus Accumbens Septi. R.L. Hakan*, and S.J. Henriksen.(SPON: N. Ling). Research Inst. of Scripps Clinic, La Jolla, CA, 92037

The nucleus accumbens septi (NAS) of the basal forebrain has been implicated through behavioral research to be critical for mediating the self-administration of opiate drugs in laboratory rats (Vaccarino, et. al., Pharm. Bioch. & Behav., 24, 61, 1986;). As part of an investigation to determine the actions of opiates at the cellular level within this structure, we have examined spontaneous neuronal activity as well as the cellular activity and field responses evoked by afferent pathway stimulation (ipsilateral fimbria). These events were recorded with single-barrel glass microelectrodes in halothane anesthetized rats. Cell firing rates were quantified using an on-line computer system for interstimulus and peristimulation histogram analysis. We then observed the effects of sytemically administered heroin and morphine (s.c.) on these electrophysiological parameters (one cell/subject; n=36). The most striking feature of single-cell activity in the NAS (n=100) was heterogeneity. That is, throughout the postero-medial, anterior and lateral regions of the NAS, both slow (<5Hz) and fast (>5Hz) spontaneously active cells were mixed with inactive cells which discharged only following stimulation. However, such regional analysis consistently found fast cells at the ventral NAS-olfatory tuberculum border. Synaptic "field" responses to ipsilateral fimbria stimulation consisted of an intial negative-going wave (≈7ms to peak) followed by a positive-going wave (≈19ms to peak). The amplitudes for either of these two field components rarely exceeded 2mv. The latencies for stimulation-evoked single cell discharges regularly coincided with both the negative and positive field components. Heroin and morphine most commonly depressed the discharge rates of spontaneously active cells in a naloxone reversible fashion (20/36 cells tested). Again heterogeneity was observed, in that other cells were excited (n=7/36) or were unaffected (n=9/36) by these drugs. "Field" responses and stimulation-evoked single cell activity were consistently unaltered by drug administration. Our results contrast with prior reports which have described homogeneous patterns of cellular activity within the NAS (Yang and Mogenson, Br. Res., 324, 69, 1984; White and Wang, J. Neurosci., 6, 274, 1986). Similarly, iontophoretically applied opioids have been previously reported to uniformly depress electrophoretically evoked cell activity in the NAS (McCarthy et. al., J. Physiol. Lond., 267, 40, 1977), while our work suggests a much more heterogeneous action. We observed that only spontaneously active cells were affected by opiate drugs while evoked cell activity was unaffected. This heterogeneity in discharge rate of NAS neurons and subsequent differential responsiveness to systemic opiate drugs is consistent with the known diversity of synaptic inputs into this region as well as the intrinsic "mosaic" of NAS cell islands (Chronister et. al., In: The Neurobio. of the Nuc. Acc., 173, 1981).

356.19 PEPTIDE FRAGMENTS DERIVED FROM THE β-CHAIN OF HEMOGLOBIN (HEMORPHINS) ARE CENTRALLY ACTIVE IN VIVO. T.P. Davis and F. Porreca. Department of Pharmacology, University of Arizona College of Medicine, Tucson, AZ 85724

Recently, Brantl et al., (Eur. J. Pharmacol. 125:309, 1986) reported that treating bovine blood with gastrointestinal enzymes leads to the formation of several opioid peptides. Two of these peptides (Tyr-Pro-Trp-Thr and Tyr-Pro-Trp-Thr-Gln), termed hemorphin-4 (H-4) and hemorphin-5 (H-5), respectively, correspond to the 34-37 and 34-38 fragments of the β-chain of bovine hemoglobin and the 35-38 and 35-39 fragments of human hemoglobin. Using the electrically stimulated myenteric plexus/longitudinal muscle preparation of the guinea pig ileum, they reported that the tetrapeptide, H-4, had an IC_{50} of 45.2μM and the C-terminally extended pentapeptide, H-5, had an IC_{50} of 46.1μM. Both of these effects were naloxone-reversible with the peptides showing unique enzymatic stability. Since hemorphins could be formed during physiological or pathological degration of hemoglobin, we were interested in determining if these peptide fragments exerted in vivo, central nervous system effects such as analgesia, inhibition of gastrointestinal transit or inhibition of the spontaneous micturition reflex as previously reported for the proenkephalin A fragments (Davis et al, Eur. J. Pharmacol., 111:177, 1985).

Solid-phase peptide synthesis of the two peptides was carried out using the Fmoc procedure. Purification was accomplished by semi-preparative HPLC with confirmation by amino acid analyses, low resolution fast atom bombardment mass spectrometry (FAB/MS) and FAB MS-MS which provided the peptide sequence. The effects in vivo of H-4 and H-5 were studied in two models of phasic and tonic pain, the mouse tail flick assay (TFA) (55^{o}C warm water, latency to rapid flick) and hindpaw formalin assay (PFA) (5% formalin, 5 μl volume, behavioral scoring), respectively. Additionally, two physiological endpoints, central modulation of bladder motility and central effects on intestinal propulsion of an oral radiolabelled marker were monitored in the mouse. Both H-4 and H-5 were given by the intracerebroventricular (i.c.v.) route in all tests. In the TFA, H-4 and H-5 (25-75 μg at +10 min) produced dose-related, naloxone-reversible analgesia with H-4 being about two times more potent. However, when H-4 and H-5 were tested in the PFA, they had no effect over the same dose range. H-5 produced an effect in the PFA only at a dose of 100 μg. Inhibition of gastrointestinal transit was not affected by either H-4 or H-5 (0.1 μg to 70 μg). H-4 or H-5 (5-20 μg) inhibited micturition contractions in a dose-related and naloxone-reversible fashion, and H-4 was once again about twice as potent as H-5. These data provide evidence that H-4 and H-5 can exert naloxone-reversible opioid actions in vivo and therefore may be physiologically important blood-borne peptides. (Supported by NIH DK36289 & NS 23710.)

356.20 ACTIVITY OF DIMERIC DERMORPHIN ANALOGS ON GASTRIC SECRETION IN THE RAT. A. Guglietta, L.H. Lazarus, B.J. Irons* and P. Melchiorri*. NIEHS, LBNT, Research Triangle Park, NC 27709, and Institute of Medical Pharmacology III, 00185 Rome, Italy.

Dermorphin (DM) (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) is an opiate peptide isolated from the skin of South American frogs and has marked affinity for μ-type receptor. DM administered icv in rats produces analgesia, catalepsy, releases PRL, inhibits LH release and gastric secretion. Structure-activity relationship studies demonstrated the importance of D-Ala^2 for its action on gastric secretion and showed that none of the analogs tested was as potent as DM in reducing gastric secretion. In this study we investigated the action of several dimeric DM analogs on gastric secretion using the pylorus-ligated rat as a biological model. The use of dimeric peptides, in which two chains of the same peptide are linked directly through their carboxy terminal amide or through CH_2 group might give insight on a particular action of DM since dimeric peptides may have different receptor affinity and selectivity than the monomer. DM_1 dimeric-tetra-DM_2, dimeric-penta-DM_0, dimeric-penta-DM_2, [Sar^4]-dimeric-penta-DM_0 and [D-Arg^2, Sar^4]-dimeric-penta-DM_0 (the subscript represents the number of CH_2 groups) were injected icv in rats and 2 h later the animals were killed and the gastric volume, pH, [H^+] concentration and gastric acid output determined. All the peptides tested significantly ($p<0.05$) reduced the gastric volume and gastric acid output. DM dimeric-penta-DM_0, [Sar^4]-dimeric-penta-DM_0 and [D-Arg^2, Sar^4]-dimeric-penta-DM_0 also reduced the [H^+] concentration and increased the gastric pH, while dimeric-penta-DM_2 and dimeric-tetra-DM_2 were inactive in modifying these parameters. The percent of activity of the dimeric DM analogs compared to that of DM is summarized in the following table:

PEPTIDE	VOL	% pH	CONC	OUT
DM	100.0	100.0	100.0	100.0
Dimeric-tetra-DM_2	35.4	40.9	11.1	35.5
Dimeric-penta-DM_0	87.3	90.5	95.3	90.9
Dimeric-penta-DM_2	45.9	19.0	13.4	50.2
[Sar^4]-dimeric-penta-DM_0	106.2	126.8	117.5	106.7
[D-Arg^2, Sar^4]-dimeric-penta-DM_0	101.1	119.0	139.3	103.7

[Sar^4]-dimeric-DM_0 and [D-Arg^2, Sar^4]-dimeric-DM_0 were more potent than DM in suppressing gastric secretion in rats on all the parameters studied. These results extend the structure-activity studies on DM analogs and show for the first time that two analogs are more potent than DM in reducing gastric secretion in rats.

MESSENGER RNA REGULATION IV

357.1 LOCALIZATION OF NEURONS CONTAINING ESTRADIOL RECEPTOR mRNA IN THE RAT BRAIN AS STUDIED BY IN SITU HYBRIDIZATION. G. Pelletier, N. Liao* and M.G. Govindan*. MRC Group in Molecular Endocrinology, Le Centre Hospitalier de l'Université Laval, Quebec G1V 4G2, Canada.

The distribution of neurons which are capable of concentrating radioactive estradiol has already been reported in a few species. The purpose of the present study was to localize estradiol receptor mRNA within the rat brain using an in situ hybridization approach. Adult male rats were perfused with 4% paraformaldehyde in 0.1M phosphate buffer and frozen 10 μm section were cut with a cryostat. A synthetic oligonucleotide probe labeled with ^{35}S complementary to the mRNA coding for estradiol receptor was used. Sections were incubated in pre-hybridization buffer to reduce non-specific labeling. Following this, 100 μl of hybridization buffer containing 300,000 cpm were applied to each section at 37C for 2 days. After appropriate rinsing, the sections were exposed to X-Ray films for one day. Selected slides were subsequently coated with Kodak NTB2 liquid emulsion and exposed for 3 days. Observation of radioautograms (films or coated slides) revealed that strong labeling occurred in several brain areas including the cerebral cortex, hippocampus, amygdala, supraoptic nucleus, paraventricular nucleus, ventromedial nucleus, arcuate nucleus and olfactorius nucleus. In the positive regions, silver grains were detected over both nuclei and cytoplasm of neurons. Preincubation with RNAse completely prevented radioautographic labeling. In general, the results obtained with the in situ hybridization agree well with previous results obtained after injection of labeled estradiol. This approach should be very useful to study the effect of different factors on estradiol receptor gene expression in individual neurons.

357.2 **Regional Variations in the Concentration of Gonadotropin-Releasing Hormone (GnRH) mRNA in the Male Macaque.** L. Vician*, L.A. Adams*, L.J. Standish, D.K. Clifton* and R.A. Steiner. Depts of Ob/Gyn and Physiology and Biophysics, University of Washington, Seattle, WA 98195.

GnRH neurons are localized in specific brain regions, especially in the hypothalamus and other limbic areas. The regional distribution of GnRH cells could be an anatomical reflection of functional differences in the GnRH system. Indeed, besides its neuroendocrine function in controlling the secretion of LH and FSH, GnRH has been implicated as a neurotransmitter in the regulation of sexual behavior. This being the case, we might expect that the synthetic capacity of GnRH cells within a region would be related to the physiological role of those cells. For example, the neuroendocrine cells projecting to the median eminence might have a greater synthetic capacity and hence more GnRH mRNA than cells serving a more classical neurotransmitter function. To test the hypothesis that the synthetic capacity of GnRH cells varies as a function of neuroanatomical locus, we used in situ hybridization to determine the GnRH mRNA content of individual cells in the septum, diagonal band of Broca (DBB) medial preoptic area (MPOA) and ventral medial hypothalamus (VMH) of the male macaque, Macaca fascicularis (n=3). Paraformaldehyde-fixed coronal sections (20 μm) were prepared for in situ hybridization and incubated with a ^{35}S-labelled RNA probe complementary to the human GnRH mRNA sequence. Following the stringent wash, slides were processed for autoradiography and read by dark-field illumination. The number of photographic grains overlying each cell was counted by computerized image processing.

Region	Grains/Cell $\pm$ SEM
Septum	137 $\pm$ 23
DBB	116 $\pm$ 14
MPOA	127 $\pm$ 20
VMH	184 $\pm$ 50

These data indicate that there is a significant difference ($p<0.05$; 2-way ANOVA) in the GnRH mRNA content of cells in various regions of the forebrain and hypothalamus. This observation is consistent with the hypothesis that GnRH producing cells found in different brain regions serve different functions and that the expression of the GnRH gene in these cells is regulated accordingly.

357.3 CUSHING'S SYNDROME IN THE HORSE: PITUITARY HISTOLOGY AND EVALUATION OF POMC GENE EXPRESSION. D.R. Gehlert[+], N.O. Dybdal[o], W.R. Millington[+], B.M. Chronwall[+]. [+]Exper. Therap. Branch, NINCDS, NIH, Bethesda, MD 20892 and [o]Dept. Vet. Pathol., Univ. of California, Davis, CA 95616

A syndrome in old horses consisting of hirsuitism (long and curly hair) with an abnormal shedding pattern, hyperglycemia and general debilitation is associated with a pituitary intermediate lobe tumor. While a normal horse pituitary weighs between 1-3g the average tumor containing gland weighs 7 g. There is no focal lesion but rather a diffuse change from an old IL to a tumor. Histologically, the tumor tissue is adenomatous, hyperplastic and non-neoplastic. The tumor cells are indistinguishable from normal cells, however, an organization into rosettes is prominent within the tumor. Both normal tissue and tumor stain immunohistochemically for alpha-MSH. EM preparations of normal and tumor tissue show typical endocrine cells with high density of mitochondria, a well developed RER and filled with a great number of secretory vesicles. In order to examine the role of the POMC in this syndrome, we have evaluated POMC mRNA, β-endorphin immunoreactivity (iβ-END) and post-translational processing of β-END.

Equine Cushing's syndrome was associated with substantially elevated levels of circulating iβ-END. The iβ-END concentration of plasma from Cushing's horses was approximately 60-fold higher than that of controls. CSF iβ-END was also correspondingly elevated. However, the concentration of iβ-END in the tumor tissue itself was not significantly different than that of control intermediate lobe tissue . Separation of the individual molecular forms of β-END by cation exchange chromatography (N-acetylated and non-acetylated β-END-1-31, -1-27, -1-26) indicated that while the total iβ-END levels were unchanged, significant differences occurred in the pattern of β-END peptides in the tumor tissue. Specifically IL tumors contained significantly higher concentration of opioid-active, β-END-1-31, and lower levels of C-terminally shortened non-opioid forms (β-END-1-27 and -1-26) of the peptide than control IL.

POMC gene expression was examined using *in situ* hybridization and a dot-blot hybridization assay. A 35-mer oligonucleotide probe complementary to POMC mRNA was 3' end labeled with either ^{35}S-dCTP (*in situ* hybridization) or ^{32}P-dCTP (dot-blot hybridization). An intense hybridization signal was seen over both the IL from control horses and tumor tissue from the Cushing's horses. No significant differences in the relative levels of POMC mRNA were detected.

Theses results indicate that while equine Cushing's syndrome is characterized by markedly increased levels of circulating and CSF β-END, the synthetic capacity of the IL tumor appears to be similar to that of normal horse IL. It also appears that the extent to which β-END-1-31 is processed post-translationally is markedly reduced in equine Cushing's syndrome.

357.4 PROOPIOMELANOCORTIN mRNA EXPRESSION IN HUMAN PITUITARIES STUDIED BY QUANTITATIVE IN SITU HYBRIDIZATION. G. Mengod[1*], M.M. Vivanco[1*], A. Probst[2] and J.M. Palacios[1]. [1]Preclinical Research, SANDOZ LTD., Basle, Switzerland, CH-4002. [2]Dept. of Neuropathology, Inst. of Pathology, University of Basle, Basle, Switzerland, CH-4003.

In situ hybridization was used to localize and quantify the expression of mRNA coding for proopiomelanocortin (POMC) hormone in human pituitary glands obtained at autopsy. A 32-base synthetic oligonucleotide complementary to the 3' untranslated region of the human POMC mRNA, ^{32}P-labeled with terminal deoxynucleotidyltransferase was used as a probe. Brain paste standards containing known amounts of the "sense" 32-base oligonucleotide were used for quantification and standardization of the hybridization reaction and autoradiography. The specificity of the hybridization reaction was determined by competition experiments, RNAage treatment, hybridization with heterologous probes and by immunohistochemistry experiments. POMC mRNA was visualized autoradiographically only in the anterior lobe of the pituitary where it was distributed in a "patchy-like" organization. No hybridization was observed in the posterior lobe. Computer-assisted microdensitometric quantification of the POMC mRNA in 43 human pituitary glands revealed 1) no effect of postmortem delay (between 230 to 66 hours); 2) no influence of gender; 3) no influence of age between 42 and 103 years old. Because of the reported effects of dopaminergic drugs on POMC mRNA expression, we examined pituitary glands from several patients dying with schizophrenia (and treated with neuroleptics), Parkinson's disease (trated with L-DOPA). In addition, patients dying from Alzheimer's disease were also examined. When compared with healthy controls higher levels of POMC mRNA expression in schizophrenia pituitary glands and lower values in Parkinsonian's pituitaries were observed. Interestingly lower levels of POMC expression were also observed in Alzheimer's disease pituitaries. In contrast, the expression of β-acting mRNA was unaltered in these populations as compared to controls. These results show that in situ hybridization can be used to study POMC mRNA expression in human postmortem materials and that changes associated with disease and/or drug treatment can also be studied and correlated with changes in animal models of disease.

357.5 PHYSIOLOGICAL INDUCTION OF PROOPIOMELANOCORTIN mRNA IN THE FROG PITUITARY. R.M. Dores, R.G. Krause II, and M.E. Lewis. Department of Biological Sciences, University of Denver, Denver, CO and Medical Products Department, Experimental Station, E.I. DuPont Co., Wilmington, DE.

The release of alpha-MSH from the frog pituitary mediates the process of adaptation to a dark background by stimulating the dispersion of pigment in dermal melanophores. Since this adaptation depends upon continued release of this peptide, there should be increased demand for the precursor, proopiomelanocortin (POMC), which has been demonstrated using biochemical assays of in vitro biosynthesis. To determine whether these changes are due to increased amounts of POMC mRNA in the intermediate lobe of pituitary, frogs (Xenopus laevis) were dark adapted for 1-7 days prior to sacrifice. Plasma was collected for alpha-MSH radioimmunoassay, and the brain and pituitary were removed for in situ hybridization histochemistry using a radiolabelled synthetic oligonucleotide (30-mer) complementary to part of Xenopus laevis POMC mRNA. Exposure to a dark background resulted in rapid increases in plasma alpha-MSH levels and in dermal pigmentation, and over several days, a greater than ten-fold increase in pituitary intermediate lobe POMC mRNA hybridization signal. These results suggest that normal pituitary stores of alpha-MSH are sufficient for transient changes in coloration due to changes in environmental illumination, but that chronic changes induce a metabolic demand resulting in a marked increase in the mRNA encoding the precursor for this peptide. This research was supported by NIH grant DK 36587.

357.6 ANALYSIS OF RAT POMC HETEROGENEOUS NUCLEAR RNA FOLLOWING CRF TREATMENT IN VIVO. D. J. Autelitano*, M. Blum, M. Lopingco* and J.L.Roberts. Fishberg Center for Neurobiology, Mt. Sinai Med. Ctr., New York, NY 10029.

Previous studies from this laboratory have shown that chronic injection of corticotropin releasing factor (CRF) results in elevated levels of anterior pituitary (AP) mRNA, and lowered levels of neurointermediate lobe (NIL) mRNA in the rat. In the present study, we have used a highly sensitive S1 nuclease protection assay to analyze the levels of POMC heterogeneous nuclear RNA (hnRNA) as well as mRNA in AP and NIL following acute in vivo treatment with CRF to determine whether the long term changes in mRNA are proceeded by alterations in hnRNA.

Adult female Sprague Dawley rats were given a single subcutaneous injection of either synthetic rCRF (20ug), or vehicle, and were sacrificed at 0, 20 and 60 min., and at 4hrs. after treatment. AP and NIL were separated, pooled, (n=3), and RNA was isolated from both cytosolic and nuclear fractions. A cRNA probe spanning the entire region of exon 1 and a small portion of intron A of the POMC gene was used in a solution hybridization assay to detect POMC hnRNA and fully processed mRNA. Following hybridization and S1 nuclease digestion, samples were electrophoresed on 6% acrylamide gels, exposed to X-ray film,and the protected bands were excised, counted and quantitated by comparison to a standard curve of radiolabelled sense and antisense RNA generated from SP6 vectors containing the POMC exon1/intron A insert.

No changes in the relative amounts of cytoplasmic POMC mRNA were detected in AP or NIL following either 20 min. or 60 min. CRF treatment. CRF administration led to a 35%-40% increase in the detectable level of POMC hnRNA in the AP nucleus at 20 - 60 min. post CRF treatment; by 4hrs. after treatment, AP POMC hnRNA levels were 100% greater than their respective controls. Associated with the CRF induced increase in POMC hnRNA in the AP, levels of nuclear mRNA in AP were elevated 40%-50% above controls following 60 min. and 4hr. CRF treatment. In contrast to the AP, no alterations in the levels of nuclear POMC hnRNA or mRNA were observed in the NIL at any of the time points examined.

These data suggest that in the rat AP, CRF increases the relative abundance of POMC hnRNA, presumably by increasing the rate of gene transcription and/or by altering hnRNA processing. The lack of an effect on NIL POMC hnRNA may reflect differences in responsiveness to the given dose of CRF by corticotrophs and melanotrophs.

357.7 TRANSLOCATION OF A POSSIBLE DNA-BINDING PROTEIN IN ANTERIOR PITUITARY CORTICOTROPH TUMOR CELLS. J.F. Bishop, D. R. Gehlert and T.L. O'Donohue, Exper. Ther. Branch, NINCDS, NIH, Bethesda, MD 20892.

In order to identify intracellular protein messengers in eucaryotic cells, a method was developed to study the correlation between the phosphorylation state of a protein and biochemical events occuring in the cell. Thus, in AtT20-D16-16 cells, the phosphorylation states of both a 19 and a 14 kDa protein were significantly correlated with immunoreactive β-endorphin secretion (Bishop et al. Mol. Cell. Endocrinol., 1987 in press). Since the onset of POMC mRNA synthesis in response to second messengers follows a similar time course as seen for these phosphorylation events--increasing in approximately 15 to 30 minutes (Regina M Knight, personal communiation)--it is possible that these events are also involved in transmission of the signal to activate POMC gene transcription. Preliminary evidence (Bishop et al. Soc. Neurosci. Abst. 12:737, 1986) indicated that only the 14 kDA protein is present in both the nuclear and cytosolic fractions of AtT20 cells.

The present work was initiated to: 1) confirm the translocation of the 14 kDa phosphoprotein; 2) examine DNA-binding proteins in AtT20 cells and determine if the 14 kDa protein is itself a DNA-binding protein; and 3) develop methods to study correlation of phosphorylative changes in the nucleus and POMC gene activation. AtT-20 cells were prelabeled with ^{35}S-methionine and phorbol ester-stimulated changes in the distribution of the 14 kDa phosphoprotein were studied using a pulse-chase approach. Although ^{35}S-methionine-labeled 14 kDa protein was present in only very low concentrations, time dependent fluctuations in its distribution were seen, with a trend toward decreasing concentrations in the nuclear fraction over time. DNA-binding proteins were examined by binding ^{35}S-methionine-labeled nuclear proteins to dsDNA-cellulose followed by step elution with 500 mM and then 2 M NaCl. The majority of the bound proteins were eluted with 500 mM NaCl and most of these proteins were between 23 and 100 kDa. Two dimensional gel analysis revealed primarily basic proteins, including a high concentration of histones in the 2 M NaCl eluate. However, an approximately 14-16 kDa acidic protein with a pI very similar to the 14 kDa phosphoprotein was also seen.

To investigate the possible involvement of these DNA-binding proteins in POMC gene transcription, a correlative method was developed. Briefly, AtT20 cells were prelabeled with inorganic ^{32}P and the lysed cells were then fractionated via CsCl gradient centrifugation to separate proteins from RNA. Proteins were then TCA-precipitated repeatedly to remove salt and run on two dimensional gels. The RNA fraction was washed and run on formaldehyde gels comprised of 1% agarose. This procedure led to excellent recovery and resolution of ^{32}P-labeled proteins and, although the recovery of ^{32}P-labeled RNA was less reliable, several bands were seen between 3 and 10 kb. It is possible that one of these bands represents POMC heteronuclear mRNA (hnRNA). Densitometric scanning of alterations in protein phosphorylation and of alterations in the concentration of POMC hnRNA may provide a convenient method to select possible key phosphoproteins for investigation of their roles in the modulation of POMC gene transcription.

357.8 EXPRESSION OF OXYTOCIN AND VASOPRESSIN GENES IN THE LEYDIG CELLS AND THEIR REGULATION BY CYCLIC AMP. J.R. Dave*, R.V. Rebois*, S.G. Culp*, L. Liu*, B.Tabakoff and P.L. Hoffman*. (SPON: G.H. de Vries) Laboratory of Physiological and Pharmacological Studies, DICBR, NIAAA, Rockville, MD 20852 and DMNB, NINCDS, Bethesda, MD 20892.

Recent studies have demonstrated the presence of immunoreactive oxytocin (OT) and vasopressin (VP), OT and VP receptors and physiological functions for these hormones in the male reproductive tract of several species. The demonstration of very high (approximately 100-fold higher than circulating levels) concentrations of OT and VP in the testis have suggested that the testis represents a possible peripheral source of these hormones. The objectives of this study were to determine if (i) OT and VP genes are expressed in rat testis and (ii) human chorionic gonadotrophin (hCG), a known regulator of steroidogenesis in the testis which works by activation of adenylate cyclase, would modify the expression of these genes. Using oligodeoxyribonucleotide probes (courtesy of Drs. M. Brownstein and W. Scott Young, NIMH) for VP (complementary to the mRNA coding for the last 16 amino acids of the glycopeptide region, which has no analogous counterpart in the OT mRNA) and OT (complementary to the last 15 bases of the 5'-untranslated region and subsequent 33 bases), and Northern blot and slot blot techniques, OT and VP mRNAs were found to be present both in rat testis and in mouse-derived Leydig tumor cells (MLTC-1). The MLTC-1 cells were treated with either 50 pM or 2 nM hCG (a concentration of hCG which optimally stimulates cAMP secretion in these cells) or 1 mM dibutyrylcyclic AMP, respectively, for 24 hr, and VP and OT mRNA levels were quantitated. The hCG treatment of MLTC-1 cells produced a dose-related decrease in OT and VP mRNA levels. Dibutyrylcyclic AMP treatment also decreased OT and VP mRNA levels in these cells. Our data, thus, suggest that the testis could be a primary site of synthesis and processing of active OT and VP peptides in the male reproductive tract, and that cyclic AMP may be one of the regulators of these genes in the male reproductive system.

357.9 OSMOTIC REGULATION OF VASOPRESSIN mRNA IN THE SUPRAOPTIC NUCLEI DURING FETAL LIFE. S.M. Reppert and G.R. Uhl. (SPON: W.J. Schwartz). Children's and Neurology Services and Howard Hughes Medical Institute, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

We recently used quantitative in situ hybridization techniques to examine the developmental appearance of hypothalamic vasopressin (prepropressophysin) mRNA in rats. We found that vasopressin mRNA levels in the supraoptic nuclei (SON) were reliably detected on day 16 of gestation, while mRNA in the suprachiasmatic nuclei (SCN) was detectable on day 21. Furthermore, we found regulated expression of the vasopressin gene in SCN on gestational day 21, as vasopressin mRNA levels exhibit a prominent day-night rhythm. In the present study, we examined the prenatal regulation of vasopressin mRNA levels in the SON by osmotically challenging pregnant rats.

Two groups of timed pregnant Sprague-Dawley rats were used. The control group was given food and tap water ad libitum throughout pregnancy. The dams of the experimental group were given food ad libitum and only 2% saline to drink starting on day 16 of gestation. On gestational day 21, dams and fetuses from both groups were weighed, decapitated, and the brains were immersion-fixed. Coronal sections (10 um) through the central portion of the SON were subjected to in situ hybridization using a 40-base ^{35}S-labeled oligonucleotide probe complementary to exon c of the vasopressin gene. Trunk blood from dams and fetuses was collected at sacrifice, and plasma osmolalities were measured by freezing point depression.

The 2% saline elicited a 9% increase in plasma osmolality in the dams (mean osmolality was 320 mOsm/kg vs 293 mOsm/kg for control dams). Similarly, serum osmolality was increased by 11% in the fetuses from the salt loaded group, compared to fetuses from control dams (means of 336 mOsm/kg vs. 304 mOsm/kg). Fetuses from salt loaded dams also weighed 28% less than fetuses from control dams. Regional analysis of the film autoradiograms showed that vasopressin mRNA levels in the SON of the osmotically stimulated fetuses were significantly higher than those in the SON of control fetuses ($p<0.005$; n=3 fetuses from each of 3 litters for each group). Similar differences were seen for SON vasopressin mRNA levels between the dams of the two groups.

These results suggest that osmotic stimuli can regulate vasopressin mRNA levels in the fetal hypothalamus well before the time that plasma vasopressin influences water balance in the developing rat. The findings also suggest that osmoreceptive tissues are responsive to stimuli while the central nervous system is at a relatively immature stage of development. Supported by PHS Grant DK-38116 and Howard Hughes Medical Institute.

357.10 DEVELOPMENT OF ARGININE VASOPRESSIN mRNA IN RAT HYPOTHALAMUS. Sandra G. McElligott and Frank Baldino, Jr. Medical Products Department, E. I. du Pont de Nemours and Co., Wilmington, DE 19898

Changes in the distribution and content of vasopressin (AVP) mRNA were compared with peptide expression during the perinatal development of the rat hypothalamus. Individual AVP mRNA-containing cells were resolved using in situ hybridization with a synthetic oligodeoxynucleotide probe complementary to the glycoprotein region of prepropressophysin mRNA.

Vasopressin mRNA was observed before birth (E16) in the magnocellular neurons of the supraoptic nucleus (SON), the paraventricular nucleus (PVN), and the nucleus circularis. In contrast, AVP mRNA was first detected at birth (PDO) in the parvocellular neurons of the suprachiasmatic nucleus (SCN). Since vasopressin neurons in the SON and PVN originate prior to the neurons of the SCN, these results demonstrate that each hypothalamic nucleus differentiates according to its own timetable regardless of its neuropeptide content. In general, the expression of AVP mRNA can be detected 1-2 days prior to peptide immunoreactivity. The time delay between the initial transcription of AVP mRNA and peptide expression may be due to low antigenicity or the immaturity of posttranslational processes. Although before birth these nuclei still lack adult architecture, the embryonic cells expressing AVP mRNA were confined to areas which express AVP in the adult brain and were not widely distributed throughout the brain.

Both autoradiographic grain counts of PVN cells and optical density measurements revealed increases in mRNA levels which correlated with the increase in postnatal peptide content. Therefore, changes in peptide content after birth may result from increased mRNA synthesis. The early differentiation of the prepressophysin products raises questions on the functional involvment of AVP in brain development. The expression of AVP mRNA is correlated with developmental trophic factors.

357.11 EXPRESSION OF NEUROPEPTIDE Y mRNA IN NEURONAL AND NON-NEURONAL TISSUE AND ITS RESPONSE TO RESERPINE TREATMENT AND STRESS - IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY. M. SCHALLING[1], A. ERICSSON*[3], H. PERSSON*[3], S.-Y. CHAI*[1], Å. DAGERLIND*[1], S. BRENE*[1], K. SEROOGY[1], J.M. LUNDBERG*[2], D. LARHAMMAR*[3], L. TERENIUS*[4], J. MASSOULIÉ*[5], M. GOLDSTEIN[6] and T. HÖKFELT*[1]. Departments of [1]Histology and [2]Pharmacology, Karolinska Institutet, P.O. Box 60400, S-104 01 Stockholm, Sweden, Departments of [3]Medical Genetics and [4]Pharmacology, Uppsala University, Uppsala, Sweden, [5]Laboratoire de Neurobiologie, Ecole Normale Supérieure, Paris, France, and [6]Department of Psychiatry, New York University, Medical Center, School of Medicine, New York, USA.

We have examined the content of neuropeptide Y mRNA and its product in cells in both neuronal and non-neuronal tissue in Sprague Dawley rat as well as in several different mouse strains. Northern blotting techniques have been used along with in situ hybridization and immunohistochemistry to define different NPY containing compartments in the adrenal gland and their response to drug treatment such as reserpine. Furthermore, the rat brain and especially the locus coeruleus have been analyzed biochemically and histochemically for their response to reserpine treatment. Computerized densitometry has been used to quantify data both from the in situ hybridizations and the Northern blotting experiments. In non-neuronal tissue, in situ hybridization has been used to identify megakaryocytes in spleen and bone marrow of the Sprague Dawley rat and several mouse strains as a site of NPY synthesis. These data have also been confirmed by immunohistochemistry and radioimmunoassay. In the adrenal gland, NPY synthesis and content was demonstrated both in chromaffin cells and in a population of ganglion cells, which were rich in acetylcholinesterase and thus probably are cholinergic. The enzyme tyrosine hydroxylase (TH) which coexists with NPY in both the rat adrenal medulla and the locus coeruleus, has been analyzed both with in situ hybridization and biochemically for its response to reserpine. A marked increase in TH and NPY mRNA was seen both in chromaffin cells and ganglion cells as early as 1 day after drug treatment. Some other peptides such as cholecystokinin (CCK) and vasoactive intestinal polypeptide (VIP) as well as the enzyme phenylethanolamine N-methyltransferase have also been analyzed along these lines.

357.12 **RAT NEUROPEPTIDE Y PRECURSOR MESSENGER RNA: CHARACTERIZATION, TISSUE DISTRIBUTION, AND REGULATION BY GLUCOCORTICOIDS, CYCLIC AMP, CALCIUM, AND NERVE GROWTH FACTOR.** H. Higuchi and S.L. Sabol. Lab. of Biochemical Genetics, NHLBI, NIH, 36/1C-06, Bethesda, MD 20892.

Neuropeptide Y (NPY) is an abundant and important transmitter-modulator in the central and peripheral nervous systems. To study regulation of the NPY precursor (prepro-NPY) gene in experimental systems, we isolated and sequenced rat brain prepro-NPY cDNA clones. The deduced structure of rat prepro-NPY (98 amino acids) consists of a 29-residue signal peptide followed by the 36-residue NPY sequence, a proteolysis/amidation site Gly-Lys-Arg, and finally a 30-residue COOH-terminal peptide. This structure is highly similar to that of human prepro-NPY (Minth et al. (1984) PNAS 81, 4577-4581), particularly with respect to the NPY and COOH-terminal peptides (100% and 93% amino acid identity, respectively). Such strong evolutionary conservation is consistent with important physiological activities of both NPY and COOH-terminal peptides.

Northern blot analysis of rat brain RNA revealed that prepro-NPY mRNA (~800 bases) is most abundant in the striatum and cortex, (7.7 and 7.2 pg/μg total RNA, respectively). The abundances in other regions, relative to that in striatum, were as follows: hippocampus 34%, hypothalamus 33%, medulla oblongata 18%, midbrain 9%, cerebellum 6%, and spinal cord 30%. These levels parallel the concentrations of immunoreactive NPY in these regions, except for the hypothalamus, which is richest in NPY. Abundant prepro-NPY mRNA was also found in the adrenal gland, spleen, heart, and lung (40%, 26%, 11%, and 7%, respectively, of the level in striatum); these tissues are known to be innervated by NPY-containing neurons.

We are studying the regulation of the prepro-NPY gene in clonal PC-12 rat pheochromocytoma cells and NG108-15 mouse neuroblastoma-rat glioma hybrid cells. Prepro-NPY mRNA abundances were measured by blot hybridization. In untreated PC-12 cells the level of prepro-NPY mRNA is low but positively correlated with cell density (0.03-0.2 pg/μg RNA). Treatment of cells for 25-48 hr with dexamethasone (Dex, 1 μM) or the adenylate cyclase activator forskolin (10 μM) increased prepro-NPY mRNA 2-5-fold, while treatment with both elicited 4-17-fold increases. The protein kinase C activator phorbol 12-myristate 13-acetate (PMA, 0.4 μM) and/or the calcium ionophore A23187 (0.4 μM) also elevated prepro-NPY mRNA levels several-fold in the presence but not in the absence of Dex or forskolin. Combined treatment with Dex, forskolin, PMA, and A23187 led to dramatic increases (40-50-fold). Treatment with nerve growth factor (NGF, 2.5 S, 50 ng/ml) for 48 hr elicited 2-4-fold elevations with or without Dex. In contrast to PC-12 cells, untreated NG108-15 cells have a high level of prepro-NPY mRNA (14 pg/μg RNA). Treatment with Dex for 48 hr elevated this level 2-fold, but treatment with forskolin, 8-bromo-cyclic AMP, or other differentiating agents (e.g. 2% dimethylsulfoxide) reduced the level to 20-30% of the control. These data reveal a complex portrait of regulation of the prepro-NPY gene by the independent but cooperative influences of cell type, differentiation state, glucocorticoids, cyclic AMP (apparently acting through different mechanisms in the two cell lines), calcium ions, and NGF.

357.13 **Effect of Hypophysectomy and Growth Hormone Administration on Pre-prosomatostatin mRNA in Three CNS Regions.** KV Rogers*, L Vician*, RA Steiner and DK Clifton*. Depts of OB/GYN, Physiology and Biophysics, and Psychology, University of Washington, Seattle, WA 98195.

Physiological evidence suggests that somatostatin (SS), released from the median eminence (ME), inhibits pituitary growth hormone (GH) release and that GH acts via short loop feedback to stimulate SS secretion. The mechanism of this feedback effect may involve changes in SS synthesis as well as release since hypophysectomy (HPX) reduces hypothalamic SS content in rats and the treatment of HPX rats with GH prevents this reduction. Lesion experiments indicate that the periventricular nucleus (PeN) of the hypothalamus and the medial basal amygdala (mAMG) are the major sources of ME SS. We hypothesized that HPX would reduce SS synthetic capacity in the PeN and mAMG, as reflected in a reduction in SS mRNA levels, and that the administration of bovine GH to HPX rats would prevent this reduction. We tested this hypothesis by using in situ hybridization and a computerized image analysis system to measure pre-proSS mRNA signal levels in individual cells of the PeN and mAMG of male rats in 4 different experiment groups: HPX, HPX + bGH, sham-HPX and sham-HPX + bGH. We also measured SS mRNA signal levels in the cortex, a CNS region that does not project to the ME. Results are shown below; grains/cell is an index of SS mRNA.

	Mean grains/cell		
Group	PeN	mAMG	cortex
HPX + vehicle	125 ± 10	120 ± 5	172 ± 15
HPX + bGH	177 ± 16	123 ± 6	178 ± 13
sham-HPX + vehicle	196 ± 31	135 ± 9	142 ± 10
sham-HPX + bGH	290 ± 29	155 ± 12	178 ± 11

In HPX rats, SS synthetic capacity was reduced in the PeN ($p<0.01$) and mAMG ($p<0.05$), but not in the cortex. GH treatment prevented this reduction in the PeN ($p<0.01$) but not in the mAMG. In addition, bGH treatment increased SS synthetic capacity in the PeN of sham-HPX rats ($P<0.01$) . We conclude that the level of circulating GH influences the expression of the SS gene in the PeN of the hypothalamus but that some other pituitary hormone, such as TSH, is necessary for the maintenance of mAMG SS biosynthesis. SS mRNA levels in the cortex are not influenced by circulating pituitary hormone levels. These data demonstrate differential regulation of SS gene expression as a function of neuroanatomical location. (Supported by NIH Grant HD-12629).

357.14 **STEROID REGULATION OF SOMATOSTATIN mRNA IN THE RAT HYPOTHALAMUS,** **H. Werner*, Y. Koch*, F. Baldino Jr. and I. Gozes.** (SPON: R. Simantov). Department of Hormone Research, The Weizmann Institute of Science, Rehovot 76100, Israel, and Biomedical Research Department, Neurobiology Group, E.I. du PONT de Nemours and Co., Wilmington, Delaware 19898, U.S.A.

The neuropeptide somatostatin (SS) plays a physiologic inhibitory role in the regulation of growth hormone secretion from the anterior pituitary gland through its secretion into the hypophyseal portal circulation. Since growth processes are sensitive to circulating steroid levels, we have investigated the involvement of gonadal steroids in the expression of the brain SS gene. The levels of SS specific mRNA in the adult rat hypothalamus/preoptic area and cerebral cortex were measured following gonadectomy and steroid replacement treatment, by using a quantitative densitometric hybridization assay.

Our results indicated that the levels of SS mRNA in the rat hypothalamus are significantly decreased following gonadectomy (72% and 64% from the intact female and male values, respectively). The levels of SS mRNA in the cerebral cortex are not significantly modified after castration. Daily injections of estradiol dibenzoate (20 μg/rat/day, s.c) to ovariectomized animals reversed the decrease in hypothalamic SS mRNA by 24 h after commencing the treatment. Testosterone administration (200 μg/rat/day) to orchidectomized rats resulted in resumption of precastration values following 7 days treatment, but not after 1 day.

The rapid increase in SS mRNA levels in response to estrogen treatment in the female rat may suggest that the steroid directly regulates the expression of the SS gene, probably at a transcriptional level. The modulation of the SS gene expression by estrogen may partly explain the estrogen inhibition of weight gain and linear growth by this hormone.

357.15 STUDIES ON AN NGF-INDUCIBLE GENE IN PC12. R.POSSENTI*°, A.LEVI*', U.DI PORZIO*", J.D. ELDRIDGE*", B.M.PATERSON*". °Dept. Experim. Medic. Univ. Tor Vergata, Rome Italy, 'Lab. Cell Biology CNR Rome Italy, "NIH, NCI Bethesda MD 20892. (SPON: G.F. Ayala)

The rat-derived PC12 cells have manteined the property of immature chromaffin cells to acquire a neuronal phenotype in response to nerve growth factor (NGF). To obtain probes to study the early regulation of gene expression by NGF in its target cells, we have screened, by a differential hybridization procedure, a cDNA library constructed from mRNA of PC12 cells exposed to NGF for 24 hours. Of the few cDNA clones defined by their property of corresponding to NGF-inducible mRNAs, the clone VGF8a was chosen for further characterization. The level of the mRNA mesaured by either S1 or Northern blot analysis, was induced up to 40-50 fold after 6 hours of NGF exposure, and remained high also in fully differentiated cells (14 days of treatment). Run-on experiments showed that the transcription of VGF8a was induced by NGF up to 5-6 fold.

Completed sequence of the protein (710 aa) has been deduced from nucleotide sequence of the cDNA. We could not find any homology with other proteins sequenced so far. The VGF8a protein shows a high per-cent of proline and cystine residues, that could provide a compact rigid structures, a highly hydrophobic NH_2 region and several target sites for trypsin-like proteases.

Furterly we subcloned two different regions of the cDNA in procariote expression vectors and immunized rabbits against the recombinant proteins. According to cellular fractionation criteria and immunofluorescence the VGF8a proteins is associated with intracellular membranes. Immunoprecipitation or Western blot analysis showed at least two distinct polipeptides of 80-85000 MW.

The final line of investigation involved the characterization of the regulatory sequence of the VGF8a gene. The genomic organization of the gene, as deduced from sequence analysis, is very simple with a single short intron in the 5' leader region of the mRNA. A tipical TATA box is situated 30 bp before the transcribed region and a CAAT box 40 bp upstream. Three distinct consensus sequences, tipical for the SP1 protein binding, are also present in the 300 bp long "promoter region". This region is sufficent to drive the transient expression of the CAT gene in PC12 cells but not in other cell lines.

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357.16 DEVELOPMENTAL APPEARENCE OF SODIUM CHANNEL PROTEIN AND mRNA IN THE RAT BRAIN R. Scheinman[1], N. Edelstein*[1], A. Goldin*[2], R. Dunn*[3], V. Auld*[3], R. Moon*[1], N. Davidson*[2], and W. Catterall[1]. [1]Department of Pharmacology, University of Washington, Seattle, WA, 98195; [2]Division of Chemistry, California Institute of Technology, Pasadena, CA, 91125; and [3]Department of Medical Genetics, University of Toronto, Toronto, Canada, M5G 1L6.

The sodium channel (NaCh) from rat brain is a complex of α (260kD), β1 (36kD), and β2 (33kD) subunits. Functional NaCh may be quantitated by their ability to bind saxitoxin (STX). Previous measurements of binding of [3H]STX or [3H]tetrodotoxin show that STX receptors are detectable in the brain by day 1 after birth and increase progressively in number until the adult level is reached at day 21 to day 28. Here we compare this time course of appearance of STX receptors to the appearance of immunoreactive sodium channel protein and mRNA. NaCh α subunit protein was measured by immunoblotting or by immunoprecipitation with affinity purified antiserum. α subunits in immunoprecipitates were quantitated by phosphorylation with the cAMP dependent protein kinase. The timing of appearence of STX receptors and α subunit protein was similar. Low but detectable levels were observed on day 1 after birth and increased to the adult level by day 21 to day 28. cDNA clones encoding rat brain NaCh α subunits were isolated from a rat brain cDNA library constructed in the expression vector λgt11 by screening with affinity purified anti-α antibody. Appearance of α_x subunit mRNA was measured by Northern blot and by dot blot analysis using a ^{32}P labeled 2500bp cDNA probe cloned into the pGEM vector. The cDNA probe hybridizes to one size class of mRNA approximately 8.9 kb in length. This mRNA species is present at low but detectable levels as early as embryonic day 16. Maximum abundance is reached by day 7 and the level declines by day 14 to adult levels. The peak of mRNA coincides with the greatest rate of increase in sodium channel number. These data suggest that modulation of mRNA abundance is a primary mechanism of regulation of developmental appearance of α subunit protein and of functional sodium channels in the developing brain.

357.17 IDENTIFICATION OF TRANSCRIPTIONAL REGULATORY ELEMENTS OF THE MUSCLE NICOTINIC ACETYLCHOLINE RECEPTOR δ-SUBUNIT GENE. S.M. Evans*, P.D. Gardner, S. Heinemann and J. Patrick. Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138.

The mammalian muscle nicotinic acetylcholine receptor (AChR) mediates the synaptic interaction of nerve and muscle. The AChR is comprised of four subunits in the stoichiometry $\alpha_2\beta\gamma\delta$. Changes which occur in AChR expression during neuromuscular junction development are due in part to changes in transcript levels for each of the subunit genes. Denervation of muscle tissue results in a large increase in AChR subunit transcript levels, suggesting that AChR genes are regulated by the presence of the nerve.

As a start towards understanding the neural regulation of AChR subunit gene transcription, we have begun to isolate transcriptional control regions for each of the four subunit genes. We isolated two clones from a mouse genomic library (vector: λCh28) which hybridize under stringent conditions to a mouse AChR δ-subunit cDNA. One of these clones, λDG77, contains an insert of approximately 12kb and was subjected to detailed restriction enzyme and Southern blot analyses. A 1.8kb Xba I fragment of λDG77 hybridizes to the extreme 5' region of a δ-subunit cDNA. This genomic DNA fragment was subcloned upstream of the structural gene encoding chloramphenicol acetyltransferase (CAT) in the expression vector, pUC19.CAT. Transfection of BC_3H-1 mouse muscle cells with this CAT construct yielded readily detectable levels of CAT activity. However, when the 1.8kb fragment was subcloned in the opposite orientation into pUC19.CAT, no CAT activity was seen following transfection of BC_3H-1 cells. Thus, the transcriptional activity is orientation-dependent indicating the presence of a promoter element in this δ-subunit genomic DNA fragment.

We have used the fusing mouse muscle cell line, C2C12, to study the developmental regulation of this putative δ-subunit promoter. The expression of AChR subunit genes in C2C12 cells increases upon cell fusion. This increase is due, at least in part, to an increase in transcription of the AChR genes. Therefore, we determined if the transcriptional activity of the 1.8kb fragment is affected by the fusion process. As expected, little CAT activity was detected in extracts of unfused transfected cells while extracts of fused transfected cells contained easily measurable levels of CAT activity. Thus, in addition to being orientation-dependent, the transcriptional activity of the 1.8kb fragment of λDG77 appears to be developmentally regulated as would be predicted for transcriptional control elements of an AChR subunit gene.

357.18 DELETION ANALYSIS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR γ-SUBUNIT GENE PROMOTER. P.D. Gardner, S. Heinemann and J. Patrick. Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138.

We are interested in understanding the molecular mechanisms of nicotinic acetylcholine receptor (AChR) gene expression. It is well-documented that innervation has a profound influence on AChR gene expression in a developing muscle. It is likely that a nerve exerts this influence at the level of transcription of the AChR genes. To investigate this possibility further we have begun identifying transcriptional control elements of the AChR genes.

Recently, we identified a 1.1kb DNA fragment, located at the 5' end of the mouse muscle AChR γ-subunit gene, that confers cell type specific transcription of a reporter gene in transiently transfected mouse muscle cells. This transcriptional activity is developmentally regulated in the fusing mouse muscle cell line, C2C12. The 1.1kb fragment contains 750 base pairs of 5' flanking DNA, the first exon (5' untranslated and leader sequences), the first intron and a portion of the second exon (mature protein coding sequence) of the γ-subunit gene. To localize sequence elements necessary for transcriptional regulation of the γ-subunit gene, deletion analysis of the 1.1kb fragment was carried out. Deletion of 400 base pairs from the 3' end of the 1.1kb fragment leads to a loss of transcriptional activity in transfected muscle cells. The most notable feature of the deleted DNA is the first intron of the γ-subunit gene. Transfection of muscle cells with an expression clone in which the intron is used to promote transcription of a reporter gene yields no measurable transcriptional activity. Thus, the transcriptional activity of the 1.1kb fragment requires both 5' and 3' sequences.

Interestingly, sequence analysis indicates that the γ-subunit first intron contains two sequence elements that have striking homology with two well-documented transcriptional control elements. The first, located 33 nucleotides downstream of the exon/intron splice junction, is a 12 out of 13 base pair match with the core sequence of the SV40 enhancer. Fifty-eight base pairs downstream of this region, but still within the intron, is a 9 out of 10 base pair match with the Sp1 recognition site. Immediately adjacent to this potential Sp1 binding site is a potential stem and loop structure (13 base pair stem and 20 base loop). The role of these sequence elements in the regulation of transcription of the AChR genes is currently being investigated.

357.19 ELECTRICAL ACTIVITY REGULATES THE LEVEL OF mRNA ENCODING ALL FOUR SUBUNITS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR IN RAT SKELETAL MUSCLE. D. Goldman*, H. Brenner and S. Heinemann (SPON. S. Fisher). Mental Health Res. Inst. and Dept. of Bioch., Univ. of Mich., Ann Arbor, Mi., 48109; Dept. of Physiol., Univ. of Basel, Basel, Switzerland; and Salk Inst., Mol. Neurobiol. Lab., San Diego, Ca., 92138.

The nicotinic acetylcholine receptor (nAchR) is a pentameric integral membrane protein with a subunit composition $\alpha_2\beta\gamma\delta$. In adult vertebrate skeletal muscle the receptor is localized to the neuromuscular junction. However, prior to innervation or after denervation of adult muscle one finds receptors throughout the muscle fibers surface. The increase in extrajunctional receptors, following muscle denervation is a result of an increase in mRNA levels coding for the extrajunctional receptor. It is clear that activity induced in muscle by the nerve plays a role in regulating nAchR levels since electrical stimulation of denervated muscle can suppress the denervation induced increase in extrajunctional receptors.

In order to test the idea that electrical activity suppresses nAchR gene expression we measured the amount of receptor specific mRNA in denervated and denervated but chronically stimulated muscle. We find that in denervated muscle activity, elicited by extracellular electrodes, prevents an increase in extrajunctional nAchRs and their corresponding mRNAs. In addition, we find that activity will reduce the increased level of extrajunctional receptors and their mRNAs in 6 day denervated muscle that was subsequently stimulated with extracellular electrodes for an additional 5 to 7 days. These results are consistent with the idea that the motor neuron controls extrajunctional nAchR gene expression by the activity it induces in muscle.

357.20 ACETYLCHOLINE RECEPTOR REGULATION IN RAT PRIMARY MUSCLE IN RESPONSE TO BRAIN EXTRACT AND ASCORBATE. O. Horovitz* and M.M. Salpeter (SPON: O. Hamill). Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

Fetal rat brain extract, when added to the growth medium of the rat-derived L5 muscle cell line, induces a 2-5 fold increase in surface acetylcholine receptor (AchR) site density (Neugebauer et al., Brain Res. 346:58, 1985). One active component in the rat brain was found to be ascorbate (Knaack & Podleski, PNAS USA 82:575, 1985), which causes a surface receptor increase (due to accelerated AchR insertion) similar to that produced by rat extract. Ascorbate also caused an increase in mRNA measured for the AchR α-subunit (Knaack et al., Neurosci. Abstr. 11:771, 1985). However, no similar significant increase in surface AchR site density has been observed in response to either brain extract or ascorbate in rat primary cells in culture, where the control levels of surface AchR density are initially more than 5-fold higher and equivalent to extrajunctional levels in denervated muscle (for review see Salpeter & Loring, Progress in Neurobiology 25:297, 1985). A question arises as to whether rat primary cells are unable to respond to these "receptor-stimulating" nerve factors or whether the lack of increase in surface receptors is controlled at a different level.

In the present study, we compared the response of rat primary muscle cells to that of L5 cells in order to evaluate the basis of their different responses to brain extract and ascorbate. Using AchR α-subunit specific RNA transcripts as a probe, we looked at the levels of m-RNA in both cell types by Northern analysis. We found that in rat primary cells, the control level of mRNA for the α-subunit of AchR (measured per unit total RNA) is about 8 times higher than in L5 cells. Yet, like L5 cells, rat primary cells respond to both rat brain extract and ascorbate by a significant (up to 4-fold) increase in the level of the mRNA transcript. Our results suggest therefore that the difference in response of L5 and rat primary cells to brain extract and ascorbate is probably regulated by post-transcriptional events.

Many studies using developing and adult innervated and denervated muscle have shown a co-regulation of receptor mRNA transcript levels and expression of surface AchR. Yet the correlation between these two variables differs widely in different cell types and under different experimental manipulations. The response of rat primary muscle cells to brain extract and ascorbate is one dramatic illustration of surface AchR levels not increasing in response to a stimulus which elevates some specific AchR mRNA transcripts. It therefore provides a good system in which regulation of surface AchR expression can be studied in a primary cell. (Supported by NIH grant GM 10422 to M.M. Salpeter)

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS III

358.1 **AMINO ACID CONCENTRATIONS IN RAT AUDITORY, OLFACTORY AND VISUAL STRUCTURES.** Judy A. Parli*, John E. Schemenaur*, Donald A. Godfrey and C. David Ross. (SPON: D. F. Peterson) Dept. of Physiology, Oral Roberts Univ., Tulsa, OK 74171.

To obtain an overall comparison of amino acid metabolism across prominent structures of 3 sensory systems, homogenates of cochlea, cochlear nucleus (CN), olfactory bulb (OB), and retina from 3-5 albino rats were analyzed for concentrations of amino acids and activities of some related enzymes. Homogenates of whole brain were also analyzed. Ortho-phthalaldehyde derivatives of amino acids were separated by reverse phase high performance liquid chromatography (HPLC) and quantified by fluorescence detection. Fluorometric enzymatic microassays were utilized to determine the activities of glutaminase, total and cytosolic aspartate aminotransferase (tAAT, cAAT), malate dehydrogenase (MDH), and lactate dehydrogenase (LDH).

	Cochlea	CN	OB	Retina	Brain
	Amino acid concentrations (mmol/kg protein)				
Asp	6.1	26.6	28.2	25.1	24.3
Glu	15.7	58.2	70.3	95.4	62.6
Asn	3.7	2.6	2.4	5.0	2.2
Ser	17.0	6.9	7.9	8.2	7.7
Gln	15.3	29.4	43.2	7.4	27.4
Gly	18.8	29.2	10.3	34.7	13.7
Thr	6.4	5.8	7.4	5.6	5.7
Arg	3.3	2.3	11.9	4.3	3.2
Tau	32.5	26.3	145.9	530.0	44.2
Ala	14.1	6.9	12.4	15.8	5.0
Tyr	1.4	0.6	1.3	3.4	1.1
GABA	1.3	11.9	82.8	39.5	33.8
Lys	7.5	5.2	6.8	13.0	3.7
	Enzyme activities (mol/kg protein per hr)				
Glutaminase	0.3	13.7	25.0	26.8	16.2
tAAT	12.6	117.7	123.1	167.9	104.4
cAAT	4.9	57.1	54.9	73.3	44.3
MDH	5.1	41.8	43.5	37.6	33.7
LDH	4.1	23.6	22.8	70.8	17.0

Other amino acids measured and found to be in very low concentrations were Met, Trp, Val, Phe, Ile, and Leu. Retina contains an extremely high concentration of Tau, as well as high concentrations of Gly and GABA. Tau and GABA levels are also high in olfactory bulb, whereas cochlear nucleus contains a high concentration of Gly. Cochlea has lower concentrations of most amino acids than the other tissues, with the notable exception of Ser. The glutaminase activities correlate well with the Glu concentrations of the tissues (correlation coefficient r = 0.96).

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358.2 Ca2+–DEPENDENT RELEASE OF AMINO ACIDS IN HIPPOCAMPAL SUBFIELDS QUANTIFIED BY HPLC. P.A. Jarvie*, J.M. Wade* and J.T. Slevin. Depts. Neurology and Pharmacology, VA and Univ. Kentucky Med. Ctrs., Lexington, KY 40536-0084.

The hippocampal formation is ideally suited for studying the localization of neurotransmitter (NT) candidates in neurons. It contains three main excitatory neuronal projections: the afferent perforant path, mossy fiber system, and Shaffer/commissural path. These three consecutive pathways are well-characterized and are organized into distinct laminae. Superimposed on this excitatory system is an inhibitory one composed of a diffuse net of short-axon neurons, concentrated around both dentate granule and hippocampal pyramidal neurons. The most reliable and selective marker for amino acids as NTs is Ca2+-dependent, electrically or chemically-stimulated release. Work to date, including release of either preloaded 3H-amino acid or endogenous amino acid (micro-enzymatic method), suggests the NT of the perforant path is L-glutamate (glu); of the Shaffer/commissural system is either L-aspartate (asp) or glu; and of the mossy fiber system may be asp, glu, or neither.

We have measured K+-stimulated (55 mM), Ca2+-dependent (2.0 mM) amino acid release in tissue slabs roughly demarcating the regions of the dentate gyrus, mossy fiber axonal arbor (regio inferior), and Shaffer/commissural axonal arbor (regio superior); these were dissected from 500 μ coronal slices of hippocampus from 225g male Sprague Dawley rats. Ten μl aliquots of Krebs buffer, in which tissue slices were floated, were directly injected onto an HPLC where the sample was automatically derivitized with o-phthaldialdehyde. Amino acid derivatives were separated and detected fluorometricaly. Neither the putatiave inhibitory NT, taurine, or the glu precursor, glutamine, were released above baseline with K+ stimulation. Asp, glu and GABA were all released in all three areas in response to K+ stimulation; > than 80% was Ca2+-dependent. GABA release was uniform in all three regions in the range of 500 pmoles/mg protein/min. Calcium stimulated glu release was 20 to 30-fold higher than asp release. Glu release was highest in the dentate gyrus at 4 nmoles/mg protein/min. These data, determined using a very simple release method with direct HPLC measurement of the bathing medium, suggest that glu or perhaps a dipeptide containing glu, is the NT for all three major excitatory neuronal projections in the hippocampal formation. Furthermore, the homogeneous distribution of GABA release is similar to what has been described immunocytochemically using an antibody to GABA (Anderson, KJ. et al, Neurosci Letts. 69:7, 1986).

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358.3 POSTEMBEDDING IMMUNOGOLD LABELLING PERMITS A SEMIQUANTITATIVE ASSESSMENT OF TAURINE CONTENTS IN DIFFERENT CELL TYPES AND PROCESSES IN RAT CEREBELLUM. O. P. Ottersen, Anatomical Institute, University of Oslo, N-0162 Oslo 1, Norway

We have used the method of Storm-Mathisen et al. (Nature, 301:517, 1983) to raise antisera against conjugated taurine (Madsen et al., Neurosci. Lett. 60:255, 1985; Ottersen et al., Exp. Brain Res. 59:457, 1985) in the belief that knowledge about the exact localization of this amino acid in normal and pathological brain tissue could provide important clues as to its functional roles. Ultrathin plastic-embedded sections of rat cerebella perfusion-fixed with a mixture of glutaraldehyde and paraformaldehyde were incubated with taurine antiserum no. 20 and subsequently treated with a secondary antibody coupled to colloidal gold particles. The densities of gold particles in different cellular profiles were determined with the assistance of a computer. In the cerebellar cortex the highest concentration of gold particles occurred in the somata, dendrites, and dendritic spines of the Purkinje cells, supporting previous light-microscopical observations. In a representative experiment (antiserum dilution 1:500) the following particle densities were found (density in Purkinje cell somata = 100%, values corrected for background): Golgi cell terminals 25%***, granule cell dendrites 23%, parallel fiber terminals 21%, granule cell somata 19%, stellate cell terminals 11%*, glial cytoplasm 8%, stellate cell somata 4%**, mossy fiber terminals 2% (asterisks represent values significantly different from preceding value; *p<0.025, **p<0.01, ***p<0.001, Student's t-test). In the cerebellar nuclei, taurine-like immunoreactivity was concentrated in boutons sharing the ultrastructural features of Purkinje cell terminals. These terminals showed a mean gold particle density that was about 60 % higher than that of the Purkinje cell somata.

Ultrathin sections containing a series of different amino acid-glutaraldehyde-brain protein conjugates were incubated in the same drops of sera as the tissue sections for simultaneous specificity control. The gold particle density over the taurine conjugate was at least two orders of magnitude higher than that over any of the other amino acid conjugates (which had densities near background level), indicating that the distribution of gold particles in the tissue was not confounded by crossreactivity with GABA, glutamate or other amino acids. The low taurine-like immunoreactivity in the stellate cell terminals does not support previous suggestions that these terminals mediate taurinergic inhibition. The present data suggest instead that taurine might play a role in the synaptic function of the Purkinje cell terminals. - Supported by NAVF.

358.4 EFFECTS OF COLCHICINE LESIONS AND KAINIC ACID ON OPIATE RECEPTOR SUBTYPES IN RAT HIPPOCAMPUS. D.C. Perry[1] and L.M. Grimes[2], [1]Department of Pharmacology, George Washington University, Washington, D.C., 20037 and [2]Curriculum in Toxicology, Univ. of North Carolina, Chapel Hill, NC 27154.

Enkephalin (ENK) and dynorphin (DYN) in hippocampal dentate granule cells and their mossy fiber projections may play a role in convulsant disorders. To determine which opiate receptor subtypes are involved, we employed quantitative in vitro receptor autoradiography to measure changes in binding to mu, lambda and benzomorphan (BNZ) sites after colchicine (COL) lesions (to selectively destroy granule cells) and/or kainic acid (KA) (which causes a depletion of ENK and DYN from mossy fibers, followed in 48 hrs by an increase in peptide levels). Anesthetized rats were given bilateral hilar injections of saline or COL (2.5 lg/0.5 ll). Two weeks later, surviving rats were given saline or KA (8 mg/kg sc); animals were sacrificed 48 hours later. Mu sites were labeled with 3H-dihydromorphine (10 lM naloxone (NAL) blank); BNZ sites were labeled with 3H-diprenorphine (DIP) in the presence of 10 lM morphiceptin and 200 nM [DAla^{2}DLeu^5]-enkephalin (2 lM DIP blank); lambda sites were labeled with 3H-NAL in the presence of 300 nM DIP (10 lM NAL blank) (Perry and Sadee, Eur. J. Pharm. 129: 147, 1986). Binding was quantitated using a Loats computerized densitometer.

Mu and BNZ binding was present over the pyramidal cell layer in CA1-3, and increased over a rostral-caudal gradient. With mu binding, COL caused a small (20%) decrease in CA1 and in caudal CA3; COL plus KA caused a further decrease (up to 40%). KA alone, however, caused a large increase (50-90%) in mu binding, especially in rostral CA3 regions. With BNZ binding, COL and COL plus KA caused decreases (30-35%) throughout, whereas KA alone increased binding (40%) in rostral sections, with no change in caudal sections. Lambda binding was located over granule cells and mossy fibers, with a rostral-caudal gradient opposite to mu and BNZ. COL decreased lambda binding 30-60%, while KA had little effect; however, the combination produced an even larger decrease (up to 80% loss in caudal sections). The anatomical distribution and response to lesions of mu receptors resemble that seen earlier for ENK while the pattern for BNZ receptor resembles that for DYN. The distribution of lambda sites is inversely proportional to the peptide densities; their response to physiological insult appears most similar to DYN. Lambda sites may represent autoreceptors on mossy fibers, or they may be located on dendrites of CA3 pyramidal cells and hilar neurons. Supported by DA04191 (DCP) and 5-T32-ES-07126 (IMG).

358.5 KAINIC ACID INJECTION INTO THE RAT SPINAL CORD DECREASES ADENOSINE A_1 AND A_2 RECEPTOR DENSITY. J. I. Choca, R. D. Green*, and H. K. Proudfit, Dept. of Pharmacology, University of Illinois at Chicago, College of Medicine, Chicago, IL 60612.

Studies in our laboratory and others have suggested separate roles for adenosine A_1 and A_2 receptors in the modulation of nociceptive stimuli at the spinal cord level. We have shown that rat spinal cord membranes contain both adenosine A_1 and A_2 receptors, which are coupled to the inhibition and stimulation of adenylate cyclase, respectively (Choca et al., 1987). In quantitative autoradiographic studies of spinal cord slices (20 um), the Bmax of [^{3}H]5'-N-ethylcarboxamide adenosine ([^{3}H]NECA) was nearly twice the Bmax of [^{3}H]N^6-(1-methyl-2-phenylethyl)adenosine ([^{3}H]R-PIA) in each of three spinal cord areas: 1)the substantia gelatinosa (SG), 2)the central canal (CC), 3)the ventral horn (VH). The binding sites characterized in spinal cord slices were identical to the sites characterized in membranes: 1)the K_d values determined in slices for [^{3}H]NECA and [^{3}H]R-PIA (Table 1) were the same as those reported for membranes, and 2)N^6-(cyclopentyl)adenosine (CPA) displaced [^{3}H]NECA from two binding sites (K_{i-1} = 0.36 ± 0.53 nM, K_{i-2} = 241 ± 277 nM) and [^{3}H]R-PIA from one site (K_i = 1.0 ± 0.4 nM; N=3). Thus, the binding characteristics of these ligands were identical in slices and in membranes. An estimate of A_2 receptor number was obtained by subtracting the [^{3}H]R-PIA Bmax (A_1 receptor number) from the [^{3}H]NECA Bmax (A_1 plus A_2 receptor number; Table 1).

Unilateral dorsal rhizotomy (DR, N=8, 4-6 roots cut), unilateral hemitransection (HT, N=9, Table 2) and complete spinal transection (not shown) failed to alter either [^{3}H]NECA or [^{3}H]R-PIA binding densities in the SG. On the other hand multiple unilateral kainic acid injections into the dorsal horn (0.2 ul 10mM solution in PBS) eliminated 37.2% [^{3}H]R-PIA and 41.7% [^{3}H]NECA binding densities (N=9, Table 2). In some spinal cord sections, kainic acid was capable of eliminating 100% of both [^{3}H]R-PIA and [^{3}H]NECA binding in the SG. These reults indicate that most, if not all, the adenosine A_1 and A_2 receptors in the SG are located on intrinsic neurons in the rat spinal cord. (Supported by PHS grant DA03980.)

TABLE 1

	$A_1 + A_2$ [^{3}H]NECA	A_1 [^{3}H]R-PIA	A_2 N minus P
SG	506 ± 20	301 ± 23	205 ± 20
CC	290 ± 18	148 ± 13	142 ± 13
VH	166 ± 9	84 ± 14	82 ± 16
K_d	11.5 ± 1.5	1.6 ± 0.3	

Bmax units = fmol/mg prot
K_d units = nM

TABLE 2

	%CONTROL [^{3}H]R-PIA	%CONTROL [^{3}H]NECA
HT	99.1 ± 1.6	98.0 ± 2.7
DR	98.4 ± 1.9	99.3 ± 3.3
KA	62.8 ± 8.1	58.3 ± 6.8

358.6 ASSOCIATION OF ADENOSINE A1-RECEPTORS WITH PUTATIVE GLUTAMATERGIC NEURONS IN THE HIPPOCAMPUS OF THE RAT. J. Deckert and M.B. Jorgensen*. Unit on Neurochemistry, BPB, NIMH and SNB, NINCDS, Bethesda, MD 20892

Lesioning of cholinergic, adrenergic and serotoninergic hippocampal afferents and subsequent measurement of adenosine A1-receptors in a membrane binding assay suggested that adenosine A1-receptors were not associated with any of these hippocampal afferents (Murray T.F. and Cheney D.L., Neuropharm., 21:575-580, 1982). About 85% of the synaptic input to the outer two-thirds of the molecular layer of the ipsilateral dentate gyrus in rat brain is provided by entorhinal cortex neurons (Matthews D.A. et al., Brain Res. 115:1-21, 1976). These neurons are thought to utilize the excitatory amino acid glutamate as a neurotransmitter (Nadler J.V. et al., Nature, 260:538-540, 1976).

In a quantitative autoradiographic study with [^{3}H]cyclohexyladenosine as the ligand probe (Lewis M.E. et al., Eur. J. Pharm., 73:109-111, 1981) we investigated adenosine A1-receptors four days after unilateral surgical removal of the entorhinal cortex (Jorgensen M.B. et al., Acta Neuropath., in press). This selective lesion of the major single source of synaptic input to hippocampal granule cells resulted in a 40% loss of [^{3}H]cyclohexyladenosine binding in the molecular layer of the ipsilateral dentate gyrus as compared to the contralateral dentate gyrus and controls ($p < 0.01$, Scheffe-F-test):

	Molecular layer ipsilateral	contralateral
lesioned (n=5)	80±8 fmol/mg	133±8 fmol/mg
control (n=5)	130±11 fmol/mg	123±12 fmol/mg

(mean±SEM, [^{3}H]cyclohexyladenosine concentration 2.5 nM). No significant differences in [^{3}H]cyclohexyladenosine binding were observed between ipsilateral and contralateral or control stratum radiatum, stratum oriens and parietal cortex.

This strongly suggests that adenosine A1-receptors are either localized on these hippocampal afferents themselves or associated with their synapses with granule cells. This localization is consistent with the reported inhibition of [^{3}H]glutamate release from rat dentate gyrus slices by an adenosine analogue (Dolphin A.C. and Archer E.R., Neurosci. Lett., 43:49-54, 1983).

358.7 LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMISTRY FOR GLUTAMATE IN THE RAT SUPRAOPTIC NUCLEUS. R.B. Meeker, D.J. Swanson* and J.N. Hayward. Department of Neurology and Neurobiology Curriculum, University of North Carolina, Chapel Hill, N.C. 27514.

While glutamate (Glu) is thought to be a major excitatory neurotransmitter in the central nervous system (McLennan, 1983), its role in the release of vasopressin and/or oxytocin remains unclear. The development of a highly specific antiserum against Glu (Hepler et al.,1986), has allowed us to examine the distributions of Glu-like immunoreactivity (Glu-LI) in the rat supraoptic nucleus (SON). Rat brains were fixed by cardiac perfusion with 4% carbodiimide (or paraformaldehyde) + 15% saturated picric acid + 0.1 - 0.2% glutaraldehyde in 0.12 M phosphate buffer, pH 7.3. Coronal sections (50 μm) were cut on a Vibratome and immunocytochemically labeled for Glu using an antiserum dilution of 1:5000 and the ABC technique with diaminobenzidine as the chromagen. Under light miscrscopy (LM), a high level of Glu-LI was found in the SON. The perikarya of these magnocellular neuroendocrine cells stained with variable intensities ranging from intermediate to low levels. A major band of dense labeling was found in the region immediately ventral to the packed cell somata of the SON, in the ventral dendritic neuropil (VDN). Heavily labeled, punctate Glu-LI was observed throughout the VDN. Ultrastructural (EM) examination of the SON revealed low levels of reaction product within the cytoplasm of the magnocellular SON neurons, often associated with the rough endoplasmic reticulum (RER). In the VDN we found heavily labeled Glu-LI dendrites, axon-like processes and terminals. The darkly stained punctate structures observed in the VDN under LM, were identified in EM as cross-sections of large, heavily labeled dendrite-like processes. These processes occasionally contained neurosecretory granules (NSG) suggesting a magnocellular origin. In addition, Glu-LI terminals generally contained round, clear vesicles but occasionally contained both clear and dense-core vesicles. Most of the Glu-LI labeled terminals showed synaptic specializations contacting Glu-LI processes.

In summary, we find the rat SON heavily labeled with Glu-LI at LM and EM. The intermediate to low level of Glu-LI in the cell somata at LM appears to be localized in the RER at EM. The high level of Glu-LI in the VDN at LM is localized at EM in: 1) Dendrite like processes occasionally containing NSG; 2) Terminals containing round, clear vesicles; 3) Terminals containing mixed round, clear and dense-core vesicles. Each type of terminal formed specializations impinging on Glu-LI processes. These observations suggest that the SON receives significant glutaminergic innervation which may be an excitatory neurotransmitter for vasopressin and/or oxytocin release.

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358.8 THE DISTRIBUTION AND MORPHOLOGICAL DIVERSITY OF GABA-CONTAINING NEURONS IN THE PREFRONTAL CORTEX OF HUMAN NEWBORN BABY Zheng Deshu, Gai Weiping*,Department of Anatomy, Hunan Medical College, Changsha, Hunan, People's Republic of China

GABA has been belived to be the principle neurotransmitter of the local neurons in the cerebral cortex.The distribution and morphological characteristics of the GABA-containing neurons are essential for understanding the function of the local neuron in the cerebral cortex.The prefrontal cortex of a human full term new born baby just after accident death was studied by means of immunocytochemistry technique with antibody directed against GABA(Immunonuclear corp.).GABA-containing neurons were found over all layers , all were nonpyramidal cells.The laminar distribution of GABA-containing neurons were not even between different layers,density in layer II was prominently higher than that in other layers.Density in layer III and layer IV was higher than that in layer V and layer VI.The tangential distribution over all layers was also uneven,but was not so obvious as that in laminar distribution.Diameters of positive neurons were between 5-16um(measured as a circle).There was a superficial to deep sequence of cell size,with the larger diameter being located in progressively deeper layers.The GABA positive neurons could be identified into three types;multipolar,bitufted and bipolar.Some of the GABA positive neurons were hard to identify,due to the poor staining of their processes.GABA positive endings were found surounding the somata and proximal dendrites of both the GABA positive and negative neurons,but not so numerous as found in the adult monkey prefrontal cortex.The result of the present study revealed that GABA-containing neurons in the prefrontal cortex of the human newborn baby have well developed,but whether laminar and tangential distribution pattern will change in a certain period after born, remains to be further studied.

358.9 REGIONAL DISTRIBUTION OF GABA-B AND GABA-A BINDING SITES IN RAT BRAIN: A COMPARATIVE, QUANTITATIVE AUTORADIOGRAPHIC STUDY. J.B. Penney, D.C.M. Chu and A.B. Young. Neuroscience Program and Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

Within the nervous system, the inhibitory effects of GABA appear to be mediated through two distinct receptor subtypes: GABA-A and GABA-B receptors. GABA-A receptors have been well characterized by various methods. The existence of GABA-B receptors in various CNS structures has been inferred from the biochemical and electrophysiologic actions of the GABA-B agonist, baclofen. Direct autoradiographic visualization of GABA-B receptors has been limited to the spinal cord and cerebellum. We have previously used [3H]GABA quantitative autoradiography to examine both GABA-B and GABA-A receptors in human postmortem tissue (Chu et al., Neurosci. Abstr. 11:725, 1985). In the present study, we used a similar assay to examine the kinetics, pharmacology and regional distribution of GABA-B receptors in rat brain. Comparison to the regional distribution of GABA-A receptors was also done.

[3H]GABA binding to GABA-B receptors at 4°C reaches equilibrium within 45 min. The association and dissociation rate constants for GABA-B binding to outer neocortical layers are $2.87 \pm 0.17 \times 10^{5}$ $min^{-1}M^{-1}$ and 0.0966 ± 0.0118 min^{-1}, respectively. A K_D value of 336 ± 40 nM is calculated for these results. Binding to GABA-B and GABA-A receptors is saturable. Analysis of Scatchard plots reveals that GABA-A receptors bind [3H]GABA with twice the affinity of GABA-B receptors. While the affinities of each type of GABA receptor are relatively uniform across regions, the B_{max} of both types of GABA receptor varies from region to region. There are roughly 2-3X as many GABA-A receptors as there are GABA-B receptors in regions where both occur. The distribution of GABA-B receptors in rat brain is different from that of GABA-A receptors in the olfactory bulb, cerebellum, thalamus, neocortex, medial habenula and interpeduncular nucleus. Other areas high in GABA-B binding include the medial and lateral geniculates, the superior colliculus and certain amygdaloid nuclei. Regions intermediate in GABA-B binding include outer cortex, striatum, thalamus and hippocampus. Low amounts of GABA-B binding occur in brainstem, basal forebrain, globus pallidus and subiculum. Binding to white matter tracts and ventricles is negligible. The distribution of GABA-B receptors is in agreement with previously postulated sites of action of baclofen.

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358.10 MODULATION OF [35]S-TBPS BINDING BY THE GABA RECOGNITION SITE: AUTORADIOGRAPHIC EVIDENCE. C.A. Wilmot, A.M. Szczepanik* and D.B. Ellis*. Dept. Biochemistry, Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ 08876.

TBPS, t-butylbicyclophosphorothionate, is a potent convulsant which blocks GABA-ergic neurotransmission by interacting with the convulsant site of the GABA/benzodiazepine/chloride ionophore receptor complex. [35]S-TBPS binding sites in cortical homogenate preparations are subject to allosteric modulation by benzodiazepines and GABA agonists. To visualize the anatomical location of these binding sites and to identify those areas modulated by the GABA recognition site, a method for [35]S-TBPS autoradiography was established. Autoradiograms were generated from rat brain sections (Wistar, male), freeze-dried, preincubated in buffer, incubated with [35]S-TBPS, 2 nM, 50-60 Ci/mmole, and exposed to Kodak X-OMAT AR film for 7 days, along with [35]S-brain mash standards, 230-382,500 dpm/mg protein. Specific binding, defined with 10 μM picrotoxin, was typically 95% of total binding. Adjacent sections incubated in the presence of 0.1 - 10 μM GABA showed a graded and complete inhibition of binding. The GABA antagonist, RU 5135, 10^{-9} to 10^{-7} M, totally reversed the effect of 10 μM GABA. In the presence of 10^{-7} M RU 5135 alone, binding was 60-70% of total specific, whereas in the combined presence of 10^{-7} M RU 5135 and 10 μM GABA, binding was increased up to 136% of total specific. Since RU 5135 itself has weak effects on [35]S-TBPS binding and potently reverses the inhibition produced by GABA, these results suggest that GABA affects [35]S-TBPS binding via an interaction at the GABA recognition site. Brain areas in which these effects were most prominent were the deep cortical layers of the motor and somatosensory areas of the frontoparietal cortex, globus pallidus, ventral pallidum, n. vertical limb of the diagonal band, anteromedial and ventrolateral areas of the thalamus, anterior pretectal areas, superior colliculus and the substantia nigra, pars reticulata. GABA-modulated [35]S-TBPS binding sites are thus site-specifically distributed in deep cortical layers, subcortical areas and mesencephalic areas.

358.11 RESOLVING GABA$_A$/BENZODIAZEPINE RECEPTORS: SUBCELLULAR LOCALIZATION IN THE CNS WITH MONOCLONAL ANTIBODIES. H. Möhler*and J.G. Richards (SPON: J.R. Martin), Pharma Res. Dept., F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basle, Switzerland.

The ability of benzodiazepines to reduce the extent of anxiety, the degree of muscle tension, the likelihood of convulsions and the level of vigilance is triggered by their selective interaction with allosteric sites on GABA receptors in neuronal membranes of the CNS. A variety of morphological techniques are currently used to determine receptor expression, their numbers, affinity, cellular and subcellular localization and their regulation in microscopic regions of the CNS. For such investigations, high affinity radioligands, monoclonal antibodies and hybridization probes are essential tools. The resolution of receptor autoradiography, while providing invaluable quantitative data about benzodiazepine receptor ligands (Richards et al., this meeting), is unable to reveal the cellular and subcellular localization of receptors. Monoclonal antibodies raised against a purified GABA$_A$/benzodiazepine receptor complex that recognize either the α-subunit (50 kD) or the β-subunit (55 kD) have been used to visualize the subcellular distribution of the receptors in rat brain, spinal cord, retina and pituitary by immunocytochemistry (Richards et al., J. Neurosci., in press). The findings support a modulatory role of benzodiazepines in GABAergic synaptic inhibition in various regions of the brain (olfactory bulb, hippocampus, substantia nigra, cerebellum), spinal cord (dorsal horn), pituitary (anterior and neurointermediate lobes) and retina (internal plexiform layer). In the near future, it will be possible to study benzodiazepine receptor expression in the CNS by in situ hybridization, once the cDNA, encoding the subunit proteins, has been isolated.

358.12 LOCALIZATION OF PERIPHERAL BENZODIAZEPINE BINDING SITES TO ERYTHROCYTE MEMBRANES. J.M.M. Olson*, B.J. Ciliax, W.R. Mancini* and A.B. Young. Depts. of Pharmacology, Neurology and Neuroscience Program, University of Michigan, Ann Arbor, MI 48104.

Benzodiazepines (BDZ) bind to two pharmacologically distinct sites. The central BDZ receptor is located on CNS neurons. The sedative, hypnotic and anticonvulsant activities of benzodiazepines are mediated by these central receptors. The peripheral BDZ binding sites are located on adrenal, kidney, glia, and other non-neuronal tissues. The function and subcellular location of these binding sites are not yet known. Pharmacologically, PK 11195 and Ro 5-4864 bind to the peripheral site with nanomolar affinities while flunitrazepam, a benzodiazepine that binds to both central and peripheral binding sites, binds with mid-nanomolar affinity. Clonazepam binds specifically to central receptors.

Using subcellular fractionation of rat adrenal cells, Anholt and coworkers report that the peripheral benzodiazepine receptor is located on the outer mitochondrial membrane (J. Biol. Chem. 261: 576, 1986). Because mature human erythrocytes lack mitochondria, we performed binding studies on these cells to determine whether there are peripheral benzodiazepine binding sites which are not associated with mitochondria.

Erythrocytes were separated from leukocytes and platelets by centrifugation. Erythrocytes were then lysed in hypotonic sodium phosphate buffer, washed, and centrifuged to remove hemoglobin and other intracellular contaminants from the membranes. Binding assays were performed using 2.5 X 10^{-9} M [3H]PK 11195.

[3H]PK 11195 bound to both intact human erythrocytes and purified erythrocyte membranes in a specific, saturable manner. Nonspecific binding represented less than 17% of total binding. At 4°, maximum levels of binding (500 fmol/mg membrane protein) occurred by 90 minutes. IC-50 values for displacement of [3H]PK 11195 by unlabelled PK 11195, Ro 5-4864, flunitrazepam, and clonazepam were similar to those reported for other tissues containing the binding site. Unlabelled competitors followed the same rank order of displacement in intact rat erthyrocytes.

Our results suggest that non-mitochondrial binding sites, pharmacologically indistinguishable from the peripheral benzodiazepine binding sites demonstrated in adrenal cells and other peripheral tissues, are located on erythrocyte membranes.

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358.13 CHARACTERIZATION AND DENSITY OF THE PERIPHERAL BENZODIAZEPINE BINDING SITES IN MITOCHONDRIA FROM DIFFERENT ORGANS. L. Antkiewicz-Michaluk, K.E. Krueger, A. Guidotti and E. Costa (SPON: W.M. Saidel). FIDIA-Georgetown Institute for the Neurosciences, Georgetown University Medical Center, Washington, D.C. 20007.

Peripheral-type benzodiazepine recognition sites are found in many peripheral organs as well as the central nervous system and exhibit a different pharmacological specificity than that of the benzodiazepine recognition sites associated with GABA receptors. Peripheral-type benzodiazepine receptors are apparently localized on the outer mitochondrial membrane, yet considerable variation exists among different tissues in the density of these sites. To examine these differences more closely, we determined the relative density of specific binding sites for PK11195 on mitochondrial preparations from several organs of the rat. The following relative proportions of PK11195 specific binding sites versus protein content were observed: adrenal gland 1.00; lung, kidney, heart 0.10; liver, testis, skeletal muscle 0.04; brain 0.01. The specific activities of two mitochondrial enzymes, succinic dehydrogenase (SDH) and cytochrome c oxidase (CCO), also showed variation in each mitochondrial preparation, however, the ratio of SDH to CCO was nearly constant from all tissues. There was no correlation between the levels of PK11195 binding and the specific activities of these enzymes. This accounted for a ratio of PK11195 binding to SDH and CCO activities being 20-100 fold greater in adrenal gland mitochondria in comparison to the other organs. To further characterize the binding of benzodiazepine to mitochondrial populations from different tissues [^{3}H]PK14105, a photoaffinity ligand specific for peripheral type receptors (Doble et al., Mol. Pharmacol. (1987) 31: 42-49), was used. Specific binding of PK14105 was blocked by the addition of 1 uM PK11195. Following irradiation approximately 40% of the bound [^{3}H]PK14105 could not be displaced by PK11195, or dissociated from the membrane by warming to 20°C. The quantity of [^{3}H]PK14105 covalently incorporated into mitochondria from different organs paralleled that of specific PK11195 binding. SDS-polyacrylamide gel electrophoresis and autoradiography of mitochondrial membranes photolabeled with [^{3}H]PK14105 revealed a polypeptide band with an apparent M_r of 16,000 daltons. From all organs examined this component was not labeled when photolabeling was performed in the presence of 1 uM PK11195. These results demonstrate that the quantity of peripheral-type benzodiazepine receptors on mitochondria varies greatly from different tissue sources with adrenal gland mitochondria exhibiting the highest levels. The binding sites from different mitochondrial populations appear to be located in a common protein component evidenced by the photolabeling of a 16,000 dalton polypeptide.

358.14 ^{3}H-ALPIDEM: A HIGH AFFINITY LIGAND FOR IMIDAZOPYRIDINE RECOGNITION SITES IN THE PERIPHERY. S. Arbilla*, S. Tan*, B. Zivkovic, G. Perrault*, P. George*, J. Allen*, A. Wick* and S.Z. Langer. Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France

Alpidem (ALP: 6-chloro(4chlorophenyl)2N,N di-n-propyl imidazo[1,2-a]pyridine-3-acetamide) displaces with high affinity (0.71 nM) ^{3}H-Ro 5-4864 binding to rat kidney. The radiolabelled compound (^{3}H-ALP 51.8 Ci/mmol, LERS Chemistry Department) binds to rat cerebral cortex membranes preincubated with Ro 5-4864 1 μM with a Kd of 1.50 ± 0.14 nM and a Bmax of 1438 ± 129 fmol/mg prot (n = 3). In the present study, ^{3}H-ALP was used to explore its binding characteristics in the rat kidney and platelets as well as human platelets. Crude membrane preparations (3 μg prot/ml) were incubated for 120 min at 25°C with ^{3}H-ALP (0.003 - 3 nM). Specific ^{3}H-ALP binding was defined in kidney in the presence of Ro 5-4864 1 μM and in platelets in the presence of PK 11195 2 μM.

Under these conditions, ^{3}H-ALP binding, in the three preparations was reversible and displaced by Ro 5-4864, PK 11195 and flunitrazepam but not by Ro 15-1788 or clonazepam.

Scatchard analysis of saturation data indicates that ^{3}H-ALP binds with high affinity to a single class of recongnition sites. The affinity constants (Kd) and maximal number of binding sites (Bmax) are shown in table 1.

Table 1 : Binding characteristics of ^{3}H-alpidem

	n	Kd (pM)	Bmax fmol/mg prot
Human platelets	3	289 ± 32	3754 ± 140
Rat platelets	3	44 ± 13	4500 ± 729
Rat kidney	5	57 ± 7	2493 ± 150

The Bmax for ^{3}H-ALP binding in the rat kidney was significantly reduced to 1228 ± 93 fmol/mg prot (n = 5, $p<0.001$) when measured 48 hr after the repeated administration of alpidem (100 mg/kg x 2 p.o., 10 days) (Table 1).

It is concluded that peripheral receptors in the kidney and platelets can be recognized not only by benzodiazepines or the isoquinolinecarboxamide PK 11195 but also by imidazopyridines like alpidem. As ^{3}H-ALP binding sites undergo down regulation, it is suggested that this recogntion site might be a functional receptor and that ALP behaves as an agonist. The high affinity of ALP for these sites indicates that ALP may prove useful in the understanding of the physiological or pharmacological functions associated with these receptors which are also present in the central nervous system.

358.15 USE OF THE SELECTIVE BENZODIAZEPINE-1 (BZ-1) LIGAND [^{3}H]-2-OXO-QUAZEPAM (SCH 15-725) TO LOCALIZE BZ-1 RECEPTORS IN THE RAT BRAIN. J.P. Yezuita*, R.T. McCabe, A. Barnett[1], L.C. Iorio*[1], and J.K. Wamsley (SPON: S.S. Stensaas). Depts. of Psychiatry and Pharmacology and Toxicology, Univ. of Utah, SLC, UT 84132, and [1]Biological Research Division, Schering-Plough Corp., Bloomfield, NJ 07003.

A metabolite of the benzodiazepine agonist quazepam, 2-oxo-quazepam (2OXOQ; 7-chloro-1-(2,2,2-trifluoroethyl)-1,3-dihydro-5-(2-fluorophenyl)-2H-1,4-benzodiazepin-2-one), has been studied to determine its binding characteristics for labeling the BZ receptor in sections of the rat brain. Autoradiographic measurements of brain regions known to bind BZ ligands indicate [^{3}H]-2OXOQ is selective for the BZ-1 receptor subtype.

Dissociation, association, and saturation experiments, as well as displacement studies, were performed to establish optimal binding conditions for use in generating autoradiograms. Specific binding was defined by incubating tissue sections taken from rat forebrain in 0.17 M Tris buffer (4°C; pH 7.4) containing 2 nM [^{3}H]-2OXOQ, while nonspecific binding was determined in the same manner except in the presence of 1 μM clonazepam.

The data obtained from these experiments indicate that the specific binding of [^{3}H]-2OXOQ is saturable and of high affinity, reaches equilibrium after 30 minutes of incubation, and has a dissociation time of approximately 10 minutes. Saturation curves reveal that the maximum specific binding reaches 40-45% at 4.5 nM [^{3}H]-2OXOQ. Scatchard analysis performed to estimate the number of receptors (B_{max}) and dissociation constant (K_d) gave results of 18.4 fmol/mg tissue and 1.6 nM, respectively. Additionally, displacement experiments demonstrate that [^{3}H]-2OXOQ is displaceable by both BZ and BZ-1 specific ligands.

Quantitation of grain densities on autoradiographic films showed differential binding in accordance with known distributions of BZ-1 and BZ-2 receptor sites. In particular, intermediate to high binding was observed in lamina IV of the cerebral cortex, substantia innominata, substantia nigra, zona incerta, and molecular layer of the cerebellum (BZ-1 sites), while low binding was measured in other layers of the cortex, as well as dentate gyrus and superior colliculus (BZ-2 sites). These results indicate that [^{3}H]-2OXOQ binds preferentially to the BZ-1 receptor subtype and has potential for use in delineating the actions of BZ-1 selective ligands. The tritiated form of this ligand, moreover, should provide a useful tool for further investigation of the density and distribution of BZ-1 sites in the CNS.

STRESS, HORMONES AND THE AUTONOMIC NERVOUS SYSTEM I

359.1 STRESS-INDUCED RENIN AND CORTICOSTERONE SECRETION IS BLOCKED BY DESTRUCTION OF CELL BODIES IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. K.D. Richardson Morton*, M.S. Brownfield+, S.A. Lorens, and L.D. Van de Kar. Dept. of Pharmacology, Loyola University Medical Center, Maywood, IL 60153 and +University of Wisconsin School of Vet. Medicine, Madison, WI 53706.

We have previously reported that electrolytic lesions in the hypothalamic paraventricular nucleus (PVN) prevent the stress-induced increase in plasma renin activity (PRA) , plasma renin concentration (PRC) and plasma corticosterone levels following a ten minute conditioned emotional (fear) response (CER) paradigm (Richardson Morton et al., Neurosci. Abst. 12: 290.15, 1986). This experiment was designed to determine whether cell bodies in the PVN or fibers of passage through the PVN are involved in mediating the stress-induced increase in PRA, PRC and corticosterone levels. Bilateral stereotaxic microinjections of ibotenic acid (10 ug/ul; 0.3 ul per side) were performed 14 days prior to the stress procedure. Ibotenic acid is a neurotoxin that selectively destroys cell bodies while leaving fibers of passage intact. Histological evaluation of the tissue revealed cell death and lysis in the PVN where there was a concomitant suppression of corticosterone secretion. Ibotenic acid injection into the PVN prevented the effect of stress on PRA, PRC and corticosterone levels. Values for PRC (production of Angiotensin I under saturating levels of exogenous substrate) were slightly higher than those for PRA (production of Angiotensin I from endogenous substrate), but the stress-induced increase in PRA, PRC and corticosteorne levels were clearly blocked by ibotenic acid injection into the PVN. This suggests that the blockade of the effect of stress on PRA is not due to changes in renin substrate levels. The results with ibotenic acid suggest that neurons in the PVN play an important role in mediating stress-induced increases in renin and corticosterone secretion. (Supported by Sigma Xi and the Bane Charitable Trust).

359.2 REPEATED STRESS INCREASES ANGIOTENSIN II RECEPTOR NUMBER IN RAT PARAVENTRICULAR NUCLEUS AND SUBFORNICAL ORGAN. E. Castrén* and J.M Saavedra. LCS, NIMH, Bethesda MD 20892.

Angiotensin II (ANG) is a circulating neurohormone, which has a modulatory role in the regulation of sympathetic activity and pituitary hormone release. There are ANG receptors in the subfornical organ (SFO), in the hypothalamic paraventricular nucleus (PVN), and in the anterior pituitary.

PVN is a key site of the central regulation for the stress response. It regulates the sympathetic activity by direct connections to intermediolateral nucleus of the spinal cord. CRF-containing cells in the parvocellular PVN regulate the ACTH release from the anterior pituitary. PVN receives direct input from SFO, an organ which is partially outside the blood brain barrier and mediates information from circulating hormones to central nervous system.

Plasma renin activity, which reflects ANG plasma levels, is elevated during repeated stress suggesting that peripheral renin-angiotensin system is activated. We have studied the effect of repeated immobilization stress on ANG receptors in PVN, SFO and anterior pituitary by quantitative receptor autoradiography using ^{125}I-Sar^1-ANG as a ligand.

Immobilization (two hours per day for ten days) significantly increased the ANG receptor concentration in PVN and SFO without any significant effect on binding affinity. The B_{max} values (fmol/mg protein, mean±SEM) were 105±11 and 63±7 ($P<0.01$) in PVN ($n=7$), and 311±14 and 230±9 ($P<0.01$) in SFO ($n=6$) for stressed rats and controls, respectively. Repeated stress did not alter the ANG receptor number in the anterior pituitary.

The increase in ANG receptor concentration in SFO may represent an upregulation by elevated plasma ANG levels during the stress. This kind of upregulation has been described after chronic dehydration in SFO and zona glomerulosa of the adrenal gland. PVN, on the other hand, is inside the blood brain barrier and thus not affected by circulating ANG. The increased ANG receptor number in this nucleus suggests an important role for putative central renin-angiotensin system in the regulation of the stress response.

359.3 REGULATION OF TYPE I AND TYPE II GLUCOCORTICOID RECEPTORS IN RAT HIPPOCAMPUS BY STEROID AND PEPTIDE FACTORS. R.E. Brinton, L. MacIsaac* and B.S. McEwen. Laboratory of Neuroendocrinology, Rockefeller University, New York, NY 10021.

Because the hippocampus is implicated in cognitive and affective processes and is also a target for glucocorticoid action, we explored factors which regulate glucocorticoid receptors in the hippocampus (HC). Previous work demonstrated an influence of high levels of corticosterone (CORT) and of ACTH and vasopressin (AVP) on glucocorticoid receptor (GR) binding but did not distinguish between receptor subtypes (for review see McEwen, DeKloet and Rostene, Phyiol. Rev., 1986). In this study we utilized both adrenal steroids and AVP in an effort to observe the dynamic adaptive capability of both GR receptor types. To explore the specificity of regulatory factors, male Sprague Dawley rats were either adrenalectomized (ADX) or hypophysectomized (HX), or both. At the time of ADX or sham surgery, rats were treated with low to moderate levels of regulatory factors by subcutaneous Alzet minipumps for 3d. Cytosol GR were assayed in the presence of sodium molybdate with 3H-dexamethasone (DEX) as ligand and 2.5 uM CORT to define nonspecific binding. Type II receptors were distinguished from Type I by means of the specific type II receptor agonist, Ru 26988 (0.5 uM). Separation of bound from free 3H-DEX was accomplished by Sephadex LH-20 gel filtration. In ADX rats, a low dose of CORT (1 ug/h) produced in 6h a marked reduction in Type I GR receptors. This reduction was even greater when CORT (1ug/h) was delivered for 72h, but a significant recovery of binding occurred 6h after ceasing the CORT treatment. The depression and recovery of Type I binding at 6h indicates a rapid and sensitive regulation of Type I receptors in the HC which may reflect nuclear translocation or inactivation of binding. Type II receptors were down-regulated following 72h of 1 ug/h CORT. However, the Type II receptors did not show the rapid regulation observed for the Type I receptors at 6h. The regulation of glucocorticoid receptors by 1 ug/h CORT was unique for the HC and was not observed in the other brain regions examined. Administration of 1 ug/h aldosterone (ALDO) for 72h depressed binding to Type I but not Type II receptors in HX rats. HX elevated Type II receptors, whereas Type I binding increased only when the HX rats were ADX. The elevation of Type I receptors following 3d ADX was prevented by 1 ug/h ALDO. These data indicate that low levels of CORT and ALDO can have a pronounced and selective influence upon availability of GR binding sites in the HC. In vivo treatment with either 0.33-1 ug/h AVP reduced Type II receptor binding in the HC and reduced the apparent Kd of the Type I receptor. From these data AVP appears to have a regulatory influence upon both types of the GR in vivo.
(Supported by NIMH Grant MH41256).

359.4 SHORT-TERM CHANGES IN GLUCOCORTICOID RECEPTOR LEVELS IN THE RAT HIPPOCAMPUS. R.L. Spencer* and B.S. McEwen. (SPON: C. Fischette). Lab. of Neuroendocrinology, Rockefeller Univ., New York, NY 10021.

Levels of the glucocorticoid receptor (GR) in the rat hippocampus (HC) vary under certain conditions such as adrenalectomy, corticosterone (CORT) treatment, stress, and aging. To study the response of the glucocorticoid system to stress and chronic alcohol consumption, we are further characterizing the binding properties and activation process of GR in the rat brain. Two distinct populations of glucocorticoid binding sites are found in HC cytosol. One, the Type I or corticosteroid receptor (CR), shows a relative binding affinity of CORT,aldosterone (ALDO) > dexamethasome (DEX), and the second site, the Type II or glucocorticoid receptor (GR), has a relative binding affinity of DEX>CORT>ALDO. Rats were adrenalectomized (ADX) prior to the binding assay in order to eliminate endogenous hormone. However, allowing only 16 hr of ADX to clear endogenous CORT may still result in some upregulation of receptor level. To examine this we compared the binding of ^{3}H-DEX (1-20 nM) to HC cytosol from 16 hr ADX or no ADX male Spraque-Dawley rats. Binding in the presence or absence of the pure GR agonist, RU26988 (.5 μM), was used to differentiate GR from CR binding, and CORT (2.5 μM) was used to assess non-specific binding. Using Scatchard analysis, the GR receptor for the 16 hr ADX rats had a Bmax of 329 fmoles/mg protein and a Kd of .73 nM; the CR receptor had a Bmax of 144 fmoles/ mg protein and a Kd of 2.0 nM. The cytosol from the non-ADX rats was divided into two equal portions. One portion was first passed through Sephadex LH-20 columns before incubation with ^{3}H-DEX in an effort to remove endogenous free CORT present in the cytosol. The Bmax for the GR receptor from the non-ADX rats was 50% of that measured in ADX cytosol. The two column step doubled apparent affinity of ^{3}H-DEX binding but did not change Bmax. There was no measurable CR binding in the non-ADX rats. To further assess if the different level of receptors present in ADX and non-ADX cytosol reflects a shuttling of receptor between cytosolic and nuclear compartments and/or a short-term upregulation of cytosolic receptor levels, we attempted to extract receptor from isolated nuclei and measure levels with our binding assay. Although we were able to measure the hippocampal nuclear uptake of an in vivo injection of ^{3}H-CORT, we were not able to measure with our in vitro binding assay specific ^{3}H-CORT or ^{3}H-DEX binding in protein extracts from HC cell isolated nuclei. Whether or not this indicates that the native receptor is unable to rebind steroid after entering into the nucleus or that the binding capability of the receptor is disrupted as a consequence of our isolation procedure is still under investigation. Supported by MH41256 and AA05256.

359.5 A METHODOLOGICAL IMPROVEMENT FOR STUDYING CHRONIC STRESS IN RATS. D.L. Pitman*, A.M. Schilling*, T. Pritzel*, C. Bashford*, J.E. Ottenweller* and B.H. Natelson. VA Med. Ctr. & Dept. of Neurosciences, New Jersey Medical School, E. Orange, NJ 07019.

We have developed a new method for studying chronic stress which avoids many earlier methodological problems. When animals can alter the intensity, duration, or frequency of shock in the chronic stress setting, the stress response is usually affected. Grid shock may be modified by postural changes or reduced by animals' excreta. Tail shock can be interrupted if the electrode slips. Additionally, rats instrumented with tail electrodes can gnaw at the apparatus; this is a coping mechanism which is known to further influence results. Additionally, investigators need to disturb animals in order to apply the electrodes. This in itself may act as a signal that stress sessions are to begin.

In our paradigm, animals live in the experimental apparatus and thus do not have to be disturbed for blood sampling or stressor administration. A leather belt (.5 in wide x 7" long) is fastened securely between the rat's hips and rib cage. The animal is placed on all fours in a wooden archway and secured to it by a loop sewed in the leather belt. The archway/belt with the animal is secured to a wire mesh tray and placed in an individually ventilated, light-tight, sound attenuated cubicle. For our on going work, each chamber has been provided with cue lights for Pavlovian conditioning.

Shock is delivered via subcutaneous flank electrodes which are implanted at the same time animals are initially placed into the apparatus. Blood is sampled via indwelling femoral artery catheters which are exteriorized out of each individual cubicle; this allows us to sample the animal without disturbing it in any way. Rats' activity is assessed by counting contact closures everytime the animal moves in the archway. With this set-up, rats can be given any pattern of unmodifiable signals/shock, can have blood samples taken, and activity monitored -- without disturbing the animal at all.

After placement in the apparatus, rats initially struggle but within 30 min become quiet. Body weight falls to approximately 90% of original weight and then stabilizes for the 2 week duration of our experiments. Both food and water intake show a similar pattern with reduced intake for the first two days and then a gradual increase over time. After one day of placement in the apparatus, plasma levels of corticosterone are elevated slightly (10-12 ug/dl) but fall by day 2 to levels comparable to a.m. levels found in free-ranging undisturbed rats (4-6 ug/dl).
Supported by VA medical research funds

359.6 STRESS-INDUCED CORTICOSTERONE SECRETION IN NEONATAL RATS:POSSIBLE NEURAL MEDIATORS. S. Clark*, S. Pachman*, Scott Lurie* S. Schanberg*, C. Kuhn. Duke University Medical Center, Durham, N.C. 27710

Although it has been suggested that the neonatal HPA axis is unresponsive to stress for up to 10 days, we have observed marked increases in corticosterone (CS) following exposure to either ether or maternal deprivation stress in rat pups as young as day 5 using a sensitive RIA for CS. To investigate possible neural mediators of these stress responses in rat pups, CS secretion evoked by several stimuli which increase CS in adult rats was compared in 10-day old and adult rats. Morphine (5 mg/kg), the cholinesterase inhibitor physostigmine (0.5 mg/kg), the alpha antagonists phenoxybenzamine (10 mg/kg) and yohimbine (1 mg/kg), and the dopamine-beta hydroxylase inhibitor diethyldithiocarbamate (400 mg/kg) were given to adult and neonatal rats and serum CS assayed by RIA. Each of these agents stimulated CS secretion in neonates from two- to four fold, although basal secretion in pups was markedly lower than that observed in adults, as previously reported. The degree to which each stimulus was suppressed by low dose (0.025 ug/kg) dexamethasone (DEX) also was similar in neonates and adults: the low dose of DEX suppressed responses to morphine and ether stress effectively, while stimulation produced by adrenergic blockade escaped from DEX suppression in both neonates and adults. A developmental difference in DEX suppression of physostigmine-induced CS secretion was observed at this dose of DEX, with suppression observed in pups, but escape in adults.

These findings suggest that many neural stimuli which alter CS secretion in the adult also are effective in neonates, despite the lower basal rate of secretion and the somewhat blunted responses. Tonic noradrenergic inhibition seems to play a dominant role in HPA regulation early in neonatal life. The inhibition of these responses by a low dose of DEX implicates the CRF neuron as the prime mediator of this response. This study also suggests that CS hypersecretion might prove to be a useful endocrine index for studying stress-induced behavioral disruption in developing animals, a possibility which has potential implications for investigation of endocrine correlates of childhood psychiatric disorders.
Supported by grant nos. DA 02739 and MH 13688

359.7 SENSITIZATION OF NEUROENDOCRINE RESPONSES TO REPEATED STRESS IN RATS. T.E. Orr*, B.N. Bunnell, E.H. Mougey* and J.L. Meyerhoff (SPON: L.J. Peacock). Dept. Psychology, Univ. of Georgia, Athens, GA 30602 and Dept. Med. Neurosciences, Walter Reed Army Inst. of Research, Washington, DC 20307.

Neuroendocrine responses to acute stress have been studied extensively while responses to chronic or repeated stressors have received less attention. Most reports from the latter studies suggest that responses are attenuated following a prolonged period of exposure to stressors. Studies from our laboratories have indicated that, in response to severe daily stressors, the neuroendocrine responses do not necessarily decrease in a linear fashion (Bunnell, B.N., et al., Neurosci. Abst., 10:95, 1984). Instead, there appears to be a period during which the responses are sensitized to some stressors. The present study sought to examine this sensitization in more detail by measuring the adrenal cortical responses to several stressors following 1, 2, 3, 4, and 5 days of exposure.

Two groups of 30 rats were placed in restraint cages and given daily 15 min exposure to intermittent 5 sec, .017 watt tailshock delivered on a 60 sec variable time schedule. One of these groups was not subjected to the two weeks of preexperimental handling that was given to all the other groups in the experiment. Another group of 30 rats was restrained in the cages, but not shocked, while a fourth group received 15 min of total immobilization each day. The last group of 30 served as home cage controls. Six rats from each group were killed each day for five days following exposure to the stressors. Trunk blood was collected in heparinized tubes and stored at -18°C until radioimmunoassay for corticosterone.

Plasma corticosterone was elevated in all four stress groups across all five days relative to home cage controls. Sensitization occurred on the second day in both the handled and nonhandled rats that received tailshock and persisted throughout the remainder of the experiment. A smaller rise in sensitivity occurred later in the restraint group, but no sensitization was observed with total immobilization.

Corticosterone ug/100ml (SEM)

Day:	1	2	3	4	5
Shock (unhandled)	27.5(2.4)	36.0(2.8)	34.9(1.7)	38.6(2.)	35.6(2.6)
Shock (handled)	21.6(2.0)	30.3(1.7)	29.2(0.5)	31.9(1.8)	31.9(2.1)
Restraint	20.3(1.6)	17.1(0.6)	25.1(2.5)	28.3(2.0)	28.7(3.0)
Immob.	24.3(2.1)	24.7(2.2)	24.6(3.7)	28.2(3.8)	25.9(3.8)
Home Cage	11.0(2.1)	8.4(2.3)	4.8(0.7)	8.1(2.0)	4.3(0.8)

359.8 HORMONAL AND BEHAVIORAL ALTERATIONS PRODUCED BY PRENATAL STRESS CONTROLLABILITY IN THE RAT. L.K. Takahashi,* C.M. Barksdale,* N.H. Kalin,* and J.A. Vanden Burgt* (SPON: M. Carnes). Dept. of Psychiatry, Univ. of Wisconsin-Madison, and Psychiatry Service, Wm. S. Middleton Veterans Hospital, Madison, WI 53705.

Female Sprague-Dawley rats were exposed to escapable shock (E), inescapable yoked shock (Y), or no-shock (C) treatments during the course of pregnancy to determine the effects of controllable and uncontrollable prenatal stress on stress-induced behavioral and hormonal responses in the offspring. Tail shocks (0.5 mA, 80 shocks per session) were given every three days throughout pregnancy in a wheel-turn box that allowed group E animals to terminate shock by spinning the wheel. Y animals received uncontrollable shocks using the same parameters as for E animals. C animals were simply restrained in the apparatus.

At 14 days of age, male-female littermates were examined under one of four conditions: separation (SP), separation control (SPC), shock (SH), and shock control (SHC). SP tests consisted of separating pups from their litter and placing them individually in a styrofoam cup for 10 min, after which they were decapitated and plasma was collected for ACTH determination. Pups in the SPC groups were decapitated immediately after removal from the litter. SH tests examined the effects of grid shock (five 1.0-s shocks, 0.5 mA, one shock per minute) on the development of analgesia using a tail-flick test. SHC conditions were identical except that shock was not delivered. Pups were decapitated for plasma collection immediately after analgesia assessment in SH and SHC tests.

The major findings were (i) SP produced a significant elevation in ACTH values (96.1 pg/ml) relative to SPC (44.2 pg/ml) treatment ($p < .001$); (ii) female pups had significantly higher ACTH values (179.2 pg/ml) than males (128.4 pg/ml) after SH treatment ($p < .01$); (iii) ACTH levels were significantly higher in pups from Y mothers (121.2 pg/ml) than in E (91.8 pg/ml) and C (98.0 pg/ml) pups ($p < .05$); and (iv) C pups exhibited significantly longer tail-flick latency scores (2.2 s) than E (1.5 s) and Y (1.2 s) pups after SH treatment ($p < .05$). These results indicate that stress-induced analgesic responsivity in offspring is diminished by both controllable and uncontrollable prenatal stress. That only pregnant rats lacking control over prenatal stress produce young with elevated ACTH levels suggests that maternal controllability ameliorates the effects of prenatal stressors on levels of stress-related hormones in the offspring.

359.9 THE NEUROPHARMACOLOGY OF SEPARATION-INDUCED DISTRESS IN NONHUMAN PRIMATES. N.H. Kalin,* S.E. Shelton,* and C.M. Barksdale* (SPON: B. Eichelman). Dept. of Psychiatry, Univ. of Wisconsin-Madison 53792, and Wm. S. Middleton Veterans Hospital, Madison, WI 53705.

Disruption of the primate mother-infant attachment bond is a naturally occurring stressor that results in marked behavioral, physiological, and endocrine activation. We studied the effects of alterations of benzodiazepine, opiate, and catecholamine systems on the behavioral and endocrine response of infant rhesus monkeys briefly separated from their mothers.

The benzodiazepine agonist diazepam (1.0 mg/kg) significantly decreased distress vocalizations and increased social behavior and activity in monkeys undergoing separation. This dose of diazepam also significantly reduced the separation-induced increase in pituitary-adrenal activity. The benzodiazepine antagonist Ro 15-1788 (10 mg/kg) significantly blocked diazepam's effect on activity and pituitary-adrenal hormones but did not affect diazepam's reduction of distress vocalizations.

The opiate agonist morphine (1.0 mg/kg) significantly reduced separation-induced distress vocalizations without affecting activity levels. When administered alone, the opiate antagonist naloxone (10 mg/kg) significantly increased distress vocalizations. A lower dose of naloxone, 0.1 mg/kg, had no intrinsic effects of its own but it blocked morphine's effect on reducing separation-induced vocalizations. When administered alone, this dose of naloxone significantly enhanced separation-induced increases in ACTH; with morphine, it blocked morphine's reduction of ACTH.

The α-2 agonist clonidine was administered in a wide dosage range (1, 10, 33, 67, and 100 µg/kg). The 1- and 10-µg/kg dosages had no effect on any of the separation-induced behaviors. Significant reductions in activity levels, locomotion, and distress vocalizations occurred with doses of 33, 67, and 100 µg/kg. That we could not find a dose that selectively reduced distress vocalizations without reducing activity levels suggests that the effects of clonidine on separation-induced vocalizations are secondary to sedation. We found that 0.1, 1.0, and 5.0 mg/kg of the β-adrenergic antagonist propanolol did not significantly affect any behavioral aspect of the separation response, but 20 mg/kg resulted in increased distress vocalizations associated with a significant decrease in activity.

Taken together, our results suggest that endogenous benzodiazepine and opiate systems play a role in modulating the separation response in infant primates.

359.10 DISSOCIATION OF TWO ASPECTS OF SEPERATION DISTRESS BY CHOLECYSTOKININ OCTAPEPTIDE. A. Weller* and E.M. Blass. Dept. of Psychology, Johns Hopkins Univ., Baltimore, MD 21218.

Acutely isolated rat pups emit ultrasonic distress vocalizations (DV) and display 'stress-induced analgesia' (SIA) as measured by a hot-plate paw-lift response. This study explored the influence of opioids and cholecystokinin (CCK) upon these responses to stress. Morphine (0.25 mg/kg i.p.) reduced DV and increased pain threshold. Exogenous sulfated CCK (1 & 2 ug/kg i.p.) selectively reduced the number of DVs, yet did not affect pain responsivity. This selectivity may imply seperate physiological pathways mediating behaviors during short-term seperation.

Opioid-CCK interactions were also examined. CCK did not antagonize the effects of morphine on either measure. On the contrary, subthreshold doses of morphine (0.125 mg/kg) and CCK (1 ug/kg as a second injection), while ineffective when injected with saline, were highly effective in reducing DV when injected in combination. CCK also strongly prevented the antagonistic effects of naltrexone on DV modified by endogenous opioids and morphine. Thus, opioid mechanisms seem to mediate both DV and SIA, while exogenous CCK selectively influences the vocalization component of the response to isolation stress.

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359.11 THE RELATIONSHIP OF HORMONAL AND IMMUNOLOGICAL RESPONSES TO STRESS IN TYPE A BEHAVIOR. K.S. BLASDELL*, P. MILLS*, R.K. WALLACE, W.L. VAN ZANDT, R. SCHNEIDER*, K. WALTON, D. HILL*. Department of Physiological and Biological Sciences, Maharishi International University, Fairfield, IA, 52556.

The multidirectional communication between the nervous system, the neuroendocrine system and the immune sytem suggests that there may be differences in the hormonal and immunological responses to stress based on behavioral type. We recently reported that the proliferation response of lymphocytes to the mitogen phytohemagglutinen (PHA) showed highly variable individual response patterns during the experimental conditions of mental stress or rest. When these same subjects were divided into Type A and Type B groups, however, a significant pattern of response emerged. While Type A behavior has been associated with hyper-responsiveness of the cardiovascular system, the lymphocytes of Type A individuals appear to react and recover more slowly from stress than the lymphocytes of Type B individuals. Further, long-term practice of Transcendental Meditation technique appeared to modify the hormonal and immunological responses of Type A meditators in the direction of Type B subjects.

The present study is an extension of the previous work with the intention of exploring hormonal correlates which might influence these patterns. Serial blood samples were drawn from 23 right-handed males before and after two sessions of mental arithmetic separated by rest periods. The subjects were independently rated on the Structured Interview for Type A behavior. The immune parameters measured included PHA stimulation, total WBC and differential cell counts. Simultaneously, plasma levels of prolactin, norepinephrine, epinephrine and luteinizing hormone were measured.

ANOVA with repeated measures showed significant differences between Type A and Type B individuals across the experimental conditions on PHA response, WBC count, prolactin and epinephrine ($p = .01; .02; .0001; .01$, respectively). Further, Type A individuals showed a highly significant correlation ($p < .001$) between baseline PHA responses and the K_D for binding of [^{125}I] Iodocyanopindolol (ICYP) to the lymphocyte receptors. The PHA proliferation rate of Type As also displayed significant correlations with the levels of several hormones while Type Bs showed no consistent relationships on any of these measures.

These data indicate that behavioral type can affect the mitogenic responsiveness of lymphocytes. Furthermore, the relationship between mitogen response, neuroendocrine measures and acute stress is also dependent on behavioral type.

359.12 CHANGES IN HERPESVIRUS LATENCY IN A STRESSED POPULATION: IMPLICATIONS FOR PSYCHOLOGICAL MEDIATION OF IMMUNE RESPONSES. S. Kennedy*, J. Kiecolt-Glaser*, S. Malkoff*, L. Fisher*, C. Speicher* and R. Glaser*. (SPON: M. Torello). College of Medicine, The Ohio State University, Columbus, OH 43210.

It is becoming apparent that the immune system does not function in isolation, but instead is influenced by neural, endocrine and psychological factors, such as environmental stressors. Although the animal literature is replete with studies documenting the immunosuppressive effects of stress, there is relatively little empirical data to support these effects in humans.

The present study examined the effects of a psychological stressor, marital disruption, on cellular immune competence in 32 recently separated/divorced (S/D) males and 32 sociodemographically matched controls. Cellular immune competence was assessed by measuring plasma antibody levels (titers) to herpesviruses; typically, when the immune system is suppressed (during certain illnesses or in cases of immune disorders, for example), antibody titers are elevated, indicating virus reactivation.

Antibody titers to two herpesviruses, Epstein-Barr Virus (EBV) Viral Capsid Antigen (VCA) and Herpes Simplex Virus-Type 1 (HSV-1) were significantly elevated in the S/D group relative to controls, suggesting reactivation of latent virus as a result of a depressed cellular immune response. Serum albumin levels were comparable for both groups, ruling out the possibility that the observed differences in antibody titer were due to differences in diet or in nutritional status. In addition, self-report data indicated significantly greater depression, anxiety and loneliness (as measured by the Brief Symptom Inventory and the UCLA Loneliness Scale) in the S/D subjects.

These data suggest that psychological stressors may have negative consequences in terms of immune function, and are consistent with epidemiological data linking environmental stressors to changes in health status. Future studies will more clearly elucidate the role of neural and endocrine mechanisms involved in the immunosuppressive effects of stress.

359.13 SALIVARY CORTISOL LEVELS FOLLOWING APLLICATION OF CORTICOTROPIN RELEASING FACTOR (CRF) AND PSYCHOLOGICAL STIMULI IN HEALTHY MEN H. Lehnert, J. Beyer*, I. Gutberlet*, D.H. Hellhammer, S. Marrè*, P. Walger*, H. Vetter*. Dept. of Endocrinology, 2. Medizinische Klinik, 65 Mainz, Dept. of Physiological Psychology, University of Trier, 55 Trier and Medizinische Universitätspoliklinik, 44 Münster, FRG

The effects of CRF on salivary cortisol levels were studied in healthy young men with and without simultaneous administration of stressful stimuli. This study was performed in order to analyse the sequelae of different stressors and to study functional aspects of the hypothalamic-pituitary-adrenal feed-back system by comparison of basal, stress- and CRF-evoked cortisol concentrations.

12 healthy young male volunteers were exposed to four different situations on four consecutive days. In situation one they received a bolus of saline, in situation two 100 µg h-CRF (both single-blind). In situation three they had to perform mental arithmetics under noise distraction receiving an additional bolus of h-CRF at the end of the stress procedure (after 30 minutes). In situation four they underwent a stressful situation only and had to speak about their private and career goals in front of a sham-recording video camera (they were told that the recording was shown to speech analysts for evaluation). Blood pressure, heart rate and salivary cortisol samples were obtained every 15 minutes (and every 30 minutes during the last hour) over a course of 150 minutes. Mood and anxiety questionnaires were given before and following the CRF and stress application, respectively. Salivary cortisol was determined by a luminescence immunoassay.

Following CRF administration, salivary cortisol concentration reached a maximum after 30 minutes (from 26,2 to 44,8 nmol/l; $p < 0,015$). A similar increase was observed in situation three (by 72%), where CRF was administered following the stress. In no case had the stressful procedure any significant effect on salivary cortisol, we also did not observe an additive effect of stress and CRF. Surprisingly however, a marked and highly significant increase in salivary cortisol levels was found immediately before the beginning of the respective task, thus very likely reflecting anticipation of the task. These anticipatory increases ranged from 30 to 110% ($p < 0,005$). The integrity of the pituitary-adrenal feed-back system was demonstrated by a strong negative correlation between baseline and subsequent cortisol concentrations.

In summary, our data strongly support the salivary cortisol determination as a means for testing the pituitary-adrenal axis (e.g. by CRF application) and further demonstrate that increases in (salivary) cortisol might rather reflect anticipation of a stress than the stressful situation itself.

359.14 EFFECTS OF EXERCISE ON PLASMA ß-ENDORPHIN-LIKE IMMUNOREACTIVITY IN HUMAN. D.F. Richards* and C.A. Cahill. University of Maryland, Sch. of Nsg. Baltimore, MD. 21201.

The effects of physical stress on plasma ß-Endorphin-like immunoreactivity (ß-END-1-i) has been documented in animals and to a lesser extent in man. Presumably, stressors activate the hypothalamic/pituitary/adrenal axis to secrete ß-END-1-i into plasma. In the human, the study of this stress effect is handicapped by a number of factors. Ethical considerations limit the selection of the stressor to natural events which are thought to be stressful such medically necessary surgery. However, the degree to which these life events are stressful may vary between individuals. Therefore, the purpose of this study was to explore the effects of a standardized stressor on plasma ß-END-1-i . Twenty women were asked to walk or run on a treadmill at a rate sufficient to increase their heart rate to 75% of the calculated maximal level (75% of 220 minus age in years) and to maintain that rate for twenty minutes. Blood was collected by acute venipuncture before exercise began and at the end of twenty minutes.

Three distinct patterns of ß-END-like changes were observed. 1. In five cases, plasma levels pre-exercise were ten fold higher than those observed in the other subjects and did not change with exercise. In all of these cases more than one attempt to obtain the plasma sample was necessary. Repeated attempts to cannulate the vein may have caused ß-END-1-i to be released. Therefore, these findings were viewed as artifact and the data for these cases were not included in the statistical analysis. 2. In three cases no change in plasma levels were detected. This pattern suggests that for these individuals, the physical stressor was insufficient to activate the hypothalamic/pituitary/adrenal/axis. 3. In the majority of cases plasma levels of ß-END-1-i increase by 50% to 100% in pre and post-exercise samples. A paired t-test to compare pre and post exercise levels was carried out on the remaining 15 subjects and a statistically significant ($t=2.42$, $d.f.=28$, $p < .01$) difference in pre and post exercise levels was observed. Therefore, physical exercise sufficient to increase heart rate to 75% of the calculated capacity activates the hypothalamic/pituitary/adrenal axis and may be a useful tool in future studies of the effects of stress in the human.

359.15 COMPARISON OF TWO ANTISERA FOR MEASURING β-LPH IN HUMAN PLASMA. E.H. Mougey*, L.M. Lambe*, M.A. Oleshansky and J.L. Meyerhoff. (Spon: C.B.G. Campbell). Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307-5100.

Various psychological and physical stressors have been shown to elicit increases in plasma levels of hormones secreted from the the pituitary (prolactin, GH, ACTH, β-EP and β-LPH). We have previously reported increases in levels of β-EP and β-LPH in plasma samples from military volunteers during oral exams. These plasma samples were assayed by RIA using a commercial kit for β-EP (NEN Corp) following an extraction procedure which separates and concentrates these peptides from 1 ml of plasma. The antibody (Ab) in this kit cross-reacts completely with β-LPH.

To verify the validity and specificity of the β-LPH assay used above we also set up an RIA method using a β-LPH Ab obtained from C.H. Li. Human β-LPH obtained from Peninsula Labs was iodinated and purified on Sephadex G-50. The standards (10-200 pg β-LPH) and samples were added in a total volume of 200 ul. After addition of trace solution and first Ab the tubes were incubated for 3 days at 4°C. Second Ab (overnight at 4°C) was used to separate free from bound antigen. Blood samples were obtained from soldiers who were appearing before a "Soldier of the Month" board. These boards are rigorous, formal interviews conducted by a group of higher-ranking non-commissioned officers. Samples were collected at two early time periods on the day of the board (X,Y), at 20 min prior to entering the interview room (A), 5 min after beginning the interview (B), 20 min after beginning the interview (C) and at 5 min (D) and 20 min (E) after leaving the interview room. Plasmas were treated with aprotinin and stored at -70°C until assayed.

Plasma levels of β-LPH remained constant at the X,Y and A time points, showed a marked increase (50%) at B, remained elevated at C and returned to pre-interview levels at time points D and E. Comparison of β-LPH levels obtained after direct assay of 100 ul of plasma vs assay of extracts of 1 ml of plasma showed that the specificity and sensitivity of the β-LPH Ab is not sufficient to allow direct assay of human plasma samples. Furthermore, the Ab exhibited considerable cross-reaction with β-EP. However, the addition of 500 pg of β-EP to a plasma pool was not detectable in the β-LPH fraction following extraction. The plasmas from seven subjects were extracted and assayed with the β-LPH Ab and compared with values obtained with the β-EP Ab. All values were corrected for recovery. At time points X,Y,A,D and E the mean values for β-LPH by the two methods were comparable. At time points B and C values obtained with the β-EP Ab were slightly higher (7-14%) reflecting some contamination of the β-LPH fraction with β-EP. We concluded that the β-LPH Ab is satisfactory for the assay of human plasma provided that an extraction resulting in concentration of the β-LPH and separation from β-EP is performed. The β-EP Ab is also useful for measuring β-LPH in extracted samples except when high levels of β-EP are encountered.

359.16 STRESS-INDUCED ALTERATIONS OF GAMMA-MELANOTROPIN IN THE RAT PITUITARY. J.H. Meador-Woodruff*, E.A. Young*, N. Ling and H. Akil (Spon: R. Bradley). Mental Health Research Institute and Department of Psychiatry, University of Michigan Medical Center, Ann Arbor, MI, 48109-0720.

Gamma-melanotropin (γ_3MSH) is a 25 residue peptide in the N-terminal domain of the β-endorphin (BE)/ACTH precursor proopiomelanocortin (POMC), capable of potentiating the steroidogenic effect of ACTH on the adrenal cortex. It exists in rat pituitary as a 5K form (fully processed, glycosylated γ_3MSH) and an 11K form (γ_3MSH extended with the extreme N-terminal fragment of POMC). Gamma-MSH has been previously shown to be co-released with BE and ACTH following acute stress or treatment with secretagogues such as CRF. As this peptide evidently has a modulating role in the pituitary-adrenal axis, we examined the effect of chronic stress on pituitary and plasma forms of γ_3MSH in the rat. Male Sprague-Dawley rats were subjected to daily morning sessions of 30 minutes of swimming in 30°C water for 8 or 14 days. 24 hours following the last session, the rats were sacrificed by decapitation. Pituitaries were rapidly removed and dissected into anterior (AL) and intermediate (IL) lobes. Trunk blood was collected in EDTA-containing tubes and plasma was separated and collected. Both pituitaries and plasma were quickly frozen on dry ice and stored at -80°C. Plasma was processed using Sep-Pak C_{18} cartridges, and tissue by acetone/HCl extraction. Aliquots of these materials were subjected to Sephadex G-50 column chromatography, and aliquots of each fraction as well as from the crude extracts were assayed for γ_3MSH-IR by a specific RIA we have developed. After 8 or 14 days of chronic swim stress, the total content of γ_3MSH-IR in IL increased by 150-200%. Column chromatography revealed that unstressed animals had predominantly 5K γ_3MSH-IR in the IL, and stressed animals had elevations of both 5K and 11K forms; the induction seen in stressed animals was attributable primarily to an increase of the 11K fraction. For comparison, the BE content in IL shifted from N-acetyl BE 1-27 to N-acetyl BE 1-31 with this same stress. The γ_3MSH-IR in AL following chronic stress was minimally elevated; only 11K and larger forms were observed in both unstressed and stressed groups, and no processing shift was appreciated. The plasma levels of γ_3MSH-IR were identical between the two groups, existing as both 5K and 11K forms, with the 5K fraction predominating in both groups. Chronic swim stress thus appears to selectively induce γ_3MSH production in the IL, without a concomitant alteration in AL stores. Further, chronic swim stress followed by a 24 hour recovery period does not alter circulating levels or forms of γ_3MSH-IR. The possibility that an acute stress following this chronic stress paradigm has manifestations in plasma or the IL is currently under investigation.

359.17 STRESS INCREASES PLASMA CYCLIC AMP LEVELS IN GOLDEN HAMSTERS. K.M. Levy, B.N. Bunnell, E.H. Mougey*, M.A. Oleshansky and J.L. Meyerhoff. Dept. Psychology, U. Georgia, Athens, GA 30602 and Dept. Med. Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

Cyclic AMP is a second messenger which mediates the cellular response to several neurohumors. Plasma levels of cyclic AMP have been reported to increase in response to stress in humans as well as in rats. As it may prove a useful indicator of physiological response to stress, we measured the plasma cyclic AMP response to stress in hamsters. Since catecholamines (which might stimulate plasma cyclic AMP increases) are increased many fold by decapitation, we took blood samples via chronically implanted jugular catheters.

Although the older literature indicates that cortisol is the primary glucocorticoid secreted by hamsters, more recent data show that their adrenal cortices secrete measurable amounts of both cortisol and corticosterone, and that acute restraint stress produces elevations in both steroids (Ottenweller, J.E., et al., Life Sciences, 37:1551, 1985). In order to compare the plasma cyclic AMP response with more established stress indicators, we also measured plasma cortisol and corticosterone responses to acute footshock stress.

Twelve adult male golden hamsters were implanted with jugular catheters under nembutal anesthesia. Following a 72 hour recovery period, 6 animals were subjected for 20 min to intermittent 4 sec, 1 mA footshock delivered on a 60 sec variable time schedule in sound attenuated shock chambers. The remaining animals served as home cage controls. Immediately following exposure to footshock, the hamsters were returned to their home cages and 3 ml of blood was drawn from each animal. All experimental procedures were completed during the first two hours of the light phase of the daily 12:12 LD cycle to minimize circadian variation. After centrifugation of heparinized blood, plasma for cyclic AMP assay was incubated for 10 min with NaAc buffer at 90°C to inactivate phosphodiesterase. Samples were stored at -18°C until assayed by RIA.

		Footshock	Control
cAMP	(pm/ml ± SEM)	68.1 ± 8.1	33.3 ± 9.9
Cortisol	(ug% ± SEM)	6.9 ± 0.6	1.4 ± 0.5
Corticosterone	(ug% ± SEM)	4.8 ± 0.7	1.8 ± 0.4

Footshock doubled plasma levels of cyclic AMP and increased plasma levels of both steroids as well. It will be important to identify the tissue source of the increase in plasma cyclic AMP. Numerous studies have shown that stress increases cyclic AMP in the anterior pituitary, but the increase in plasma cyclic AMP may reflect increases in other tissues, possibly mediated via enhanced sympathetic nervous system activity. Plasma cyclic AMP levels may prove a useful indicator of the physiological response to stress in hamsters.

359.18 PSYCHOLOGICAL STRESS INCREASES HEART RATE AND PLASMA LEVELS OF CYCLIC AMP IN MAN. J.L. Meyerhoff, M.A. Oleshansky, E.H. Mougey*, C.B. Wormley*, H.R. Smith*, L.K. Wittig*, T. Maxwell-Irving* and R.C. Perez*. Neurochemistry & Neuroendocrinology Branch, Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

We have previously reported that physical exertion produces increases in plasma levels of catecholamines and cyclic AMP. The present study was undertaken to determine whether cyclic AMP would also rise during the purely psychological challenge of a competitive oral examination. The oral exam is a rite of passage in several professions. As part of their training and preparation for promotion boards, soldiers are encouraged to appear before "Soldier of the Month" boards. These are contests held monthly to select and honor an outstanding soldier based on rigorous competitive interviews conducted by a panel composed of higher-ranking non-commissioned officers. The winner is selected on the basis of knowledge of job-related subject matter, personal appearance and quality of oral presentation. Twelve contestants volunteered to be studied during these competitions and gave written informed consent for heart rate monitoring and blood sampling during the 30 min interview. Heart rate was monitored using a PMS-8 portable physiological monitoring system (Vitalog Corp., Redwood City, CA). An i.v. needle was inserted in the median cubital vein and blood was collected through non-thrombogenic tubing attached to a Cormed pump (Medina, NY) which withdrew blood continuously at 0.1 ml/min between samples and at 5.0 ml/min at discrete sampling times. Blood samples (10 ml) were collected at 5 time points: after 20 minutes of sitting in a quiet waiting room prior to the interview (REST 20); 5 minutes after sitting in the exam room and beginning the interview (EXAM 5); 20 minutes after beginning the interview (EXAM 20); 5 minutes after returning to the waiting room and being seated (POST 5); and after sitting in the waiting room for 20 minutes (POST 20). Samples were centrifuged at 4°C and an aliquot of plasma was heated at 90 C for 10 min to inactivate phosphodiesterase. After centrifugation to remove the precipitate, the supernatant was frozen at -70°C until cyclic AMP levels were determined by RIA.

	REST 20	EXAM 5	EXAM 20	POST 5	POST 20
H.R.	86 ± 2.5	114 ± 5.4*	101 ± 4.0*	93 ± 3.4	89 ± 3.3
cAMP	32 ±3.6	51 ± 6.2*	39 ±4.0	35 ± 3.3	30 ± 3.6

Heart rate (HR) given in bpm, cyclic AMP in pm/ml, mean ± s.e.m.
Data analyzed by ANOVA, then by paired t-tests. N = 12, * = $p<0.001$.

Heart rate increased 33% in the first 5 min of the oral exam and remained significantly elevated throughout the exam. Plasma levels of cyclic AMP increased 59% in the first 5 min of the exam, but unlike heart rate, did not remain significantly elevated throughout the exam. We have recently reported increases in plasma catecholamines during oral exams, and the plasma cyclic AMP response may be related to sympathetic nervous system responses.

360.1 RETINAL ACTIVITY MODULATES EYE GROWTH: EVIDENCE FROM REARING IN STROBOSCOPIC ILLUMINATION. Michael D. Gottlieb and Josh Wallman. Biology Department, City College of CUNY, New York, NY 10031.

Various visual deprivations cause increased vitreous chamber growth resulting in myopia. Providing vision to the rapidly growing eye stops the excessive elongation. Does this effect of vision involve subtle neural processing of the visual information or does it result from the increased retinal activity that unimpaired vision brings? If the latter is the case, increasing the retinal activity in an animal deprived of form vision should reduce the growth toward myopia.

In chicks, visual field restriction produces eye enlargement and myopia only in the region of the retina that has been deprived of form vision. We infer that retinal activity in the deprived region is lower than normal since the retina views a homogeneous field rather than the heterogeneity of the real world. We here report on the effects of artificially increasing the activity level of a form-deprived region of the retina by rearing animals with stroboscopic illumination.

Chicks were raised for 1 week from hatching with an occluder over one eye that deprived the nasal retina of form vision. Ambient lighting was either a 10 Hz strobe, incandescent, or both, for 14 hours daily. All eyes were refracted at 30° temporal to the optic axis, i. e., in the deprived region of the retina.

Stroboscopic illumination greatly reduced the degree of experimental myopia in the region of the retina that had been deprived of form vision. The partially occluded eyes of chicks that had been subjected to stroboscopic plus incandescent light were hardly myopic in the region of the retina that had been deprived of form vision (median: -2.0 diopters, D, n=10); similar results were obtained after 2 weeks (-0.9 D, n=6) and with stroboscopic illumination alone after 3 weeks (-2.6 D, n=6). In contrast, the partially occluded eyes of chicks raised with incandescent light were myopic in the region of the retina that had been deprived of form vision (-13.5 D, n=11), as were those raised with a 100 Hz strobe plus incandescent light for 2 weeks. The unoccluded eyes, whether raised with incandescent light or incandescent plus stroboscopic light, were not myopic (+2.2 D and +2.9 D, respectively).

Two interpretations of these results are that some class of retinal neurons actively inhibits growth of the vitreous chamber, or that this growth depends on the overall activity of the retina. In either case, one can speculate that, during normal growth, the increased retinal activity elicited by a sharply focused image may stop the enlargement of the vitreous chamber when emmetropia is reached. (Supported by NIH EY 02727)

360.2 DENDRITIC DEVELOPMENT OF CHICK RETINAL GANGLION CELLS. S.M. Fraley. Dept. of Ophthalomology, New York Medical College, Valhalla, NY 10595

The development of ganglion cell dendrites in embryonic chick retina was studied in flatmounted retinae labeled retrogradely with horseradish peroxidase (HRP) and reacted with cobalt intensification of diaminobenzidine. Although the retrograde HRP transport method has the advantage of selectively labeling retinal ganglion cells, their dendritic trees often remain incompletely labeled when the enzyme is applied at some distance from the retina. In the present studies, an *in vitro* labeling procedure has been developed which permits HRP to be applied directly to the optic nerve immediately behind the eye. In addition to Golgi-like filling of the dendritic arbors, this procedure also affords control over the density of ganglion cell label. Thus ganglion cell classes were identified in retinal preparations labeled from HRP application to the whole optic nerve and the dendritic morphology of individual cells was analyzed in detail in preparations labeled relatively sparsely following HRP application to selective portions of the nerve.

Ganglion cells examined at the fourth day of incubation (E4) showed axons without discernible dendrites. Dendrites at E8-E9 were characterized by their thick-stemmed arbors, growth cones, and initial extension in the ganglion cell layer. Subsequent profuse growth was characterized by an increase in dendritic fields and extensions into the inner plexiform layer, and by E12-E13 ganglion cells showed distinctive dendritic morphologies. Preliminary analyses have identified at least four classes of ganglion cells: 1) a cell type located preferentially near central retina with primary dendrites extending from a unipolar soma, 2) a cell type with small round soma and dendritic field whose thin arbors extend radially from the soma, 3) a cell type with two or three thick primary dendrites extending bilaterally from an elliptical soma, and 4) displaced ganglion cells with large dendritic fields whose arbors extend radially from the soma.

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360.3 CENTRIFUGAL FIBER PATTERNS IN LATE EMBRYONIC CHICK RETINA. V.M. Berthoud and S.M. Fraley. (SPON: F.E. Horvath) Dept. of Ophthalmology , New York Medical College, Valhalla, NY 10595.

Previous studies of centrifugal fibers in the avian retina have shown two types of terminal arborizations, described as divergent and convergent, that make synaptic contact primarily with amacrine cells. It is generally believed that terminal arborization in the inner nuclear layer emanates from a single parent axon. The present report describes the existence in chick of extensive intraretinal collateralization of the centrifugal fibers. It focuses on the patterns observed at hatching (E20-E21), a time when retinal morphology appears relatively mature.

It has been possible to fill the fine processes of centrifugal axons and the dendritic arbors of retinal ganglion cells using an *in vitro* technique to label the optic nerve with horseradish peroxidase (HRP). Analyses of labeled flatmounted retinae indicate that many centrifugal fibers arborize in a manner similar to that described by earlier workers. Thus single axons descending through the inner plexiform layer may form either compactly tufted arbors (convergent-type) or extensive, looping arbors (divergent-type) in the inner nuclear layer. In addition, many axons leaving the optic fiber layer issue collaterals which course obliquely for 1 cm or more through the inner plexiform layer. The collaterals in turn give off multiple short terminal processes which end in clusters of bouton-like swellings.

The function of extensive centrifugal fiber collaterals is as yet unknown although it may be speculated that they serve to enhance lateral interaction capabilities. Indeed, collateralization may provide an anatomical substrate for a physiologically defined peripheral effect similar to that described by McIlwain (J. Neurophysiol. 27: '64) in mammalian retina.

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360.4 DEVELOPMENT OF SEROTONIN N-ACETYLTRANSFERASE ACTIVITY IN CHICKEN RETINA: LIGHT-DARK DIFFERENCE, CIRCADIAN RHYTHMICITY, AND EFFECT OF DIBUTYRYL CYCLIC AMP. P. Michael Iuvone, Dept. of Pharmacology Emory Univ. Sch. Med., Atlanta, GA 30322.

Serotonin N-acetyltransferase (NAT) is a key regulatory enzyme in the biosynthesis of melatonin in retina. In retina, as well as in pineal, NAT activity is regulated as a circadian rhythm, with peak activity at night. The nocturnal increase of NAT activity appears to occur by a cyclic AMP-dependent mechanism. To examine the development of regulation of NAT activity in chick retina, eggs were incubated under a 12 h light-dark cycle. NAT activity was low from embryonic (E) day 7-17. Daytime levels increased to a maximum at about day E20 and then declined slightly to post hatch (PH) day 3. No significant light-dark differences were observed until E20, when activity in the dark-period was 60% higher than that in the preceeding light period. The light-dark difference increased in magnitude markedly on the next day, the day of hatching, and continued to increase to a 6-fold difference by PH day 3. No further increase in magnitude was observed up to 4 weeks PH.

Circadian rhythmicity of NAT activity appears to develop at or prior to hatching. The mid-day vs mid-night difference in retinal NAT activity of chicks exposed to constant darkness was approximately 2-fold at PH day 2, PH day 6 and PH 4 weeks. Chicks exposed to constant darkness from hatching (exposed to light only *in ovo*) also displayed a 2-fold day-night difference on PH day 1.

To determine if the molecular mechanisms for increasing NAT activity develop prior to the development of light-dark differences, E14 retinas were incubated in vitro for 5 hours with or without dibutyryl cAMP and IBMX. Dibutyryl cAMP and IBMX elicited a significant (approx. 6-fold) increase in NAT activity.

These studies indicate that light-dark differences in NAT activity and entrainment of the circadian clock that regulates the NAT activity rhythm develop prior to hatching. Development of the cyclic AMP-dependent mechanism for increasing NAT activity appears to significantly preceed that of rhythmicity, suggesting that the onset of rhythmicity may be related to the onset of photoreception or development of the clock in the chick retina.

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360.5 DEVELOPMENTAL CHANGES IN ULTRAVIOLET PHOTOSENSITIVITY IN RAINBOW TROUT. Craig W. Hawryshyn, Margaret G. Arnold*, Duane J. Chiasson*, and Patti C. Martin*. Department of Psychology, McMaster University, Hamilton, Ontario CANADA L8S 4K1.

A number of recent studies have reported the presence of ultraviolet (UV) photoreception (mediated by UV-sensitive cones) in vertebrates. Further work has shown that the ocular media in some of these species transmits UV radiation and that this transmission can greatly influence UV photosensitivity. The present study examines the changes in UV photosensitivity that occur during the growth of Rainbow trout.

We used the heart-rate conditioning technique (Hawryshyn and Beauchamp 1985) to measure spectral sensitivity in immobilized Rainbow trout (Salmo gairdneri). The Rainbow trout (Spring Valley Trout Farm, New Dundee, Ontario) utilized in the present study belonged to the same age cohort but varied slightly in size (40-90 grams body weight, 16.0-19.5 cm standard length). We attempted to "isolate" the sensitivity (340-540 nm range) of the UV-sensitive mechanism with a yellow (550 nm long pass) plus blue (460 nm 10 nm half max) colored background.

Two fish (44 and 60 g) exhibited a UV peak in the 380-400 nm region (λ max 390 nm visual pigment absorption curve vitamin A_2 correlates well), and a blue-sensitive mechanism shoulder in the 440-540 nm region (λ max 453 nm visual pigment absorption curve vitamin A_2 correlates well). On the other hand, two additional fish (both 90 g) showed blue-sensitive mechanism activity and lacked any evidence of a UV-sensitive mechanism peak. Spectral sensitivity of the two fish showing UV photosensitivity was remeasured (using same colored backgrounds as above) approximately one month later (average weight gain 25%). Both fish exhibited a loss of the UV peak and had a sensitivity that now conformed to the blue-sensitive visual pigment absorption curve.

There are several hypotheses which can account for this loss of UV photosensitivity in trout: (1) Increases in ocular media pigment density with size. (2) Selective photoreceptor damage with increasing vulnerability related to size or metabolic age. (3) Changes in the pattern of the retinal mosaic with development. Lyall (1957) and more recently, Bowmaker and Kunz-Ramsey (personal communication) have reported changes in the retinal mosaic of trout with age, especially with the accessory single cone which they believe contains the UV-sensitive visual pigment.

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360.6 DOPAMINE INDUCES LIGHT-ADAPTIVE RETINOMOTOR MOVEMENTS IN BULLFROG CONES AND RPE VIA D2 AND D1 RECEPTORS. Allen Dearry, Sheldon Miller, and Beth Burnside. Department of Physiology-Anatomy and School of Optometry, University of California, Berkeley 94720.

We have been investigating the mechanisms of light and circadian regulation of retinomotor movements in lower vertebrates. We previously reported that dopamine induces light-adaptive retinomotor movements in green sunfish by acting on D2 receptors on photoreceptors and retinal pigment epithelial (RPE) cells in this species. Since dopamine is the predominant catecholamine in amphibian as well as in fish retinas, we have conducted similar experiments to examine the effect of dopamine on retinomotor movements in the bullfrog Rana catesbiena.

Bullfrogs were maintained on a 12 hr light/dark cycle, and experiments were begun 3 hr into the dark period. Retinas and eyecups with attached RPE were isolated from dark-adapted bullfrogs and cultured separately for 60 min in constant darkness. Following fixation, cone and RPE pigment retinomotor positions were determined.

In Ringer solution alone, cones contracted spontaneously to their light-adapted positions. We previously reported that such light-independent cone contraction also occurs in isolated fish retinas. This light-independent cone contraction could be prevented by adding IBMX or forskolin to the Ringer solution. In the presence of IBMX, dopamine ($\geq 10^{-6}$M) induced light-adaptive cone contraction. Dopamine-induced contraction was inhibited by sulpiride (a D2 antagonist) but not by SCH23390 (a D1 antagonist). In addition, LY171555 (a D2 agonist) induced cone contraction, whereas SKF38393 (a D1 agonist) was ineffective. These results suggest that dopamine induces light-adaptive cone contraction in bullfrog retinas by acting on D2 receptors.

RPE maintained its dark-adapted retinomotor position during culture in constant darkness. Dopamine and SKF38393, but not LY171555, induced light-adaptive pigment dispersion. Dopamine-induced dispersion was inhibited by SCH23390 but not by sulpiride. Furthermore, cAMP, IBMX, and forskolin also induced light-adaptive pigment dispersion. These results suggest that dopamine induces light-adaptive pigment dispersion in bullfrog RPE by acting on D1 receptors. Elevated levels of cAMP in bullfrog RPE are associated with light-adaptive retinomotor movement.

In summary, our results indicate that dopamine elicits light-adaptive retinomotor movements in fish and bullfrogs. In both species, dopamine induces cone contraction via D2 receptors. In both, dopamine has a direct effect on the RPE. However, dopamine induces pigment dispersion in bullfrog RPE through an interaction with D1 receptors, whereas dopamine interacts with D2 receptors on fish RPE. [Supported by NIH grants EY03575, EY02205, EY03176, and RCDA EY00242 to SM.]

360.7 EVIDENCE THAT LIGHT STIMULATES ENDOGENOUS DOPAMINE RELEASE AND METABOLISM IN RETINAS OF FROGS (XENOPUS LAEVIS). Jeffrey H. Boatright*, Martha J. Hoel*, and P. Michael Iuvone (SPON: F.A. King). Emory Univ. Sch. Med., Dept. of Pharmacology, Atlanta, Georgia 30322.

Recent pharmacological studies on the regulation of a melatonin synthesizing enzyme, serotonin N-acetyltransferase (NAT), in frog retina, suggest that the dopamine (DA) receptors that regulate NAT activity are occupied in light-exposed retinas but not in dark-adapted retinas (Iuvone et al., Brain Res., in press). To obtain a more direct measure of the effect of light exposure on DA activity in frog retina, we examined retinal tissue levels of DA and 3,4-dihydroxyphenylacetic acid (DOPAC), a principal DA metabolite, and the release of endogenous DA in light and dark. DA and DOPAC were measured by HPLC with electrochemical detection. Postmetamorphic Xenopus laevis were maintained on a 12 h light-dark cycle. In the first experiment, DA and DOPAC levels were determined in retinas of frogs dissected during the last 30 min of the light period or 2 hrs into the dark period. The concentrations of DA and DOPAC in light were 41% and 170%, respectively, higher than those in darkness. In the second experiment, eye cups were prepared during the last hour of the light period. Beginning at the time of light offset, eye cups were preincubated in darkness for 30-45 min. Following preincubation, they were transferred to fresh medium and incubated for 1 hr in light or dark. DA and DOPAC levels of retinas incubated in light were significantly higher (16% and 100%) than those in darkness. Analysis of DA in incubation medium following extraction and concentration indicated that endogenous DA overflow from eye cups was 3 fold higher in light than in dark. These studies suggest that DA release and metabolism in frog retina is stimulated by light exposure.
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360.8 DO NEUROFILAMENT PROTEINS PLAY A ROLE IN CLOCKWISE GROWTH OF XENOPUS RETINAL NEURITES? P. Grant and Y. Tseng. Institute of Neuroscience, University of Oregon, Eugene, ORE 97403.

In an earlier in vitro study we found that Xenopus retinal fibers undergo developmental changes in growth behavior (Grant, P. and Tseng, Y., Abst. Soc. Neurosci. 12:119, 1986). While young embryonic neurites from explanted optic vesicles display no clockwise growth under any conditions tested, neurites from progressively older retinal explants show clockwise growth on laminin and fibronectin in serum-free medium. One hypothesis for this difference is that only mature neurites with cytoskeletons of helical neurofilament (NF) bundles are capable of clockwise growth. This implies that embryonic neurites, which show no clockwise growth, either lack all or some NF proteins, or are unable to assemble them if present. The former possibility seemed testable since synthesis of NF proteins in rat retina is developmentally regulated: the large 200K NF protein appears several days after the 140K and 68K (Shaw, G. and Weber, K., Nature, 298:277, 1982). Accordingly, Xenopus retinas of different stages (25 to 50) were cultured under conditions promoting clockwise growth. After the initial neurite outgrowth, explants were tested immunocytochemically with monoclonal antibodies against mammalian NF proteins. Antibodies to vimentin, GFAP, actin and tubulin were also tested. All but the 68K antibody reacted positively with retinal neurites at all stages. Contrary to the predictions of the hypothesis, the two large NF epitopes are present throughout development while the 68K epitope is undetectable suggesting that clockwise growth does not depend on a NF cytoskeleton. It is uncertain whether the antibodies are reacting with soluble NF antigens or proteins assembled into NF bundles. Alternatively, other non-NF antigens with similar epitopes may be present in Xenopus retinal neurites from the very beginning of ganglion cell differentiation. The data do not resolve these alternatives.
Supported by NSF grant BNS-8516317 awarded to P. Grant.

360.9 AN IN VITRO MODEL FOR QUANTITATIVE ANALYSIS OF RETINAL NEURON GROWTH IN THE CAT. P. Read Montague* and M.J. Friedlander
Neurobiology Research Center and Dept. of Physiology & Biophysics, University of Alabama at Birmingham.

The morphology of retinal ganglion cells (RGC) has been extensively studied in the intact retina. These cells form distinct morphological classes with regular arrangements of dendritic territories. The mechanisms by which these morphological types are established and the interactions that determine territorial boundaries of dendritic fields are not fully understood. We have developed an in vitro system to quantify and model RGC growth and development in a well controlled environment. Cat RGCs are particularly suitable for this type of study since a wealth of data on the distribution of projections of the various morphological types exist and the distribution of dendrites within the retina is essentially planar - thus a 2-dimensional cell culture environment does not necessarily force the RGC to assume aberrant morphological features. RGCs are obtained from cats between 3-14 months using a modified "peeling" procedure (Shiosaka et al., J. Neurosci. Meth. 10:229-235, 1984.) and/or enzymatic dispersion. RGC enriched fractions of cells are grown at low density on coverslips above a pre-established glial culture monolayer or directly on the monolayer on an inverted microscope stage. The development of individual cells is tracked for up to 2 weeks using time-lapse video phase microscopy. The continued growth of the glial monolayer and the growth of retinal glia is prevented by pulsed application of the mitotic inhibitor, cytosine-arabinofuranoside. Cultures are examined for immunoreactivity to neurofilament and glial fibrillary acidic protein (GFAP). All cells identified as neurons on morphological criteria were GFAP negative and neurofilament positive. The elaboration of neurites by these cells is being studied with respect to 1) pattern development of isolated individual cells' neurites in real time, 2) the effects of neighboring cells on an individual cell's neuritic development and 3) the degree of similarity of in vitro neuronal form to that observed in the intact retina. A predictive constrained diffusion model that includes aspects of the method of Mandelbrot ('fractal analysis') is used to characterize the cells' neurite coverage. This method assigns a numerical value to describe the plane filling nature of an individual cell's neuritic structure. Experiments are currently underway to pre-label the RGCs with fluorescent markers prior to the establishment of the cultures - this will allow definitive identification of the RGCs as distinct from the other retinal neurons in the culture.

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360.10 RELATIONSHIP BETWEEN SMALL PUNCTATE LESIONS AND IMMEDIATE SHIFTS IN VISUAL ACUITY IN THE PRIMATE RETINA. D.O. Robbins, H. Zwick*, and R.C. Long*. Department of Psychology, Ohio Wesleyan University, Delaware, OH 43015 and Division of Biorheology, Letterman Army Institute of Research, San Francisco, CA 94129

Both long-term and transient shifts in visual acuity have been observed following exposure of the primate retina to CW and Q-switched lasers. Our previous results using Argon, HeNe, and Krypton CW lasers have shown that the duration of the initial deficit following foveal exposure was related to the energy of the 100 msec flash. Typically, immediate postexposure acuity was depressed by as much as 60% from its pre-exposure level and often this deficit lasted 45 minutes or longer for energies significantly below the ED50 level for distinct fundoscopic damage. Permanent shifts in acuity under a variety of viewing conditions have been shown to exist below the ED50 energy level and without evidence of fundoscopic changes.

More recently we have examined the consequences that small, but distinct, punctate foveal lesions have on acuity. Using minimal diameter, single, 15 nsec, Nd/YAG (514.5 nm) pulses only very slight and transient changes in acuity were noted although the animal's ability to maintain a consistent threshold acuity in the exposed eye was often affected. In the case of CW exposures, each individual flash was 100 msec in duration and while it was of lesser energy, the energy was presented for a much longer duration resulting in involuntary eye movements spreading the minimal diameter spot over a larger retinal region than that which occurred when using 15 nsec pulses. The increased variability in threshold acuity of animals exposed to single, Q-switched pulses could be explained in terms of a problem the animals might experience in localizing the target on an area of fovea outside that exposed by the laser pulse. We have also examined the effects that multiple pulses, producing larger areas of disruption, have on the ability of animals to maintain a stable, threshold acuity. As expected, as the total area of foveal involvement increases, the animal's threshold acuity decreases and becomes more reminiscent of those observed using CW flashes. A morphological verification of the area of involvement and its relationship to acuity will be presented.

We have also explored the extremely transient and immediate changes acuity during the first several seconds following both CW and Q-switched exposure as well as those more permanent changes which resulted from exposure to energies the ED50 level.

360.11 IMPAIRED LIGHT SENSITIVITY IN HYPOPIGMENTED ANIMALS. G.W. Balkema and U.C. Dräger. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

In previous work we have shown that pearl mutant mice, as well as albino mice and rats, have elevated visual thresholds in the dark-adapted state. Now we extend these findings to several other hypopigmentation mutants of the mouse, albino rats and rabbits. We compared incremental visual thresholds of normal black mice (C57BL/6J +/+) and the following mutants on the same background: beige bg/bg); pale ear (ep/ep); albino (c^{2J}/c^{2J}); in addition, we recorded thresholds from steel (Sl/Sl^{D}) and W-mice, two mutants with normal neural-tube derived pigmentation of the retinal pigment epithelium (RPE) but without any neural-crest derived pigment in the choroid or the rest of the body. Thresholds were measured, using tungsten-in-glass electrodes, from single units in the superior colliculus. The dark-adapted thresholds of the normally pigmented mice were similar to those reported in earlier studies. The albino mice were 25 times less sensitive than black mice; the pale ear mutants were 20 times less sensitive; beige mutants were 11 times less sensitive; and steel mutants were five times less sensitive than black mice. The mean threshold of dark-adapted black mice was 0.0092 cd/m^2. (SEM = 0.0062 < 0.0092 < 0.013); the mean threshold of albino mice was 0.23 cd/m^2 (SEM = 0.19 < 0.23 < 0.28), and the other mutants fell between black and albino. Cell counts from the outer nuclear layer in albino mice were not significantly different from those in black mice, and no outer-segment damage was found in albino retinas, thus excluding overt light damage as an explanation for the elevated thresholds. Incremental thresholds were also measured in hooded rats and compared to those in albino rats. The albino rats were 64 times less sensitive than the pigmented hooded rat in the dark-adapted state. The mean threshold for the hooded rat was 0.0012 cd/m^2 (SEM = 0.00082 < 0.0012 < 0.0016), and the mean threshold for the albino rat was 0.073 cd/m^2 (SEM = 0.049 < 0.073 < 0.11). Similarly, incremental thresholds were found to be elevated 40 times in albino rabbits (New Zealand-White) compared to pigmented (Dutch-Belted) controls; the mean dark-adapted threshold for the albino was 0.0008 cd/m^2 compared to 0.00002 cd/m^2 for the pigmented rabbit.

The light sensitivity deficit was roughly proportional to the reduction of melanin pigmentation both in the animals' RPE and choroid. This allows the following two tentative conclusions: first, the melanin pigment external to the photoreceptors influences retinal sensitivity; and secondly, this influence depends not only on the melanin content of the RPE, which is in close contact with the photoreceptors, but also on the melanin content of the choroid, which contains the blood vessels for nutrient supply to photoreceptor outer segments. Supported by EY01938 and EY05777.

360.12 **CYTOCHROME OXIDASE ACTIVITY IN THE DIABETIC RAT RETINA.** R.B. Caldwell, S.M. Slaprick*, and C. Uphoff*. Department of Anatomy and Neurobiology, The University of Tennessee, Memphis, TN 38163.

In the retina, a vascular barrier system analagous to the blood-brain barrier is present, and in diabetic patients alterations in this system contribute to a variety of visual problems. Permeability increases have been documented in both diabetic patient and animal retinas, but relatively little is known about the specific sites and cellular mechanisms of such alterations. Therefore, we have investigated the possibility that changes in active transport may contribute to increased permeability across the blood-brain barrier in diabetic retinas. Since activity of mitochondrial cytochrome oxidase has been demonstrated to be a reliable indicator of cellular metabolic activity and can therefore be correlated with energy requiring transport activity, we have used quantitative cytochemical techniques to localize this enzyme in retinas of spontaneously diabetic and control rats. In a previous study (ARVO, '87), we found that cytochrome oxidase activity is substantially increased within the retinal pigment epithelium (RPE) of diabetic rats. We have now compared the changes in mitochondrial cytochrome oxidase activity levels within the RPE with those in retinal photoreceptor inner segments.

Mitochondria were counted and assigned levels of cytochrome oxidase reactivity as dark, moderate, or light (Carroll and Wong-Riley, '84). In RPE cells of diabetic retinas, dark mitochondria totaled 52% compared with 34% in control retinas ($X^2 = 89.44$, $p < 0.001$). In photoreceptor inner segments of diabetic retinas, dark mitochondria totaled 17% compared with 14% in the controls ($X^2 = 2.30$, $p > 0.05$). This similarity of activity within the photoreceptor inner segments suggests that the increases in activity in the RPE are due to an alteration in blood-brain barrier function rather than a generalized increase in retinal metabolic activity.

A second study investigated the possible relationship between the increase in cellular metabolic activity and changes in RPE basal membrane surface area known to occur in diabetic rats (Grimes & Laties, '80; Grimes et al., '84). Stereological techniques were used to measure plasma membrane surface area at the basal aspect of the cells. This measure was then correlated with mitochondrial cytochrome oxidase activity within the same cell. RPE cells with significant increases or decreases in membrane surface areas were observed in the diabetic retina; however, the relationship between mitochondrial cytochrome oxidase activity and surface area was the same in the diabetic RPE ($r = 0.20$) as in the control ($r = 0.18$).

In summary, the demand for ATP appears to be increased in the diabetic RPE, suggesting that an increase may occur in energy requiring transport activity across this component of the blood-retinal barrier. This change does not appear to be due to alterations in membrane surface area which occur in diabetic RPE cells. (NIH EY-04618 and The Juvenile Diabetes Foundation).

360.13 ACETYLCHOLINE STIMULATES PHOSPHATIDYLINOSITOL TURNOVER IN RAT RETINA. S.M. Moroi*, M.H. Neff and N.H. Neff. Departments of Pharmacology and Pathology, The Ohio State University College of Medicine, Columbus, OH 43210.

Receptor activated phosphatidylinositol (PI) hydrolysis is a common mechanism for transmembrane signalling by various neurotransmitters and neuromodulators in brain. We report that acetylcholine (ACh) stimulates inositol 1-monophosphate (IP_1) accumulation two- to threefold above basal level in the rat retina. Retinas were isolated from male Sprague-Dawley rats and placed individually in Krebs buffer containing 10 mM lithium chloride and 1 uCi myo-[2-^{3}H]-inositol for one hour. Various cholinergic agonists were added for another hour and the stimulation was stopped with choloroform/methanol. Antagonists were present 20 minutes before the addition of agonists. IP_1 was separated by anion exchange chromatography and counted by liquid scintillation spectrometry. Carbachol causes a twofold maximal stimulation in a concentration- and time-dependent manner with an EC_{50} of about 15 uM. The stimulatory effect of carbachol was blocked by atropine. Pirenzepine, a selective muscarinic type 1 (M_1) receptor antagonist, also inhibited the carbachol-induced accumulation of IP_1. In contrast, the selective muscarinic type 2 (M_2) receptor antagonist, gallamine, did not block this effect. Other cholinergic agonists, oxotremorine, methacholine, arecoline, and McNeil A-343 stimulated IP_1 accumulation, but were significantly less efficacious than carbachol. The oxotremorine analogs, Oxo-2 and Oxo-4, also induced IP_1 formation. These results suggest that in the rat retina ACh stimulates PI metabolism by a M_1 cholinergic receptor.

360.14 VISUAL SENSITIVITY OF ADULT GOLDFISH AFTER 3 MO. CONSTANT LIGHT OR DARK. M.K. Powers, C.J. Bassi* and P.A. Raymond (SPON: J.S. McReynolds) Vanderbilt University, Nashville, TN 37240 and University of Michigan, Ann Arbor, MI 48109.

We have recently shown that the visual sensitivity of the rod system in goldfish (Carassius auratus) is related to (a) the planimetric density of rods in the retina and (b) the length of the rod outer segments (ROS). In previous experiments we found that adult fish housed in constant light (LL) or constant dark (DD) for 1 wk had elongated ROS and increased visual sensitivity. Thus, changes in visual sensitivity could be predicted from changes in ROS length (Bassi and Powers, Invest. Ophthalmol. Vis. Sci. 27 (Suppl), 236, 1986). In the present study we exposed adult fish to 3 mo. LL or DD, and recorded dark-adapted frequency-of-seeing functions by means of a classical conditioning paradigm (Powers and Easter, Vision Res. 18, 1137, 1978). Absolute thresholds were measured for 12 goldfish, 8-10 cm body length. Six were placed in constant light at 840 lux, and 6 were placed in constant dark. When threshold was re-measured 3 mo. later, it was decreased in all DD fish but highly variable in LL fish. On average, however, fish kept in LL were 0.2 log unit less sensitive than control (LD) fish, while fish kept in DD were 0.5 log unit more sensitive than controls. These changes were transient: thresholds returned to control levels within 1 day to 1 wk of the first post-exposure test. The results suggest that exposures of 1 wk to 3 mo. can influence visual sensitivity in goldfish, and that such effects are transient. More interestingly, they suggest the possibility that short and long exposures to LL may have different visual consequences, because thresholds after 1 wk were lower (Bassi and Powers, ibid) while thresholds after 3 mo. tended to be higher. In contrast, the effects observed at short and long exposures to DD were quite similar. The effects of long term exposure observed in this study could be explained either by known visual adaptation phenomena or by changes in ROS length. Histological analysis is currently underway to investigate the latter possibility. Supported by NIH grants EY03352, EY04318, K04-EY00246, and NSF grants BNS 8203268, BNS 8200981.

360.15 ELONGATION OF ROD OUTER SEGMENTS IN ALBINO RATS MAINTAINED IN CONSTANT LIGHT. D. J. Bare* and W. K. O'Steen. Department of Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

The role of ambient lighting in the regulation of vertebrate rod outer segment length and therefore, in membrane addition and loss has been documented in both mammalian and non-mammalian species. Albino rat rod outer segments (ROS) respond to constant darkness (DD) by increasing their length and rhodopsin content (Battelle et al., 1978). Similarly, exposure of frogs and goldfish to continuous illumination (LL) results in ROS lengthening (Currie et al., 1978; Bassi et al., 1986) due to an inhibition of normal disc shedding and acceleration of disc assembly. However, changes in ROS dimensions due to LL in the rodent are unclear. A lack of any significant change in ROS dimensions in pigmented mice for both LL and DD contrasts with the DD lengthening of the albino rat (Besharse and Hollyfield, 1979).

Lengthening of rod outer segments by constant light exposure as seen in the frog and goldfish has now been demonstrated in the albino rat. Male, 8-week-old, albino rats were obtained from Harlan Sprague-Dawley labs and housed in polycarbonate cages with an ambient lighting intensity of 5 to 2 lux from the front to the back of the cage. An 8 a.m.:10 p.m. (14L:10D) lighting schedule was maintained for control animal adaptation to this low lighting environment. An experimental group was exposed to constant light of identical illuminance. Following a 7, 14 and 21 day adaptation period, a group of animals (n=5) was removed from each lighting regime and overanesthetized. The superior surface of each eye was marked with indelible ink, and the tissue was prepared for light microscopy. In a 7 μm thick section passing through the optic nerve, morphometric analyses were made of outer nuclear layer thickness and ROS lengths. Analyses for each group revealed no significant loss of photoreceptor nuclei in LL-maintained animals for the 3 week period examined. Similar analyses of ROS length for the 7 day group revealed no significant difference between cyclic and constant regimes. However, significantly longer outer segments existed across the circumference of the retina in the 14 day group ($p<0.001$) and the 21 day group ($p<0.01$) relative to cyclic controls. The greatest increase in ROS length occurred in the superior central retina. These results indicate that exposure of albino rats to constant light exerts a significant effect on membrane assembly-loss equilibrium which results in elongation of ROS. An exposure time between 7 and 14 days is apparently necessary for a shift in the equilibrium which is reflected in ROS length. Therefore, elongation of ROS in both LL and DD as demonstrated in amphibians appears to exist also in albino rats. Supported by NIH grant EY02359 (NEI).

360.16 DURATION OF RETINAL RESISTANCE TO DAMAGE FOLLOWING STIMULATION OF HEAT SHOCK PROTEIN SYNTHESIS. M. F. Barbe and M. Tytell, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

Synthesis of heat shock proteins (HSPs) in response to potentially lethal stress is a highly conserved fundamental cellular response to injury. Many investigators have shown a temporal correlation between the induction of thermal resistance and synthesis of HSPs (Gerner et al., Cancer Res., 36: 1035, 1976; Subjeck et al., Brit. J. Radiol., 55: 579, 1982). Heat shock protein production correlates with protection of the retina against light damage (Barbe et al., Soc. Neurosci. Abstr., 12: 638, 1986; Barbe and Tytell, Invest. Ophthal. Vis. Sci., (in press) 1987.

In order to investigate the duration of that protection, we have examined the degree of retinal damage after different time periods between the heat shock and the light exposure. Pentobarbital-anesthetized adult male Sprague-Dawley rats had their body temperatures raised to 41°C for 15 minutes. They were then left at room temperature for 0, 2, 4, 8, 18, 24, or 50 hrs, after which they were exposed to 250 ft-c of light for 24 hrs in a box kept at 30°C. The rats were sacrificed 2 weeks post-light exposure. Light microscopic analyses of the photoreceptor layer (ONL) revealed that the protection from damage became significant at 4 hrs post-heat shock and lasted until 24 hrs post-heat shock, with the greatest protection being at the 8 hr interval. By 50 hr post-heat shock, the retinas were no longer protected from light damage. These results are consistent with the kinetics of synthesis of the heat shock proteins, which is greatest at 4 hr after hyperthermia. Our observations indicate that at the time when accumulation of the HSPs is the greatest in the retina, the photoreceptors are most resistant to light damage. Thus, there appears to be a temporal correlation between HSP synthesis and the protection of the retina from acute damage. Supported in part by NSF grant BNS 85-20838 to M.T.

360.17 TRANSPLANTATION OF RETINAL PHOTORECEPTORS TO LIGHT-DAMAGED RETINA: SURVIVAL AND INTEGRATION OF RECEPTORS FROM A RANGE OF POSTNATAL AGES. M. Silverman* and S. Hughes* (SPON: D. Eldredge). Central Institute for the Deaf and Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Because several forms of blindness are related to the loss of the retinal photoreceptors work was initiated in our laboratory to investigate the possibility of restoring some level of visual function through the transplantation of photoreceptors. In addition we wished to determine the effect of developmental age of the transplanted photoreceptors on their survival and ability to integrate with the host retina. Photoreceptors for transplantation were taken from neonatal rats ranging in age from 7 to 15 days. Within this period photoreceptor cells undergo a developmental sequence initiated by their segregation from other cells of the retina. They then form synapses within the outer plexiform layer, develop mature morphological characteristics such as inner and outer segments and finally become functional at 12 days. In order to maintain the organization of the photoreceptor layer, we devised a method for separating the intact photoreceptor matrix, the outer nuclear layer, from the donor retina. This method consists of vibratome sectioning of the flatmounted retina to isolate the outer nuclear layer. Hosts were adult albino rats blinded by two to four weeks of constant illumination, which destroyed the photoreceptors and left the remaining neural retina intact. Transplantation of the photoreceptor matrix to the host retina was accomplished using a transcorneal approach to the subretinal space, which minimized vascular trauma to the eye. We found that this approach does not appear to disrupt the integrity of the retina, which spontaneously reattaches to the back of the eye with the transplanted photoreceptors interposed between the retina and the underlying tissues. The photoreceptors at all ages studied survive transplantation, showing appropriate growth and development as well as apparent physical integration with the blinded host retina. These findings indicate that immature as well as fully developed retinal photoreceptor matrix can be successfully transplanted to form a new outer nuclear layer with mature blind retina. It is of interest that the degree of maturity at which retinal photoreceptors can be transplanted is in marked contrast to the immature developmental stage required by other CNS tissue for successful transplantation.

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360.18 WHOLE CELL RECORDINGS OF ISOLATED RETINAL PIGMENT EPITHELIAL CELLS OF THE FROG. B.A. Hughes*, J. Immel*, and R.H. Steinberg (SPON: D.R. Copenhagen). University of California, Departments of Physiology and Ophthalmology, San Francisco, CA 94143.

We used the whole cell recording configuration of the patch-clamp technique to investigate voltage-dependent currents in single cells isolated from the retinal pigment epithelium (RPE) of the frog (Rana pipiens). The RPE-choroid was dissected from dark-adapted frog eyes and incubated in low calcium solution containing papain for 10 to 20 minutes to disperse cells. The morphology of the isolated RPE cells retained many characteristics of RPE cells in situ. Cells were columnar or cuboidal in shape and distinctly polarized. The apical end of the cell contained many melanin granules and long microvilli while the basal end was devoid of pigment and had a normal appearing surface. The standard extracellular solution consisted (in mM) of: 82.5 NaCl, 27.5 $NaHCO_3$, 2 KCl, 10 glucose, 1.0 $MgCl_2$ and 1.8 $CaCl_2$, and was saturated with 95% O_2 / 5% CO_2. In most experiments, the patch pipette contained (in mM): 75 $KMeSO_4$, 25 KCl, 17 $KHCO_3$, 5.5 EGTA-KOH, 2 $MgCl_2$, and 0.5 $CaCl_2$.

Seals in the range of 2 to 20 gigaOhms were made with patch pipettes on the basal membrane and the patch was ruptured by gentle suction. In 28 cells, the membrane potential ranged from -10 to -65 mV and averaged -25.6 +/- 13.7 mV (mean +/- SD). The input resistance and capacitance of these cells averaged 236.6 +/- 109.8 megaOhms and 37.3 +/- 18.9 pF, respectively. Extracellular Ba++ increased the Ohmic component of the input resistance by a factor of 1.84 +/- 0.44 (mean +/- SD, n=9). Voltage clamp of the membrane potential revealed a small active inward current. This current developed at potentials more hyperpolarized than -70 mV in approximately 30% of the cells and was not affected by extracellular Ba++. All cells exhibited an active outward current that appeared at potentials more depolarized than 30 mV. The outward current was carried largely by K+ since the magnitude of the current was sensitive to changes in extracellular [K+], and was blocked by the addition of extracellular Ba++ or the replacement of intracellular K+ with Cs+.

360.19 EFFECTS OF ADAPTATION ON TREE SHREW ERG SPECTRAL SENSITIVITY. H.M. Petry, B.R. Wooten*, J.P. Kelly*, and S. Agarwala*. Department of Psychology, S.U.N.Y. at Stony Brook, NY 11794 and Hunter Laboratory, Brown University, Providence, RI 02912.

The tree shrew retina is heavily cone-dominated, containing only about 3-4% rods (Immel, 1981). Psychophysical studies have shown this animal to possess dichromatic color vision of the deutan-type, presumably mediated by short-wave-sensitive (SWS) and long-wave-sensitive (LWS) cone mechanisms. This conclusion has been supported by microspectrophotometry (Petry & Harosi, 1985) and ERG flicker photometry (Jacobs & Neitz, 1986). To further study the retinal interactions underlying tree shrew color vision, we measured the spectral sensitivity of the b-wave component of the transient ERG waveform under differing conditions of adaptation. This component has been found to reflect activity of ON-bipolar neurons in other mammalian species (Knapp & Schiller, 1984).

Electroretinogram recordings were obtained from tree shrews (Tupaia belangeri) anesthetized with ketamine and xylazine. Signals were recorded using a gold foil corneal electrode referenced to a similar electrode on the other, unstimulated, eye. Monochromatic flashes of light (200 msec duration) were produced in one channel of a Maxwellian-view optical system using a 1000 W xenon source and a grating monochromator. Background illumination was produced by a second channel in which narrow-band interference filters were placed to produce the chromatic adaptation lights. Spectral sensitivity functions were obtained by varying the intensity of the monochromatic flashes to evoke a criterion b-wave response of 100 V. Data were obtained under dark-adapted conditions or on backgrounds of blue (451 nm), yellow (569 nm) or white light. Stimuli were presented at 20 nm intervals across the spectrum from 430 nm to 650 nm.

Under dark-adapted conditions, b-wave spectral sensitivity displayed a two-peaked function, with a broad major peak occurring at approximately 550 nm and a minor peak at 470 nm. A prominent notch was consistently located at 490-500 nm. Functions obtained under all conditions of light adaptation (i.e., blue, yellow and white light) were similar in shape and differed from the dark-adapted curves in that they failed to display the minor short-wave peak and the prominent notch. This suggests that the tree shrew's small population of rods make a substantial contribution to the ERG b-wave. The fact that differential chromatic adaptation failed to shift the long-wave peak or change the shape of the spectral sensitivity curve suggests that the SWS cones have weak or no input to the neurons that generate the b-wave, presumably the ON-bipolars. This is in contrast to the monkey retina where the SWS cones contribute primarily to the ON-bipolars (e.g., DeMonasterio, 1979).

360.20 REACTION TIME AND SENSITIVITY OF THE CAT TO BRIEF WHITE AND SPECTRAL INCREMENTS. M. S. Loop and G. Hortan*. Dept. of Physiological Optics, School of Optometry, Univ. Alabama at Birmingham, Birmingham, AL 35294.

Cats (4) were trained in a reaction time procedure to respond to luminance increments of various durations and spectral composition. Reaction time training required about 6 weeks and proved to be a very efficient psychophysical testing procedure characterized by 100% detection above threshold, 200-300 responses, and 20 threshold settings in a 30 min. testing session. Furthermore the animal's threshold variance was surprisingly uninfluenced by false positive rate and threshold settings were about three times more sensitive than results obtained in a Berkley Box two-choice apparatus.

Because the influence of stimulus duration is difficult to evaluate with other animal psychophysical techniques, and figures prominently in the physiological and psychophysical analysis of visual system function, we explored its influence on the cats' sensitivity, i.e. we took the targets-of-opportunity. All testing was conducted upon a photopic white background (30 cd/m^2) for a 10° increment. White increments of 25, 50, 100, 200 and 500 msec. indicated a critical duration of around 100 msec. Spectral sensitivity for 1000 and 50 msec. flashes both produced a two-peaked function with less sensitivity to the 50 msec. flashes. Reaction times at equivalent luminances from threshold were faster and less variable for a 50 msec. flash than for a 500 msec. flash, for both 455 nm and 561 nm spectral stimuli; the cats detected both wavelengths with equal speed.

These data indicate that the cat's psychophysical critical duration is compatible with electrophysiological estimates based upon retinal ganglion cell responses. The similar shape of the spectral sensitivity function for 50 and 1000 msec. flashes indicates that flash duration cannot account for the frequently reported absence of blue cone influence on cat ganglion cells. The reaction times to 50 msec. and 500 msec. flashes indicate that stimuli which produce relatively fast and brief ganglion cell discharge produce relatively fast and brief stimulus detectability. Supported by EY05576, EY03039, SO7RR0587, SO3RR03400.

361.1 PROENKEPHALIN-DERIVED PEPTIDES AND CATECHOLAMINES IN THE ADRENAL MEDULLA AND SUPERIOR CERVICAL GANGLION OF SPRAGUE-DAWLEY, SHR, AND WKY RATS. R. Rigual*[a], M.K. Stachowiak*[b], and O.H. Viveros[a] (SPON: R. Ferris), Department of Medicinal Biochemistry, The Wellcome Research Labs., RTP, NC 27709[a] and NIEHS, RTP, NC 27709[b].

The involvement of peripheral opioid peptides (OP) in blood pressure regulation is controversial. Parenteral administration of enkephalins have a biphasic action, producing hypotension by directly decreasing peripheral resistance and hypertension by reflex stimulation of J-receptors. A report by DiGuilio et al (Nature 278:646, 1979), indicated that levels of enkephalin immunoreactivity were decreased by 50% in the sympathoadrenal system of SHR as compared to WKY rats. We reexamined this strain difference in opioid activity using a radioreceptor assay, and investigated changes in enkephalin precursors and catecholamines (CA) at two stages of development. The dissected adrenal medulla of adult, age matched (6 months), male SHR, WKY and Sprague-Dawley (SD) rats had similar protein content. Native OP activity in SHR was not different from SD rats (2.90 ± 0.51 and 1.92 ± 0.69 pmol/mg protein respectively) but WKY had twice as much native OP (6.18 ± 1.75) Similary, WKY showed a marked increase in total medullary opioids (after trypsin and carboxypeptidase B digestion) as compared to SHR and SD rats (increases of 6.5 and 7.6-fold, respectively). SHR rats showed a moderate increase in CA as compared to WKY (dopamine (DA), norepinephrine (NE), epinephrine (E): 25%, 11% and 27% respectively); SD had NE and DA levels that were not different from WKY but E levels were increased by 57%. In the superior cervical ganglion (SCG) mean values for total and native OP were higher, but not significantly different in WKY as compared to SHR and SD rats. No differences were found in NE levels of SCG in any of the strains. In younger rats (7-8 weeks), the increase in total medullary OP levels in WKY was smaller than in adults (2.3-fold when compared to SHR) while native OP had twice the values of SHR. No strain differences in OP were found at this age in the SCG, but NE was higher in SHR. In conclusion, there appears to be no obvious correlation between the levels of blood pressure and the CA and opioid peptides content in the sympathoadrenal system of SD, WKY and SHR.

361.2 AGONISTS AT BOTH SIGMA RECEPTORS AND AT PHENCYCLIDINE RECEPTORS IN THE MOUSE VAS DEFERENS ACT VIA AUGMENTATION OF ELECTRICALLY EVOKED NOREPINEPHRINE RELEASE. B.G. Campbell[1]*, D.H. Bobker[1]*, F.M. Leslie[2], I.N. Mefford[3]* and E. Weber (SPON: W. Woodward). Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201, [2]Department of Pharmacology, University of California, Irvine, CA 92717 and [3]Section on Clinical Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

Sigma receptors and phencyclidine (PCP) receptors are two recently characterized sites in the mammalian central nervous system that have been proposed to mediate the psychotomimetic effects of benzomorphan opiates and of PCP, respectively. Studies attempting to determine the receptor that mediates the psychotomimetic and other effects of these drug classes have been hindered because PCP and many of the benzomorphans, including the prototypic sigma receptor ligand SKF 10,047, are not specific and interact with both sites. Furthermore, no in vitro model system containing sigma receptors has previously been described.

We report here that both the sigma receptor-selective ligand (+)-3-[3-hydroxyphenyl]-N-(1-propyl)piperidine [(+)3-PPP] and the PCP receptor-selective ligand 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP) were active in the electrically stimulated in vitro mouse vas deferens. Both compounds produced an increase in the twitch amplitude, with a maximum increase of 251.0 ± 17.8 % (n=15) for (+)3-PPP and 338.2 ± 27.4 % (n=12) for TCP. The EC_{50} values were 57.2 ± 3.1 uM and 27.6 ± 3.2 uM, respectively. Other ligands with high affinity for either the sigma receptor or the PCP receptor also augmented the twitch amplitude.

Studies were carried out to determine the mechanism of this augmentation of the twitch amplitude. Systematic studies excluded the potential mediation of these effects through any previously characterized receptor (such as α_2) or through the mechanisms of inhibition of uptake or of norepinephrine(NE)-metabolizing enzymes.

Measurement of NE overflow in response to (+)3-PPP and to TCP revealed that the electrically evoked NE overflow was significantly increased at 200 uM for each compound ($p<.005$ and $p<.01$, respectively. Experiments in which isolated vasa were loaded with tritium-labelled NE confirmed that these compounds act via augmentation of NE release in the mouse vas deferens.

Data regarding competitive antagonists at the sigma receptor will be presented.

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361.3 **ALTERATIONS IN MICTURITION REFLEXES AND SPASTICITY FOLLOWING CONTINUAL INTRATHECAL INFUSION OF MORPHINE IN HUMAN SUBJECTS WITH SPINAL CORD LESIONS.** R. M. Herman, D.W. Coombs*, R. Saunders*, M.C. Wainberg*. (SPON: H.L. Borison) Catholic Med. Ctr., Manchester, NH 03102 and Dartmouth/Hitchcock Med. Ctr., Hanover, NH 03756.

Morphine (MO), administered intraspinally by bolus injection, enhances bladder capacity in normal subjects, Ss (Rawal, N. et al., Anesth. Analg., 62:641, 1983) and Ss with chronic complete (CSCL) and incomplete (ISCL) cervico-thoracic spinal cord lesions. Further, in Ss with SCL, systemic and epidural MO, e.g. 200 µg/kg, and 3000 µg, respectively, depress nociceptive flexor reflexes and spasticity (Willer, J.C. and Bussel, B., Brain Res., 187:212, 1980; Struppler, A. et al., Life Sci., 33:607, 1983). These spinal actions of MO are reversed by i.v. naloxone. Such results convey a potential therapeutic advantage for chronic intraspinal delivery of MO. Subsequently, it was established that continual intrathecal infusion (c.i.t.i.) of MO, e.g. 500 µg/day, attenuates chronic spinal spasticity for periods greater than one year (Erickson, D.L. et al., Neurosurgery, 16:215, 1985). However, there is little evidence that this form of therapy modifies micturition reflexes. Our study examined the effect of bolus injections of i.t. MO (200 µg) and of c.i.t.i. MO (via an implanted Shiley INFUSAID^R constant rate pump; 500-900 µg/day for 4 mos. duration) on hyperactive vesical and somatic reflexes among 3 SCL Ss (2 CSCL, 1 ISCL). Micturition, or vesico-vesical and vesico-somatic, reflexes were assessed during slow and medium fill saline cystometry by measuring intravesical, intrarectal, and detrusor pressures, and EMG discharges from the external ano-urethral sphincters (EAUS) and various lower limb muscles; somato-somatic, e.g. flexor, reflexes were elicited by noxious and innocuous cutaneous stimulation of the foot. C.i.t.i. MO (and bolus i.t. MO) caused an increase in bladder capacity by altering the rate of detrusor reflex contraction, or the threshold and magnitude of the detrusor reflex response, and by intensifying vesico-sphincter activity; in effect, a 'low capacity' bladder (60-100 cc) was transformed to a 'moderate capacity' bladder (200-300 cc). Spontaneous EMG discharges, defined as flexor spasms or spasticity, were markedly suppressed in all 3 Ss despite the presence of strong motor reactions during cutaneous and vesical stimulation, and detrusor instability during vesical contraction. Tolerance and side-effects were not apparent. In contrast, bolus i.t. MO induced considerable inhibition of spontaneous discharges and all stimulus-evoked reflexes. Pruritus was the principal side-effect. These actions by bolus i.t. MO were antagonized by i.v. naloxone. Summarizing, c.i.t.i. MO at doses below 1000 µg/day may provide therapeutic benefit to Ss with SCL when a hyperreflexive, low capacity, micturition system and/or spasticity interfere with the S's quality of life and when these phenomena are refractory to routine oral medication. (Partial Support: Spinal Cord Soc.; Dartmouth/Hitchcock, Catholic Med. Ctrs.)

361.4 **THE EFFECT OF LOW DOSE INTRATHECAL MORPHINE ON BLADDER CAPACITY IN HUMAN SUBJECTS WITH SPINAL CORD LESIONS.** M.C. Wainberg*, R.M. Herman, M.K. Willscher*, P.F. delGiudice*. Catholic Med. Ctr., Manchester, NH 03102

The role of endogenous opioid peptides in the central control of micturition has recently received considerable attention (e.g. de Groat, W.C. and Kawatani, M., Neurourol. Urodyn., 4:285, 1985; Maggi, C.A. and Meli, A., J. Auton. Pharmacol., 6:133, 1986). In normal animals, (Bolam, J.M. et al., J. Auton. Nerv. Syst., 16:261, 1986) and man (Aoki, M. et al., Masui, 31:939, 1982; Rawal, N. et al., Anesth. Analg., 62:641, 1983), intraspinal administration of the exogenous opioid morphine (MO), a mu agonist, leads to naloxone sensitive increase in bladder capacity and retention of urine. Our single blinded study examined the outcome of randomly delivered bolus intrathecal (i.t.) injections of MO (50, 100, 200, 400 µg) upon bladder capacity among 6 subjects (Ss) with suprasacral spinal cord lesions (SCL) secondary to trauma (3) and multiple sclerosis (3). Micturition reflexes were investigated utilizing slow and medium fill saline cystometry accompanied by EMG recordings from the external ano-urethral sphincters (EAUS) and lower limb muscles. Within 10-15 mins. of the injection, bladder capacity (defined as the volume at the initial occurance of leakage per urethram, the urgent desire to void, or a capacity of 700 cc) increased markedly in all Ss (190-475%), modifying a 'low capacity' bladder (30-120 cc in 5 Ss) to a 'moderate capacity' bladder (200-525 cc). The mechanisms responsible for this behavior appeared to be lesion dependent. During bladder filling, Ss (3) with complete lesions exhibited a reduced rate of detrusor reflex contraction with little change in reflex threshold and magnitude of reflex contraction. In contrast, Ss (3) with incomplete lesions revealed a higher detrusor reflex threshold, and reduced strength of detrusor contraction. This discrepancy may be ascribed to MO's action on the afferent limb of two distinct micturition reflex pathways, spinal and supraspinal, respectively. Soon thereafter (1-2 hrs.), volume evoked enhancement of EAUS EMG discharge and of vesico-sphincter dyssynergia appeared without a change in the threshold of EMG firing. Detrusor instability and vesical induced limb motor discharges during filling and contraction, both prominent features of the hyperreflexive micturition reflex system in SCL, were abolished. Alterations in bladder capacity persisted for 12-22 hrs., while augmented vesico-sphincter and suppressed vesico-limb motor reflexes, and detrusor stability endured considerably longer. Among both groups of Ss, the described effects on micturition reflexes were dose insensitive but were antagonized by i.v. naloxone (20 µg/kg). These observations suggest that i.t. MO interferes with vesico-vesical reflexes and vesico-somatic integration by binding to mu receptors, located at sacral spinal cord sites involved in regulating micturition reflexes.
(Partial Support: Spinal Cord Society and Catholic Medical Center)

361.5 CARDIOVASCULAR, CATECHOLAMINE AND ENKEPHALIN RESPONSES TO RESTRAINT STRESS: EFFECTS OF ADRENAL DEMEDULLATION AND/OR GUANETHIDINE. B.A. Barron, K. Pierzchala* and G.R. Van Loon, Department of Medicine, University of Kentucky and VA Med Center, Lexington, KY 40511.

Adrenal and sympathetic nerve contributions to plasma levels of Met-enkephalin immunoreactivity (ME) and catecholamines (CA) were studied using adrenal demedullation (DMED) and chemical sympathectomy. Adult male Sprague-Dawley rats were divided into four groups: Sham-operated/saline, Sham-operated/ guanethidine (GUAN) 25 mg/kg/day, DMED/saline, and DMED/GUAN. After 4 weeks of GUAN treatment or demedullectomy, rats were restrained for 30 min while blood samples were taken for CA and ME and cardiovascular parameters monitored. DMED decreased basal plasma epinephrine (EPI) and eliminated the increase seen during restraint stress. GUAN lowered plasma norepinephrine (NE) and decreased the stress-induced elevation. DMED plus GUAN eliminated the stress-induced plasma NE increase. These results indicate that the stress-induced increase in plasma EPI is derived from the adrenal medulla, whereas the stress-induced increase in plasma NE is derived from both the adrenal and sympathetic nerves. GUAN and GUAN plus DMED resulted in elevated basal plasma ME. Restraint stress caused an increase in plasma ME which was potentiated by DMED and eliminated by GUAN treatment. From these results we conclude that the source of basal plasma ME was not simply the adrenal medulla or the sympathetic nerves. However, the plasma ME response to restraint stress appears to derive from sympathetic nerves. GUAN treatment decreased the tachycardia seen in response to restraint stress. DMED had no effect on the heart rate response; therefore only the sympathetic nerves are involved in the tachycardia seen during restraint stress. GUAN attenuated the initial response in mean arterial pressure (MAP) and eliminated the prolonged pressor response to restraint stress. DMED potentiated the pressor response, while DMED plus GUAN produced an initial hypotensive response and totally eliminated the pressor response. Sympathetic nerves appear to be primary in mediating the pressor and tachycardic responses to stress, whereas the adrenal medulla may buffer the pressor response through vasodilatation. Possible roles for ME in altering these cardiovascular responses to stress are postulated.

361.6 PLASMA MET-ENKEPHALIN RESPONSES TO STRESS: COMPARISON OF MILD RESTRAINT WITH IMMOBILIZATION. K. Pierzchala,* P. Zeman,* R. Kvetnansky and G.R. Van Loon. VA Medical Center and Dept. of Medicine, University of Kentucky, Lexington, KY 40511 and Institute of Experimental Endocrinology, Centre of Physiological Sciences, Slovak Academy of Sciences, Bratislava, Czechoslovakia.

Met-enkephalin and related peptides derived from proenkephalin A are colocalized with catecholamines in the chromaffin cells of the adrenal medulla, in sympathetic ganglia and in sympathetic nerve terminals. However, regulation of the in vivo secretion of Met-enkephalin and these related peptides is poorly understood. We have examined the stress-induced secretion into plasma of Met-enkephalin, present both in free native form and in association with larger peptides (cryptic) Met-enkephalin. Rats received an indwelling carotid cannula for blood sampling, and blood was collected in citrate, EDTA and aprotinin for measurement of plasma Met-enkephalin by radioimmunoassay. We have compared the plasma Met-enkephalin responses to a mild restraint stress in plastic cylinders with the more severe stress of immobilization. In Sprague-Dawley rats exposed to a mild restraint stress, plasma Met-enkephalin increased from a basal level of 7.8±0.6 pM to a peak of 30.8±0.9 pM at 0.5 min, and the peak was maintained for only 2 min before gradually decreasing to basal level by 10 min in spite of continued restraint. Plasma concentration of cryptic Met-enkephalin was about 500 fold higher at 3738±251 pM and increased ($p<0.01$) to 5410±548 after 0.5 min of restraint. Of considerable interest, thirty min of restraint produced a second peak of both free and cryptic Met-enkephalin just before the end of stress. In a separate study, using Wistar rats, animals were completely immobilized for 150 min. In contrast with the transient response to milder restraint, immobilization produced somewhat greater increases in plasma concentrations of both native and cryptic Met-enkephalin by 1 min and these increases were maintained throughout 150 min of stress. Thus, plasma levels of both free and cryptic Met-enkephalin increase in response to acute stressful stimuli, but show differences in the pattern of responses when exposed to different stressors.

361.7 ENDOGENOUS OPIOID PEPTIDE MEDIATION OF THE ANTINOCICEPTIVE RESPONSE IN RATS WITH STREPTOZOTOCIN-INDUCED DIABETES MELLITUS. M. Kolta,* K. Pierzchala,* and G.R. Van Loon (SPON: J.A. Roy). Dept of Medicine, Univ of Kentucky and VA Med Center, Lexington KY 40511.

Alteration of pain perception represents a complication of diabetes mellitus in man and laboratory animal models. The present study was designed to examine possible mechanisms mediating this disordered pain perception in rats with diabetes induced by streptozotocin (STZ, 50 mg/kg, iv). We examined the time course of development of the antinociceptive response, the effects of treatment with insulin, and whether diabetes alters CNS and peripheral Met-enkephalin (ME) levels. STZ-induced diabetes in adult male Sprague-Dawley rats resulted in the gradual development over 6-7 weeks of a significant increase in the pain threshold as tested by the hot plate and/or tail flick latency tests. Insulin replacement therapy initiated after development of the hypoalgesic response normalized not only glucose levels and body weight but also the increase in hot plate latency in diabetic rats. This hypoalgesic response appears to be mediated through opioid receptors since naltrexone at either 1.0 or 0.3 mg/kg ip reversed completely the increase in hot plate or tail flick latency. In addition, the concentration of native ME in spinal cord of STZ diabetic rats was 5-fold higher than that of nondiabetic controls. Native, cryptic (after digestion with trypsin and carboxypeptidase B) and total ME concentrations were decreased in neurointermediate lobe and anterior pituitary, whereas none of these forms of ME were altered quantitatively in nucleus accumbens, neostriatum or hypothalamus of diabetic rats when compared with nondiabetic controls. There is a 3-fold increase in native ME concentration in plasma of diabetic rats. The concentration and content of cryptic and total, but not the native ME, were significantly reduced in the adrenal medulla. Concentration of native ME in the pancreas was significantly reduced in the diabetic rats. This study provides evidence for opioid peptide mediation in diabetic rats of antinociception which can be prevented by insulin treatment. It also provides evidence for altered synthesis and/or release of ME in some CNS regions and peripheral tissues of STZ diabetic rats. However, it remains to be determined whether ME is the endogenous opioid mediating this antinociception and whether the effect is mediated in CNS or periphery.

361.8 ENDOGENOUS OPIOID MODULATION OF CARDIOVASCULAR RESPONSES TO STRESS. L. Marson and G. R. Van Loon. VA Medical Center and Department of Medicine, Univeristy of Kentucky, Lexington, KY, 40511.

Under nonstressful conditions, systemic or central administration of naloxone does not alter mean arterial blood pressure (MAP) or heart rate (HR). However, naloxone increases MAP during haemorrhagic or endotoxic shock. Injection of naloxone directly into the paraventricular nucleus of the hypothalamus of conscious restrained rats potentiates the epinephrine response to restraint stress without affecting MAP or HR responses to stress. To further investigate the role of endogenous brain opioids on the regulation of MAP and HR during restraint stress, we compared the effects of naloxone injected acutely (5 or 50 nmoles in 10ul) with naltrexone administered chronically (54 nmoles/hour for 10 days via Alzet® minipumps) into the lateral ventricle of conscious rats. Rats were implanted with cannulae into the lateral ventricle and carotid artery. Blood samples for measurement of plasma epinephrine (EPI) and norepinephrine (NE) were taken at intervals during the experiment, and systolic pressure (SYS), diastolic pressure (DIAS), MAP and HR were recorded continuously. Prior to restraint stress, injection of naloxone had no effect on SYS, DIAS, MAP, HR or plasma EPI or NE concentrations. Restraint stress increased SYS, DIAS, MAP, HR and plasma CA levels. Naloxone, 5 nmoles, potentiated the tachycardia response to restraint, but attenuated the restraint stress-induced increase in DIAS, SYS, and MAP. Naloxone, 50 nmoles, attenuated not only the increases in SYS, DIAS and MAP, but also the stress-induced tachycardia. In the chronically treated rats, naltrexone attenuated the stress-induced tachycardia and blocked the hypotensive response which occurs following the transient pressor response to restraint seen in the chronic saline-treated rats. Thus, both acute and chronic administration of an opioid antagonist reduces the arterial blood pressure and HR changes seen in response to restraint stress. These findings support the involvement of an endogenous opioid peptide in modulating the acute changes in arterial blood pressure and HR during stress. (Supported by the Veterans Administration and the University of Kentucky Tobacco and Health Research Institute.)

361.9 NALOXONE-REVERSIBLE DIURNAL INCREASES IN NOCICEPTIVE THRESHOLD IN THE EQUINE. S.G. Kamerling, C.A. Bagwell*, and J.G. Hamra*. Dept. of Vet. Physiol. Pharmacol. and Toxicol., Sch. of Vet. Med., Louisiana State Univ., Baton Rouge, LA 70803.

A diurnal variation in nociceptive threshold has been observed in rodents and other species (Frederickson et al., Science 198:756, 1977). Some studies have correlated this time-dependent elevation in pain threshold with increases in brain opioid levels (Wesche and Frederickson, Life Sci. 24:1861, 1979). Recent studies from this laboratory demonstrated a diurnal rhythm in pain sensitivity in performance horses (The Pharmacologist 28:169, 1986). Nociceptive thresholds were significantly elevated at 0900h, compared to thresholds obtained at 0600h, 1200h, 1800h and 2400h. Plasma beta-endorphin levels were also significantly higher at 0900h than at any other time of day. Diurnal variation of other physiologic parameters was also observed. Cardiac rate and pupil diameter were significantly greater, while respiratory rate was significantly lower at 0900h than at most other times of the day. These data led to the hypothesis that the diurnal rhythm in nociceptive sensitivity and autonomic responses in the equine are endorphin/opioid-receptor mediated. To test this hypothesis, naloxone (0.75mg/kg, i.v.) was administered to 8 horses at 0900h. Three baseline nociceptive thresholds were determined at 10 minute intervals, after which naloxone or saline was administered according to a single-blind crossover design. Three post-treatment observations were made at 10 minute intervals for 30 minutes. Nociceptive thresholds were quantified by measuring the latency to onset (sec) of the skin twitch and forelimb flexion reflexes following noxious thermal stimuli. Naloxone produced a slight but significant reduction in the forelimb withdrawal reflex latency ($p<0.05$), and a similar reduction in the skin twitch reflex latency which approached statistical significance ($P = 0.05$). These decreases were only significant at 10 minutes postinjection. However, marked sustained increases in respiratory and cardiac rates were observed for 30 minutes following naloxone administration. A marked increase in the frequency of defecation was also observed during this period. These data extend previous observations and support the hypothesis that nociceptive threshold and autonomic function are under 'opioidergic' control in the equine.
Supported by a grant from the LSU-SVM Organized Research Fund.

361.10 EXERCISE AND PHYSICAL CONDITIONING ALTER BETA-ENDORPHIN RELEASE AND NOCICEPTIVE THRESHOLDS IN PERFORMANCE HORSES. Jena G. Hamra*, Steven G. Kamerling, Cleo A. Bagwell* and John F. Freestone* (SPON: Steven A. Barker). Dept. of Veterinary Physiology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803.

Intense physical exercise produces a stress-induced increase in plasma beta-endorphin levels in man (S.R. Gambert et al, Proc. Soc. Exp. Biol. Med 168:1, 1981). Recent studies have not demonstrated a correlation between exercise intesnsity and endorphin release nor have they shown alterations in pain threshold following exericse. The present study endeavored to demonstrate a relationship between intense physical exercise, beta-endorphin release, and nociception in horses subjected to racing conditions. The effect of physical conditioning on the response to intense exercise was also evaluated. Ten Thoroughbred racehorses were subjected to a 2 furlong gallop at maximal speed. This procedure was repeated after a 10 day interval with subjects receiving naloxone (0.75 mg/kg) prior to the gallop. The 2 furlong gallop was repeated again after a nine week conditioning period which consisted of a regimen of galloping, swimming and walking. Exercise intensity was then increased to a 5 furlong gallop with a pretreatment of saline or naloxone (0.75 mg/kg) prior to exercise. Nociceptive thresholds were quantified by measuring the latency (sec) to onset of the skin twitch reflex following thermal cutaneous stimulation. Plasma beta-endorphin levels were determined using radioimmunoassay. Measurements were obtained before and over a thirty minute period following the 2 and 5 furlong gallops. Intense exercise produced a significant prolongation of the skin twitch reflex latency which was blocked by naloxone administration. Physical conditioning attenuated the increase in pain threshold following the 2 furlong gallop. However, following the 5 furlong gallop there was again a significant increase in pain threshold which was blocked by naloxone. Plasma beta-endorphin levels were significantly increased following the 2 furlong gallop and returned to baseline faster in the conditioned animal. Significantly higher levels were noted following the 5 furlong gallop. Naloxone administration significantly prolonged the increase in beta-endorphin following both the 2 furlong and 5 furlong gallops. These data suggest that intense physical exercise produces analgesia in the horse which may be mediated by beta-endorphin and/or opioid receptors and that this response to exercise is modified by physical conditioning. (Supported by a grant from the LSU-SVM Organized Research Fund.)

361.11 TRANS-CRANIAL ELECTRO-STIMULATION INCREASES TAIL FLICK LATENCIES AFTER NOCICEPTIVE CHALLENGE. M. Skolnick, C.D. Collard#, R. Hamilton#, L. Hudson-Howard#, C. Hymel# and O. Wilson#. Neurophysiology Center, Univ. of Texas Health Sciences Center, Houston, TX 77030. I. Capel#. NE London Polytech. London, England.

The analgesia induced by Transcranial Electrostimulation treatment (TCET) in male 200 g Sprague-Dawley rats was measured in three experiments. Recent work suggests that this effect is mediated by endogenous opioid systems (Murray, J.B. et. al. this volume).

Electrical stimuli consisted of biphasic charge-balanced rectangular pulses with positive amplitudes of 10 microamperes and repetition rate of 10 hz. Stimulation was either continuous with a positive pulse width of .1 msec (Mode 1), or intermittent with a positive pulse width of 2 msec and an 8 sec pause between successive packets of 256 pulses (Mode 2).

The animals were placed in plastic restrainers and stimulated bilaterally through gold plated, stainless steel electrodes previously inserted into each pinna at the vertex of the antihelix. Analgesia was assessed using the modified tail flick test. Each animal's tail was immersed (1 inch) in water at a controlled temperature of 50°C. The time, averaged over three trials, from submergence to the first coordinated motor response was taken as the tail flick latency (TFL). The difference in TFL before and after TCET was taken as a measure of analgesia.

In Experiment One, rats stimulated with mode 1 or 2 were compared to a control group that did not receive TCET. Both modes 1 and 2 were found to produce statistically significant increases in TFL (45% and 25%, respectively) as compared to a control group that did not receive TCET ($n = 42$, $p < .001$). Mode 1 produced significantly greater increases in TFL than Mode 2 ($p < .05$).

The relationship between the duration of TCET and resultant analgesia was investigated in Experiment Two. On successive davs the animals were stimulated for 0, 10, 20, 30, 40, 50, or 60 min. with either Mode 1 or 2. Both Modes 1 and 2 produced analgesia which increased with stimulation time, reaching a maximum after 30 min. ($n = 24$, $p < .001$) and decreasing thereafter.

Experiment 3 measured the duration of analgesia following 30 min of TCET with either Mode 1 or 2. The degree of analgesia was assessed immediately, 40, 80, 120, 160, 200, and 240 min after stimulation. Mode 1 stimulation was found to produce a significantly longer lasting analgesia than mode 2 ($n = 31$, $p < .05$) with Mode 1 lasting 200 min and Mode 2 lasting 80 min. (Sponsored by NMR Centers, Inc.)

361.12 TRANSCRANIAL AURICULAR ELECTROSTIMULATION (TCET): NALOXONE-REVERSIBLE ATTENUATION OF PAIN SENSITIVITY AND OPIATE ABSTINENCE SYNDROME. J.B. MURRAY*, F.C. SCHWEITZER*, G.G. LOCKE*, L.A. SWEET*, S.G. CRON* AND D.H. MALIN. Univ. of Houston - Clear Lake, Houston TX 77058.

Certain parameters of electrostimulation delivered bilaterally to the ear have been reported to induce analgesia in the rat (Skolnick, M. et al, this volume). Experiment 1 attempted to confirm this effect and test whether it is naloxone-reversible and thus presumably endorphinergically mediated.

Under chloral hydrate anesthesia, 36 male 175g Sprague-Dawley rats were bilaterally implanted through the apex of the antihelix with gold plated stainless steel electrodes. Four days later, rats were placed in cylindrical plastic restrainers and connected through 200 k ohm to a computer-controlled stimulator. Rats received 30 min. of either zero stimulation ("sham treatment") or 10 Hz, 10 microamp, 2 msec rectangular pulses, grouped in packets of 256 pulses separated by 8 sec. pauses. Each pulse was constructed so that there was no net polarizing current. Fifteen min. before termination of stimulation, rats were injected s.c. with 3 mg/kg naloxone or with saline vehicle alone.

Each rat was tested on a "blind" basis for pain sensitivity (50°C wet tail flick) immediately before and after stimulation. Rats receiving TCET plus saline were the only group to show a significant pre/post increase in tail flick latency (0.93 secs ± 0.29 secs., $p<.01$). According to Dunnett's Test, the TCET + saline rats showed significantly greater increase than the TCET + naloxone, sham + saline or sham + naloxone groups, $p<.05$.

Since these results suggest an endorphinergic effect of TCET, it seemed possible that TCET might modulate opiate abstinence syndrome. In Experiment 2, 12 225g rats were rendered dependent by s.c. infusion for 7 days with 0.89 mg/kg/hr morphine sulfate via Alzet 2ML1 osmotic minipumps. A day after pump removal, animals received either TCET (as above, except with no pauses) or sham treatment. They were then placed in a clear plastic chamber and observed for 15 minutes under "blind" conditions for abstinence signs. The predominant signs were wet-dog shakes and genital licking/seminal ejaculation. TCET-treated rats showed 11.3 ± 2.6 overall signs as compared with 25.7 ± 2.7 signs for the sham-treated controls. This difference was highly significant, $p<.005$. TCET-treated rats also exhibited significantly fewer wet-dog shakes and genital licks. The anti-abstinence effect appears to be naloxone-reversible, since TCET failed to reduce abstinence signs when compared with sham treatment in rats injected with 3mg/kg naloxone s.c. The results suggest that TCET might possibly be of value in the management of opiate abstinence syndrome. (Supported by NMR Centers, Inc.)

361.13 THE ROLE OF SEROTONERGIC PATHWAYS IN ALFENTANIL-INDUCED MUSCLE RIGIDITY IN THE RAT. M.B.Weinger*, N.T.Smith*, T.L.Yaksh, and G.F.Koob.(SPON:D.A.Mac Neil).Dept.of Anesthesiology, Univ. of Calif., San Diego,Dept. of Neurosurg. Res.,Mayo Clinic, and Div.of Preclin. Neurosci., Scripps Clinic, La Jolla, CA 92037.

Muscle rigidity is a potentially life-threatening complication of high-dose opiate anesthesia. Recent work has implicated the brainstem raphe nuclei in the expression of opiate-induced rigidity. Direct injections of methylnaloxonium (MN), a quaterary opiate antagonist, in the area of the nucleus raphe pontis (RPn) significantly attenuated alfentanil (ALF) rigidity [Brain Res. 386:280,1986]. Like the other raphe nuclei, the RPn contains serotonergic cell bodies with both ascending and descending projections. Systemic pretreatment with ketanserin, a selective serotonin antagonist, prevents ALF-induced rigidity [Anesthesiol. 65:A344,1986]. The present studies were designed to further characterize the role of other brainstem sites in opiate rigidity as well as to assess the importance of descending serotonergic systems in ALF-induced rigidity.

Male Wistar rats were implanted with 23-gauge chronic guide cannulae aimed at discrete brainstem nuclei. Following intracerebral pretreatment with MN (0.125 μg) or saline, muscle rigidity was measured by electromyographic (EMG) recordings from the gastrocnemius muscle. After obtaining baseline measurements, ALF (0.5 mg/kg s.c.) was injected and data were collected for 60 min. In another study, rats were chronically implanted with intrathecal catheters. The effects of intrathecal injections of ketanserin (25-37.5 μg), methysergide (15-45 μg), or saline on ALF-induced changes in EMG activity were determined. Other animals were intrathecally pretreated with either 75μg of 5,6-dihydroxytryptamine or ascorbic acid vehicle and had EMG activity in response to ALF measured one week later.

MN injections in the region of the periaqueductal gray and deep layers of the superior colliculus as well as those in the region of the RPn and the nucleus reticularis tegmenti pontis significantly attenuated ALF rigidity. MN injections in the area of the raphe magnus, raphe pallidus, or the dorsal tegmentum had no effect. The intrathecal injection of the serotonin antagonists ketanserin and methysergide failed to attenuate ALF rigidity. In addition, a greater than 80% reduction in spinal serotonin levels after pretreatment with 5,6-dihydroxytryptamine did not blunt the rise in EMG activity due to ALF.

The results of these studies suggest that discrete hindbrain regions known to contain serotonergic and GABAergic pathways are involved in mediating ALF-induced muscle rigidity in the rat. Additionally, the site of serotonin's modulatory role appears to be supraspinal. Further studies will lead to an improved understanding of the central sites of opiate action.

361.14 ENKEPHALIN IN THE A10 REGION INCREASES DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS AS ASSESSED BY IN VIVO VOLTAMMETRY. D. Jackson, J.O. Schenk*, and P.W. Kalivas* (SPON: R. Kuczenski), Department of Veterinary and Comparative Anatomy, Pharmacology and Physiology, and Department of Chemistry, Washington State University, Pullman, WA.

A number of studies have shown that the injection of enkephalin analogues into the A10 dopamine (DA) region activates DA neurons projecting to the nucleus accumbens. Thus, intra-A10 injection of enkephalin analogues produces a motor stimulant action that is blocked by injection of DA receptor antagonists into the nucleus accumbens. Furthermore, this motor stimulant effect is associated with an increase in the postmortem levels of DA metabolites. In the present study, in vivo chronoamperometry was employed in the nucleus accumbens to evaluate a change in catecholamine release following injection of the mu opioid enkephalin analogue, DAGO, into the A10 region.

Male Sprague-Dawley rats were anesthetized with chloralose, mounted in a stereotaxic apparatus, and a carbon fiber (40 μm diameter) working electrode implanted into the nucleus accumbens. The reference and auxiliary electrodes were placed in contact with the dura, and a stainless steel injection cannula was implanted into the A10 region ipsilateral to the working electrode. An oxidation potential of 0.5 V was applied to the working electrode for 5 sec every 10 sec to oxidize catecholamines. After a stable baseline had been obtained (1-2 hr), a microinjection of DAGO (0.15 nmole in 0.5 μl) was made into the A10 region. Following a 90 to 120 sec lag, a large increase in the signal obtained from the nucleus accumbens was measured corresponding to 8 to 10 μM DA in the external calibration. After 30 to 90 sec the signal was reduced by 50%, and remained elevated at this level for 30 to 45 min. Although experiments are now underway to verify the content of the electrochemical signal measured in the nucleus accumbens, the large amount of DA in the accumbens and the previous postmortem data support the possibility that this signal is comprised predominately of DA or its metabolite DOPAC. Thus, these data corroborate the earlier postmortem studies demonstrating that intra-A10 injection of opioids activates DA transmission in the nucleus accumbens, and they illustrate a method whereby a detailed time course of this activation can be quantified.

361.15 INHIBITION OF EVOKED STRIATAL DOPAMINE RELEASE BY SELECTIVE KAPPA RECEPTOR AGONISTS. D.W. Clow* and K. Jhamandas. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada.

Kappa opioid receptor subtypes have been found to exist in the striatum of several species, but their functional significance is largely unknown. It has been reported that exogenous and endogenous kappa agonists are capable of depressing both the spontaneous and potassium-evoked efflux of ^{3}H-dopamine (Mulder et al., Nature, 308: 278, 1984); however, little is known about kappa agonist action on endogenous dopamine (DA) release.

In the present study we have addressed this question using both a highly selective synthetic kappa agonist, "U-50,488H" as well as a putative endogenous kappa agonist, "dynorphin (1-13)". Brain slice superfusion, coupled with an alumina/perchloric acid extraction, was used to collect and isolate the DA. Separation and quantitation of the DA was carried out by HPLC coupled to electrochemical detection. U-50,488H produced a concentration-dependent inhibition of potassium-evoked DA release which was insensitive to naloxone. A putative kappa receptor antagonist, Win 44,441-3, produced a time-dependent reversal of the U-50,488H mediated inhibition. Neither Win 44,441-3 nor naloxone (1.0 μM) influenced the spontaneous or evoked release of DA when applied alone. Dynorphin (1-13), in the presence of bestatin (20 μM), produced a concentration-dependent inhibition of potassium-evoked outflow (to a maximum of 60% of control) while having no effect on the spontaneous release.

These findings suggest that the striatal release of endogenous dopamine is modulated by kappa opioid receptors.

(Supported by the Medical Research Council of Canada and a Fellowship from the Ontario Mental Health Foundation)

361.16 OPIOID MU RECEPTOR AGONIST AND NMDA RECEPTOR ANTAGONIST PRODUCE SIMILAR DEPRESSION OF SYNAPTIC TRANSMISSION IN THE RAT SPINAL DORSAL HORN: AN IN VITRO STUDY. S. Jeftinija, Dept. Vet. Anatomy, Iowa State University, Ames, Iowa, 50011.

Much evidence has accumulated to suggest that excitatory amino acids (EAA) are produced by small sensory neurons and mediate excitatory neurotransmission in the mammalian spinal cord. Even more evidence has accumulated to suggest an inhibitory role of opioids in the superficial laminae of the dorsal horn. For both opioids and EAA a heterogeneity of receptors have been disclosed. In order to provide more direct information on the physiological role and mechanism of action of EAA and opioids in the dorsal horn we developed an in vitro preparation. The horizontal spinal cord slices (400-500um thick) and the functionally connected dorsal root with the dorsal root ganglion were obtained from 18-25 days old rats following lumbosacral laminectomy. Conventional intracellular recording from dorsal horn neurons and dorsal root ganglion neurons using 3M K-acetate-filled electrodes was employed. Dorsal roots were electrically isolated from the spinal cord slice and stimulated with pulses of different intensity and duration to evoke afferent volleys monitored intracellularly from dorsal root ganglion neurons. Low intensity stimulation (5-10V; 0.02ms) of the dorsal root activated only large myelinated fibers and evoked fast excitatory postsynaptic potentials (e.p.s.p.) in spinal dorsal horn neurons. Higher intensity stimulus (20V;0.2ms -35V;0.5ms), sufficient to excite small myelinated and unmyelinated afferent fibers and corresponding small dorsal root ganglion neurons resulted in a large and more complex e.p.s.p. Thus, the initial burst of action potentials was followed by a prolonged depolarization with or without subsequent firing of action potentials. Bath application of 2-amino-5-phosphonovaleric acid (2-APV; 1 to 2x10^{-4}M), which reversibly abolished N-methyl-D-aspartate (NMDA) responses, produced a pronounced depression of the e.p.s.p. Synaptic activation by volleys in low threshold fast conducting presumably Aß fibers was not affected by 2-APV. Depression of a portion of the e.p.s.p. induced by activation of small diameter primary afferent fibers was also produced with (D-Ala^{2}, N-Me-Phe^{4}, Gly^{5}-ol)-enkephalin (DAGO; 10^{-7}-5x10^{-6}M) in concentrations that did not have a measurable effect on resting membrane potential or conductance. However, concentrations of DAGO that hyperpolarized the neurons depressed the e.p.s.p. induced by activation of large myelinated primary afferent fibers. These findings are consonant with involvement of NMDA receptors in synaptic transmission from small diameter primary afferents. In addition, the data suggest that opioids modulate NMDA receptor mediated EAA excitatory synaptic action in the dorsal horn by acting at mu receptor sites. Supported by NIH Grant 2 S07 RR07034.

361.17 THE EFFECTS OF THE KAPPA OPIOID RECEPTOR AGONIST U 50488H ON KAINIC ACID NEUROTOXICITY. W. Lason*, J. N. Simpson* and J. F. McGinty. (SPON: W.R. Wooles) Department of Anatomy, School of Medicine, East Carolina University, Greenville, N.C. 27858.

The specific kappa opioid receptor agonist, U 50488H has recently been shown to possess anticonvulsive and antiischemic properties (Tortella, et al., J. Pharmacol. Exp. Ther. 237:49, 1986; Tang, A.H., Life Sci. 37:1475, 1985). We investigated the ability of this compound to prevent kainic acid induced neuronal loss in the rat hippocampus. All intracerebroventricular injections (i.c.v.) were performed through chronically implanted cannulae in conscious male Sprague Dawley rats. For histological analysis, rats were perfused transcardially with a 4% paraformaldehyde solution 72 hours after injection and microtome sections were stained with Richardson's Nissl stain. I.c.v. injection of 1 nmole of kainic acid evoked behavioral seizures and neuronal loss exclusively in CA3 and CA4 fields of the hippocampus. U 50488H (up to 200ug, i.c.v. or 10 and 20 mg/kg, i.p.) had no effect either on behavioral seizures or on neuronal loss resulting from i.c.v. kainic acid administration. In contrast to i.c.v. injection, systemic administration of kainic acid (10 mg/kg, i.p.) caused severe neuronal damage in several brain regions, notably in the CA1 fields of both hippocampi. Pretreatment of the rats with U 50488H (10 or 20 mg/kg, i.p.) delayed, in a dose dependent manner, the appearance of seizures, significantly decreased the number of wet dog shakes, and almost completely protected the CA1 hippocampal neurons from degeneration. In contrast, U 50488H did not protect these animals against status epilepticus, body weight loss, or against extensive neuronal degeneration in other brain regions, particularly entorhinal cortex. Our data indicate that U 50488H has a protective effect on the CA1 hippocampal neurons most vulnerable to anoxic-ischemic damage following systemic kainic acid. Supported by DA 03982.

361.18 **EXCITATORY AMINO ACID ANTAGONISTS INHIBIT THE EFFECTS OF INTRATHECAL DYNORPHIN (1-13).** **R.M. Caudle* and L. Isaac. Dept. of Pharmacology, Univ. of Ill. Col. of Med., Chicago, IL 60612.**

Previous work suggested that elevation of tail-flick latency in rats following an intrathecal injection of dynorphin (1-13) (DYN) resulted from neuronal death. An electrophysiological study of DYN's effect on spinal reflexes was undertaken to ascertain the specificity of this effect.

Male Sprague Dawley rats were anesthetized with pentobarbital. The spinal cords were exposed by laminectomy and severed at L1. Dorsal and ventral roots from spinal segment L5 were isolated. The dorsal root was placed over platinum stimulating electrodes while the ventral root was placed over platinum recording electrodes. DYN (80 nmols, N=3) applied *in situ* onto the spinal cord resulted in a potentiation of the C-fiber initiated reflex followed by a loss of the reflex. Reflexes initiated by afferents from groups I-III were not affected. In another group of rats DYN (80 nmols, N=5) or saline (20 microliters, N=10) was administered intrathecally 1-30 days prior to recording. A C-fiber initiated reflex could not be evoked in DYN treated animals that had lost the tail-flick. The group I-III initiated reflexes in these animals, however, were present. All reflexes were clearly evoked in the saline treated animals. These findings indicate that intrathecal DYN initiates an excitotoxic event resulting in loss of the C-fiber reflex which is expressed behaviorally as an irreversible elevation of the tail-flick latency.

To test the hypothesis that an excitatory amino acid mediates the toxicity observed after intrathecal DYN, varying doses of excitatory amino acid antagonists were co-injected with DYN (80 nmols). The N-Methyl-D-Aspartate (NMDA) receptor specific antagonist 2-amino-5-phosphonovalerate blocked the effects of DYN on the tail-flick at a dose an order of magnitude lower than the non-specific excitatory amino acid antagonist gamma-D-glutamylglycine.

These data demonstrate that dynorphin-induced neurotoxicity, in rat spinal cord, is mediated through the NMDA subclass of excitatory amino acid receptors.

361.19 PROTECTION AGAINST MORPHINE-INDUCED EXPLOSIVE MOTOR BEHAVIOR BY THE EXCITATORY AMINO ACID ANTAGONIST, 2-AMINO-7-PHOSPHONOHEPTANOIC ACID. Y. F. Jacquet. Behavioral Neuropharmacology Laboratory, Nathan Kline Institute, Orangeburg, NY 10962.

2-amino-7-phosphonoheptanoic acid (2-APH) is a potent antagonist of excitatory amino acid receptors, preferentially those activated by N-methyl D aspartic acid (NMDA). In the present study, an injection of (±)-2-APH (17.5 nmol/0.5 uL) bilaterally in the rat periaqueductal gray (PAG) 10 min prior to a bilateral (-)-morphine (53 nmol/0.5 uL) injected at this same site potently blocked the occurrence of the explosive motor behavior (EMB) that is normally seen after morphine (53 nmol) injected in this site.

We (Jacquet et al, Science 198:842,1977) previously reported that an injection of (+)- or (-)-morphine in the rat PAG resulted in an EMB (characterized by rapid and repeated high leaps and shrill distress vocalizations) that was not blocked by the potent opiate antagonist, naloxone. Recently, we (Squires et al, Neurosci.,12:660,1986) found that this nonstereospecific morphine action in the rat PAG could be mimicked by GABA antagonists (e.g., bicuculline methiodide, picrotoxin), and blocked by GABA or the GABA potentiator, diazepam; in receptor binding studies, morphine dose-dependently reversed the inhibition by GABA of the binding of ^{35}S-t-butylbicyclophosphorothionate (TBPS) to picrotoxin binding sites, similar to the action of othr GABA antagonists. Based on these findings, we concluded that morphine-induced EMB was due to GABA receptor antagonism. The present findings of 2-APH antagonism of morphine-induced EMB suggest that in addition to GABA receptor blockade in the PAG, an additional mechanism underlying EMB consists of the resulting unopposed excitatory actions of endogenously-occurring excitatory amino acids in the PAG.

An injection of N-methyl DL aspartic acid (NMDLA) (50 nmol/0.5 uL) in the rat PAG resulted in wild running, convulsions and occasional deaths (similar to the syndrome reported for intracerebroventricularly-injected mice by Croucher et al, Science 216: 899,1982). Since all behavioral components of EMB were not mimicked by NMDA injected at this site, we concluded that EMB is the result of the unopposed excitatory actions of other excitatory amino acid receptors in the PAG in addition to the NMDA receptor. Phencyclidine (PCP) given 10 min prior (10-25 nmol/0.5 uL) failed to antagonize, but potentiated, the actions of NMDLA (50 nmol/0.5 uL) at this site.

361.20 EARLY KAINATE-INDUCED CHANGES IN HIPPOCAMPAL GABA AND ENKEPHALIN METABOLISM. P. Hudson*, L. Grimes, P. Lee, S. Li* and J. Hong. (SPON: M. Bonner) Lab. of Behavioral and Neurological Toxicology, NIEHS/NIH, Research Triangle Park, NC 27709 and Toxicology Curr., UNC-CH, Chapel Hill, NC 27514

Systemic kainic acid (KA) causes a rapid increase in hippocampal metabolic and EEG activity (Lothman, E. W., Brain Res, 218: 299, 1981). Both KA and enkephalin (ENK) are thought to enhance hippocampal epileptiform activity by blocking release of GABA (Zieglgänsberger, W., Science, 205:415, 1979; Fisher, R. S., J. Neurosci., 4:1312, 1984), but the extent to which ENK mediates a KA-induced effect on GABA and the extent to which these interactions are involved in the initiation of behavioral seizures are unknown. It is clear, however, that lesions of the opioid-containing granule cells, which reduce hippocampal ENK by 50%, do not block KA-induced convulsions (Grimes, L., Neurosci. Abstra., 12:412, 1986), suggesting that ENK contained in another population of neurons may be responsible for putative actions on hippocampal excitability. The purpose of the present studies was to determine whether systemic KA affects hippocampal GABA or ENK metabolism prior to the onset of behavioral convulsions and whether such effects also occur following lesions of the opioid-containing dentate granule cells. Male Fischer-344 rats were used in these studies. Rats (30) were injected with KA (8 mg/kg, sc) or saline (SAL) and killed after exhibiting wet dog shakes (WDS) or seizures. Limbic brain regions were assayed for amino acids using high performance liquid chromotography (HPLC). Rats (30) were given unilateral injections of colchicine (COL) (1 ug/site) into dorsal and ventral hippocampus, treated with KA or SAL 2 weeks later, and killed prior to the occurrence of WDS or after seizure onset. Brain regions were taken for amino acid analyses or blot hybridization studies using a cDNA probe for proenkephalin mRNA. Brain regions from 32 rats, injected with a lower dose of KA (6 mg/kg) and killed 2.5 hr later, were dissected for radioimmunoassay of opioid peptides or blot hybridizations studies of proenkephalin mRNA. Increases in hippocampal GABA and proenkephalin mRNA were correlated with the number of WDS. These changes occurred from the onset of shaking behavior. Hippocampal ENK rose at the onset of shaking behavior and then gradually declined, indicating that the rate of precursor processing and peptide utilization were increased. These findings suggest that KA-induced interactions between hippocampal ENK and GABA are possible, but critical appraisal requires completion of lesion studies.

361.21 OPIOID MU AND DELTA RECEPTOR ANTAGONISTS REDUCE WET DOG SHAKING ELICITED BY PERFORANT PATH STIMULATION IN RATS. C.L. Mitchell, M.I. Barnes*, S. Rahmaan*, P.M. Hudson*, and J.S. Hong. Lab. Behav. Neurol. Toxicol., Nat'l. Inst. Environ. Health Sci., NIH, Research Triangle Park, NC 27709.

Wet dog shaking (WDS) elicited by perforant path stimulation (PPS) produces a decrease in hippocampal levels of methionine-enkephalin (Mitchell et al., Fed. Proc. 46: 6597, 1987). Moreover, *intrahippocampal injection of either μ or δ, but not K, opioid receptor agonists elicit* WDS (Obie et al., Fed. Proc. 46: 6598,1987). The present study was undertaken, therefore, to determine if either μ or δ receptor antagonists, or both, would reduce WDS elicited by PPS.

Three separate experiments were conducted. These compared effect of (1) β-FNA (μ antagonist) vs. vehicle; (2) ICI 174864 (δ antagonist) vs. vehicle; and (3) combination of β-FNA and ICI 174864 vs. vehicle. Each group of animals was used for only one experiment. Male Fischer-344 rats were implanted unilaterally with bipolar PPS electrodes. Forty-eight hours after surgery all animals were stimulated to determine the threshold for eliciting WDS. The animals were then paired according to this threshold. One of each pair was placed in the vehicle group and the other in the drug group. All injections were made intraventricularly in a volume of 5 μl. The vehicle was artificial cerebrospinal fluid. β-FNA was injected in a dose of 10 μg, 24 hrs. prior to testing. ICI 174864 was injected in a dose of 2 μg, 10 minutes prior to testing. Vehicle control animals were stimulated to the point of exhibiting approximately 75 WDS. Each animal in a drug group was stimulated the same number of times as the animal to which it was yoked in the control group.

The threshold for eliciting WDS was not altered by the antagonists. Both β-FNA and ICI 174864 significantly reduced WDS. For β-FNA the mean number of shakes was 49 vs. 76 for the vehicle group (VG). For ICI 174864 the mean was 53 vs. 79 for the VG. The combination also reduced WDS (mean of 53 vs. 78 for the VG) but was not more effective than either agent alone.

These results lend further support to the notion that endogenous opioid peptides play a role in regulation of hippocampal excitability. Moreover, they suggest that both μ and δ receptors may be involved in this type of WDS.

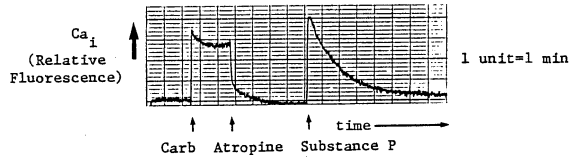
361.22 INTRAHIPPOCAMPAL INJECTION OF OPIATE PEPTIDES PRODUCE WET DOG SHAKES, CONVULSIONS AND CHANGES IN THE LEVELS OF AMINO ACIDS. P.H.K. Lee, J. Obie*, P. Hudson* and J.-S. Hong. Laboratory of Behavioral and Neurological Toxicology, NIEHS, NIH, Research Triangle Park, NC 27709.

Opiate peptides have been demonstrated to produce wet dog shakes (WDS) and epileptiform discharges in rats when administered intraventricularly. The purpose of this study is to obtain further information to support the hypothesis that opioid peptides in the hippocampus may play a role in mediating WDS and seizure activities. Injections of specific mu receptor agonist, [N-MePhe^3, D-Pro]$_2$morphicaptin (PLO17, 1-10 μg), and delta receptor agonist, [D-Ala^2, D-Leu^5]-enkephalin (DADLE, 1-10 μg), to the ventral hippocampus produced convulsive seizures and numerous WDS, suggesting that both receptors are involved in the mediation of these behaviors. However, injections of high dose of PLO17 (10 μg) to the dorsal hippocampus, frontal cortex and striatum produced neither WDS nor convulsions, therefore indicating that the ventral hippocampus is a sensitive site for these actions of opiate peptides. The levels of hippocampal glycine and gamma-aminobutyric acid (GABA) were increased but aspartate was reduced after PLO17 injection. Pretreatment with Beta-funaltrexamine hydrochloride (B-FNA), an irreversible mu receptor blocker, attenuated PLO17-induced WDS and convulsions. It also restored the PLO17-induced changes in the levels of hippocampal amino acids to that of control values. These results suggest that opiate-induced WDS and convulsive seizures are receptor mediated and may be acting through a disinhibition mechanism by attenuating the release of inhibitory amino acids in the hippocampus. Nevertheless, the degeneration of hippocampal granule cells by intrahippocampal injection of colchicine attenuated PLO17-induced WDS but potentiated the severity of convulsions, therefore suggesting that these two behaviors may be mediated by different pathways in the hippocampus. These data give further evidence to the idea that opioid peptides in the hippocampus may play an important role in regulating hippocampal excitability.

PEPTIDES: PHYSIOLOGICAL EFFECTS II

362.1 SPECIFIC-DESENSITIZATION OF SUBSTANCE P RESPONSES. B.R. Talamo, S. Soltoff, and M.K. McMillian. Depts. of Neurology and Physiology, Tufts Med. School, 136 Harrison Ave., Boston, MA 02111

Three types of receptors mediate increases in cytoplasmic calcium (Ca_i) in rat parotid acinar cells, but only the response to substance P rapidly desensitizes. Dissociated acinar cells loaded with Fura 2 show a sharp increase in Ca_i on addition of the muscarinic agonist carbachol, the α-adrenergic agonists phenylephrine and norepinephrine or substance P. Ca_i remains



elevated for many minutes in the presence of carbachol or norepinephrine, but drops off precipitiously after substance P addition, falling to 50% of the maximaum value within 2 min. The tachyphylaxis to substance P cannot be overcome by the subsequent addition of more substance P. Furthermore, it is specific to substance P; carbachol added subsequently can still stimulate an increase in Ca_i. Desensitization appears to occur more rapidly than depletion of the intracellular pool of calcium. In the presence of extracellular EGTA, carbachol can completely deplete this intracellular pool so that subsequent addition of substance P has no effect. However, when substance P is added first, Ca_i rises transiently and falls to resting levels, but subsequent addition of carbachol can still elevate Ca_i.

Other responses to substance P also show tachyphylaxis. The generation of ^{3}H inositol-phosphates by substance P in cells preloaded with ^{3}H inositol is similar to that generated by carb at early times (1 min or less), but reduced relative to that by carb at all times after 3 min. These results indicate that the specific desensitization to substance P is not due primarily to inactivation of the peptide and must be at an early step, possibly at the receptor or as a result of its interaction with Plipase C and mobilization of Ca^{++}. It appears that the substance P response shows important differences from that to muscarinic and α-adrenergic agonists in spite of the strong parallels in mechanisms of cellular activation. Further studies characterizing the calcium requirement for desensitization and the time dependence of desensitization and recovery are in progress.

362.2 BLOCKADE OF THE PRESSOR AND TACHYCARDIC RESPONSES TO SUBSTANCE P IN ANESTHETIZED RATS. J.C. Hancock and T.W. Smith*, Dept. Pharmacol., East TN State University, Quillen-Dishner College of Med., Johnson City, TN 37614.

At high doses, substance P (SP) evokes a pressor response in anesthetized rats (Hancock and Hoover, Pharmacologist, 1983). The present study was directed at determining the mechanism of that effect. Experiments were conducted in Sprague-Dawley rats anesthetized with 1.2 g urethane/kg. Arterial pressure was recorded from the left femoral artery. Drugs were injected into the rt. jugular sinus. Intravenous injection of 2.5 to 50.0 μg substance P/kg caused short duration, dose-dependent increases in peripheral arterial pressure and heart rate which were not blocked by the ganglion blocking agents, hexamethonium or chlorisondamine in doses that prevented the blood pressure and heart rate response to bilateral carotid occlusion. Following ganglion blockade, the threshold for initiating the pressor response was lowered from 4.3 ± 0.04 μg SP/kg (n=12) to 1.6 ± 0.15 μg/kg (n=10), ($p < 0.5$). The magnitude of pressor response to 10 μg SP/kg was increased from 27 ± 4.8 mmHg to 33 ± 2.4 (n=7 : $p < .01$). Pretreatment of rats, 24 hrs. prior to the experiment, with 1 mg reserpine/kg i.p. totally prevented the pressor and tachycardic responses to 10 g SP/kg (n=6).

Propranolol 0.5 to 1.0 μg/kg i.p. significantly reduced the tachycardic ($p < .05$ n=4) but not the pressor response to 10 g SP/kg. Higher doses (5-20 mg/kg) i.v. selectively reduced the tachycardic response in only 4 of 7 rats. Phentolamine, 1 to 7 mg/kg, significantly decreased the pressor ($p < .001$) but not the tachycardic response to 10 μg SP/kg. Phenoxybenzamine prevented both the pressor and tachycardic response to 10 μg SP/kg (n=5).

It is concluded that SP has an effect on autonomic ganglia to increase sympathetic nervous system activity. This effect is not evident with lower doses of SP (.005 to 1 μg/kg) because of the prominent action of SP in these doses to cause vasodilation. A central nervous system site of action is unlikely since nicotinic ganglion blockade in doses that prevented the pressor and tachycardic response to carotid occlusion did not prevent the pressor and tachycardic response to SP. The blockade of the pressor and tachycardic responses caused by SP by phenoxybenzamine is consistent with the observation that phenoxybenzamine blocks tachykinin receptors (Buck and Burcher, Neuropeptides, 1987, 9, 33-39). (This study was funded by a grant from the East Tennessee State University Research Development Committee).

362.3 NEUROTENSIN FACILITATES RELEASE OF SUBSTANCE P FROM PRIMARY AFFERENT NERVE TERMINALS IN THE GUINEA PIG INFERIOR MESENTERIC GANGLION. W.H. Stapelfeldt*, V.L.W. Go* and J.H. Szurszewski (SPON: E. Richelson). Department of Physiology and Biophysics and GI Unit, Mayo Medical School, Rochester, MN 55905.

We previously reported that neurotensin (NT) or a related transmitter of central preganglionic nerves facilitates peripheral sympathetic reflex activity through the guinea pig inferior mesenteric ganglion (IMG) by presynaptically releasing a non-cholinergic excitatory transmitter from peripheral afferent nerve terminals (Soc. Neurosci. Abst. 12: 1496, 1986; Gastroenterology 92: 1651, 1987). Since substance P is known to function as an excitatory afferent transmitter in the IMG, the present study was designed to determine the possibility of substance P mediating this response to NT. The IMG and attached lumbar colonic nerve trunks (LCN) were dissected from normal and capsaicin treated (100 mg/kg s.c.) guinea pigs, pinned down in an organ bath and superfused with oxygenated Krebs solution at 37°C. Intracellular recordings were obtained from ganglion cells using 3M KCl filled borosilicate glass micropipets (35-55 MΩ), LCN trunks were stimulated with bipolar platinum electrodes, and peptides were administered by addition to the superfusion medium. While 61% of neurons studied in ganglia from normal untreated guinea pigs exhibited a transient membrane depolarization (5.1 ± 1.2 mV) during superfusion of NT^{8-13} (5 μM), no such response was observed in ganglia from capsaicin-treated animals. Slow EPSPs in response to repetitive LCN stimulation were still evoked in most of the neurons tested indicating integrity of capsaicin-insensitive noncholinergic afferent pathways in these ganglia. Subsequent radioimmunological analysis of the neuropeptide content of these ganglia and also of the distal colon, celiac-superior mesenteric plexus, dorsal root ganglia (L1-L4), and lumbar spinal cord of the same animals revealed that material coeluting with synthetic porcine substance P on HPLC (10-60% acetonitrile, 0.1% morpholine) was depleted from the prevertebral and dorsal root ganglia, reduced by 50% in the spinal cord and by 30% in the colon of capsaicin treated animals, while the peptide content of other excitatory afferent transmitter candidates such as VIP, CCK, and bombesin was not different in these tissues compared to untreated normal animals. NT-induced transient membrane depolarizations and associated increases in conversion of evoked (LCN) subthreshold fast EPSPs to action potentials in normal ganglia were abolished or markedly attenuated after desensitization of ganglion cells to exogenously superfused substance P. In conclusion, these findings suggest that the facilitatory action of NT on peripheral afferent synaptic input to ganglion cells is mediated by release of substance P from nerve terminals of primary afferent neurons in the IMG. (Supported by DK 17632 and DFG Sta 252/1-1.)

362.4 INHIBITORY ACTIONS OF GALANIN AND SOMATOSTATIN 28 ON RAT SPINAL DORSAL HORN NEURONS. M. Randic, G. Gerber*, P. D. Ryu*, and I. Kangrga* (SPON: W. Steele). Dept. Vet. Physiol. and Pharmacol., Iowa State University, Ames, IA 50011.

Galanin, a 29 amino-acid peptide and somatostatin 28 (SS-28) are widely distributed in the rat central nervous system, including the spinal dorsal horn as demonstrated in immunohistochemical and radioimmunological studies. High density of galanin binding sites was shown in the superficial layers of the rat spinal dorsal horn. The effects of dorsal rhizotomy and capsaicin treatment suggested that galanin occurs in unmyelinated primary afferent fibers. SS-28 immunoreactivity is present in small dorsal root ganglion cells. Since actions of galanin and SS 28 on spinal dorsal horn neurons are still unknown, we examined the effects of these peptides on membrane properties and fast and slow excitatory synaptic transmission in the rat spinal dorsal horn.

Rats 16 to 22 days were used. Transverse or horizontal spinal cord slices, the latter with attached dorsal roots and ganglia were made. Cells were activated either directly with current injection via the bridge circuit, or synaptically by electrical stimulation of a lumbar dorsal root or dorsal root ganglion neurons. Peptides were applied by bath perfusion. Standard techniques were used for intracellular recording from dorsal horn neurons.

Both galanin (10^{-7} to 10^{-6}M for 1 to 7 min) and somatostatin 28 (10^{-7} to 10^{-6}M for 1 to 2 min) hyperpolarized dorsal horn neurons and caused reduction in frequency of presumptive spontaneous synaptic potentials. In 2 cells, however, a distinct depolarization was observed with galanin. While the hyperpolarization produced by galanin appears to be associated with an increase in neuronal input resistance, in the case of somatostatin 28 a fall in resistance was recorded. In the presence of TTX and TEA, galanin produced a reversible decrease in the duration of calcium spike. In the same cells apamin (5 x 10^{-8}M for 2 min), an 18 amino-acid polypeptide constituent of bee venom known to cause a block of $g_{K(Ca)}$ in a variety of tissues including spinal motoneurons, depolarized dorsal horn neurons and produced an increase in the duration of calcium spikes.

Galanin caused a marked, dose-dependent decrease in the amplitude and duration of fast and slow excitatory synaptic potentials. The depressant effect of galanin on the fast and slow excitatory synaptic transmission was reversible and the full recovery was observed within 10 to 20 min after removal of the peptide from the bath.

Our results are consistent with a neuromodulator role for galanin and somatostatin 28 in the rat spinal dorsal horn. The data obtained with apamin are consistent with the possibility that apamin increases the duration of the Ca spike by blocking $g_{K(Ca)}$ of dorsal horn neurons. Supported by NSF and USDA.

362.5 SOMATOSTATIN CAUSES HYPERPOLARIZATION OF CA1 PYRAMIDAL NEURONS BY ACTIVATION OF A MEMBRANE K^+ CONDUCTANCE. T.W.J. Watson and Q.J. Pittman. Neuroscience Research Group. University of Calgary, Calgary, Alberta, Canada T2N 4N1.

The cyclic tetradecapeptide somatostatin (SS_{14}) is a putative CNS neurotransmitter. We consistently observe a hyperpolarizing action of SS_{14} on rat CA1 pyramidal neurons and the purpose of these experiments was to examine the ionic mechanism underlying this hyperpolarization. 400 μm thick coronal rat hippocampal slices were placed in the well of a recording chamber and completely submerged and continuously perfused with oxygenated (95% O_2/5% CO_2) artificial CSF (conc. mM: NaCl 124, NaH_2CO_3 26, glucose 10, $MgSO_4$ 2, KCl 1.8, KH_2PO_4 1.25, $CaCl_2$ 1.5, pH 7.4) at a rate of 1.5-2.0 mls/min and maintained at 32-24°C. Intracellular voltage recordings from CA1 pyramidal neurons were obtained using high resistance (100-180 mΩ K^+-acetate) glass capillary microelectrodes and conventional recording techniques. Known concentrations of SS_{14} were added to the bath via an alternate perfusion line linked through a 4-way stop cock. 85% of CA1 pyramidal neurons exposed to 2μM SS_{14} exhibit a 3-8 mV hyperpolarization and suppression of spontaneous firing with response threshold being in the nanomolar range. This hyperpolarization is associated with an increase in membrane conductance and persists in 1 μM TTX and low Ca^{++} (0.5 mM) high Mg^{++} (3.5mM) where synaptic transmission is blocked. The response is blocked by membrane hyperpolarization but does not show a clear reversal potential. This SS_{14} induced hyperpolarization is not altered by recording with 2M KCl filled electrodes and thus is not Cl^- dependent. The action of SS_{14} persists in 10 mM extracellular tetraethylammonium (TEA) and following intracellular Cs loading using Cs acetate electrodes; under these conditions, in addition to hyperpolarization, SS_{14} also reduced the duration of the characteristically broadened action potentials. No change in the AHP was noted. In 5 out of 5 cells the hyperpolarizing response to SS_{14} was completely blocked by 1 mM tetrabutylammonium and was significantly reduced in 8 cells by 1 mM extracellular $BaCl_2$. We conclude that in rat CA1 pyramidal neurons, SS_{14} exerts a direct hyperpolarizing action mediated by activation of a membrane K^+ conductance that is relatively resistant to TEA and Cs.

362.6 SOMATOSTATIN REDUCES EXCITABILITY BY ENHANCING THE INWARD RECTIFICATION IN CULTURED LOCUS COERULEUS NEURONS. M. Inoue*, S. Nakajima and Y. Nakajima, Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN 47907.

Somatostatin is a neurotransmitter which produces neuronal hyperpolarization through an increase in K^+ conductance (Katayama & North, J. Physiol., 303:315, 1980); however, it is not clear what type of K^+ channels are involved. Dissociated neurons from the locus coeruleus of newborn rats were cultured for about 3 weeks (Masuko et al., J. Neurosci., 6:3229, 1986). Whole cell clamp experiments revealed that application of 0.1 μM somatostatin by pressure ejection produced a slow outward current when $[K^+]_o$ was 2.5 mM and the holding potential was -70 mV. Somatostatin-sensitive currents at various potentials were computed by subtracting control currents from currents during somatostatin application. The relationship between the somatostatin-sensitive currents and potentials revealed an inward rectification, currents increasing in the hyperpolarizing direction and decreasing in the depolarizing direction. The somatostatin-sensitive current reversed its polarity near the K^+-equilibrium potential (-105 and -86 mV at $[K^+]_o$ = 2.5 and 5 mM, respectively). Cs^+ and Ba^{2+}, each at 0.1 mM suppressed the somatostatin-induced conductance in a voltage-dependent manner. Calcium-dependent K^+ conductance does not seem to be involved in the somatostatin-induced response because: (1) apamin at 20 nM had little effect on the response, while apamin abolished the slow after-hyperpolarization produced by repetitive action potentials; (2) TEA^+ (2 mM) had only a small effect on the somatostatin response, while the TEA^+ considerably inhibited the outward currents produced by depolarization to -20 mV. The somatostatin-induced response was almost completely blocked by treating the neurons with pertussis toxin (500 ng/ml) for at least 18 hours. When the patch pipette was filled with GTP-γ-S (500 μM), somatostatin application produced an almost irreversible outward current. We conclude that the somatostatin-induced hyperpolarization, which occurs over a high membrane potential range (say, more negative than -70 mV), is caused by an enhancement of the inwardly rectifying channels, and the response is mediated through a G-protein (possibly Gi or Go) that is sensitive to pertussis toxin. Supported by NIH grant AG06093.

362.7 **INTRATHECAL SOMATOSTATIN CAUSES HINDLIMB PARALYSIS AND REDUCES SPINAL CORD BLOOD FLOW IN RATS.** J.B. Long, A. Martinez-Arizala*, J.M. Kraimer[1,]* and J.W. Holaday. Neuropharmacol. Br, Dept. of Med. Neurosci., Div. of Neuropsych., and [1]Div. of Surg., Walter Reed Army Inst. of Res., Wash., D.C. 20307-5100.

Somatostatin (SOM) is distributed throughout the CNS and has been linked to diverse neuronal functions. When injected into the subarachnoid space of the rat lumbar spinal cord (SC), SOM causes loss of hindlimb (HL) motor and nociceptive function. Intrathecal (i.t.) dynorphin A (1-13) and arg[8]-vasopressin also produce flaccid HL paralysis in rats which is associated with striking reductions in lumbosacral SC blood flow (BF) (Long et al., NIDA Monograph 75: 524, 1986 and Fed. Proc. 46: 1125, 1987). In these experiments we determined whether paralytic i.t. doses of SOM also alter SC BF, and examined SOM effects on HL function and SC perfusion following pretreatment with the SOM receptor antagonist cyclo [7-aminoheptanoyl-Phe-D-Trp-Lys-Thr(Bzl)].

HL motor function was examined following intervertebral injection of SOM through 30 ga needles into the L4-L5 subarachnoid space of halothane anesthetized 300-350 g SD rats. Neurological evaluations using a 0-3 grading scale were made at varied intervals following injections. SC BF was examined following SOM injection through PE 10 i.t. catheters (8.5 cm) into the lumbar subarachnoid space of rats anesthetized with ketamine and xylazine (50 and 10 mg/kg, i.m., respectively). Two sequential injections of radiolabeled microspheres (10 min preceding and 5, 10 or 60 min following i.t. SOM injections) provided pre- and post-treatment measurements of cardiac output (CO) and BFs to the brain and cervical, thoracic, and lumbosacral SC.

SOM (1-25 nmoles) produced a dose-related HL paralysis within 1-3 min of injection. With lower doses of SOM (1-6 nmoles), rats recovered some degree of HL motor function within 48 hrs of injection. Higher SOM doses (12-25 nmoles) produced persistent motor impairment. SOM (6.2 and 12.5 nmoles) also significantly reduced BF to lumbosacral SC (41 and 80%) without altering CO or reducing BFs to the brain or other SC regions. The SOM antagonist (5 min pretreatment with 0.3 nmoles) blocked the HL paralysis produced by 3.1 and 6.25 nmoles of SOM, and significantly improved neurological recovery 24 hrs following i.t. injection of 12.5 nmoles of SOM. At this dose, the SOM antagonist also blocked lumbosacral BF reductions produced by 6.25 moles of SOM, and did not by itself alter SC perfusion. However, higher doses of the antagonist (0.6-2.5 nmoles) produced HL paralysis, indicating that this compound is a potent partial agonist at the receptor sites underlying these neurological deficits. Thus, SOM produced HL paralysis and reduced SC BF through interactions with a recognition site which could be at least partially blocked by the SOM antagonist. These results indicate a need for concern over the clinical application of i.t. SOM for analgesia (Chrubasik et al., The Lancet 2: 1208, 1984), and also suggest a possible role of endogenous SOM in SC injury mechanisms.

362.8 PROLONGED EXERCISE ELEVATES NEUROTENSIN LEVELS IN BLOOD AND ADRENAL GLANDS IN FEMALE RATS. J. F. Axelson and C. F. Ferris. Psychology Department, Holy Cross College, Worcester, MA 01610, Physio. Dept., Univ. Mass. Med. Cent., Worcester, MA 01520.

To examine the effects of prolonged exercise on neurotensin levels in adrenal glands and in systemic circulation, samples from female rats forced to SWIM were compared to control animals subjected to different treatments for the same duration as SWIMMERS. These control groups consisted of animals: 1) forced to wade in water; 2) RESTRAINED in small plexiglass chambers; and 3) living in SEDENTARY conditions. Daily SWIMMING, WADING, and RESTRAINING manipulations were increased in 15 minute increments until animals reached a maximum of 2.5 hours and then continued for one month. At the completion of the last day of exercise or control manipulations, animals were decapitated to obtain adrenal glands and blood samples. SWIM and RESTRAINED groups had three and two-fold increases in corticosterone, respectively, when compared to both WADING and SEDENTARY animals, which did not differ. A six-fold increase in mean adrenal neurotensin-like immunoreactivity (NTLI) content was seen in SWIMMERS when compared to all other groups.

In a second experiment, animals forced to SWIM were compared to SEDENTARY animals. After four weeks these animals had reached 2.5 hours of daily swimming and all animals were anesthesized with ether, the adrenals were removed, and blood collected from the abdominal aorta. Pools of adrenal glands and plasma were extracted and processed for the chromatographic (HPLC) and immunochemical characterization of neurotensin for the respective tissue. SWIMMER's pooled adrenal glands showed a pronounced elevation of NT (655 fmol/gm) when compared to SEDENTARY animals (209 fmol/gm). Following HPLC, NT and its' N-terminal metabolites, were two-fold higher in abdominal aorta blood samples in SWIMMERS when compared to controls. Recently, we have found that although adrenal NT levels appear to be chronically elevated in animals exercising for prolonged periods of time, NT levels immediately following swimming are elevated from those seen prior to exercise manipulations. In summary, forced swimming appears to be a reliable method for inducing a pronounced increase in the level of NT in both the adrenal gland and systemic circulation.

362.9 THE NEUROTENSIN (NT) ANALOGUE XENOPSIN ALSO EXCITES NIGRAL DOPAMINE NEURONS AND INDUCES HYPOTHERMIA IN RATS. S. Bischoff*, M. Heinrich*, E. Küng*, M. Pozza*, M. Schaub*, K. Stöcklin* and A. Vassout* (SPON: G.E. Fagg). Pharma. Res. Dept., Ciba-Geigy Ltd., 4002 Basle, Switzerland.

The tridecapeptide NT exerts many peripheral and central biological activities in rats and other species. Several natural structurally-related analogues have been isolated, among them Xenopsin, discovered in the skin of the frog Xenopus Laevis. This octopeptide presents analogies with the C-terminal end of NT and possesses a Trp residue in the corresponding 11 position of NT.
In this study, we compared the effects of Xenopsin with NT, the fragments NT_{8-13}, NT_{1-8}, and the analogue D-Trp^{11} a) in NT radioligand binding assays, b) on nigral DA neuronal firing rate, and c) on body temperature, all tests being performed in rats.
Xenopsin competed with NT binding sites in brain with IC_{50}'s of 300 and 9.5 nM in ^{3}H- and ^{125}I-NT radioligand binding assays respectively. In comparison, the IC_{50}'s of the other peptides were the following for competition with both radioligands: NT: 15/2.2 nM; NT_{8-13}: 2/1.5 nM; D-Trp^{11}: 1000/1570 nM. NT_{1-8} was inactive.
In electrophysiological studies, Xenopsin excited DA neurons in the substantia nigra (SN) pars compacta. The recordings were made on 400 µm coronal sections of the SN at 0.1 µM. Increased DA neuron firing rate was also found with NT, NT_{8-13} and D-Trp^{11} at 0.1 µM whereas NT_{1-8} was inactive. Compared to NT, all active peptides were equally or even more potent than NT.
Finally, effects on thermoregulation were evaluated by icv injection in rats implanted with cannula into the left lateral ventricle. Xenopsin produced hypothermia comparable to NT in its amplitude (-3°C), duration of action (about 2 h) and estimated ED_{50} (10 µg). D-Trp^{11} was more and longer active (ED_{50}: 0.17 µg), NT_{8-13} much less (ED_{50}: 130 µg) and NT_{1-8} inactive.
Although the existence of Xenopsin in rat brain is still a matter of controversy, our data confirm an interaction with low and high affinity NT binding sites and demonstrate that this analogue of NT can mimic both in vitro and in vivo biological activities of NT. Whether Xenopsin acted via its own or via NT receptors remains to be clarified.

362.10 SPECIES DIFFERENCES IN THE EFFECTS OF BENZOTRIPT, A CHOLECYSTOKININ ANTAGONIST, IN THE HIPPOCAMPAL CA3 REGION. D.B. Jaffe*, P.G. Aitken, J.V. Nadler (SPON: M.M. Okazaki). Depts.of Physiology and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

The rat and guinea pig differ in the distribution of neuronal cholecystokinin-like immunoreactivity (CCK-IR) in the hippocampal CA3 region. In the rat, CCK-IR is found only in interneurons, whereas in the guinea pig CCK-IR is found in the mossy fibers as well as in interneurons. We undertook the present study to determine if these species differ in the effects of CCK and of benzotript (BZT - a CCK antagonist) on CA3 electrophysiology.

Transverse hippocampal slices were prepared from Sprague-Dawley rats or Hartley guinea pigs and maintained at 35.5°C in an interface chamber. An extracellular recording electrode was placed in stratum pyramidale of CA3b, and a monopolar stimulating electrode was positioned to activate the mossy fibers. Electrode positions were adjusted to maximize the mossy fiber (MF)-evoked response. Input/output (I/O) curves were generated by plotting the amplitude of the MF-evoked response versus stimulus current over a range of stimulus intensities. Drugs were applied by dissolving them in the perfusion medium. CCK octapeptide, sulphated, was applied at 100nM and BZT was applied at 200µM. These concentrations were chosen to achieve a maximal effect based on the reported affinities of these compounds for CCK receptors. I/O curves were generated before drug application, after 30 minutes of perfusion with drug-containing medium, and after 30 minutes of drug washout. During drug application, slices were monitored for the occurrence of spontaneous synchronized activity ("bursts"). To provide a single statistic indicative of any drug-induced changes in I/O curves, a control value was first determined as the average area under the pre-drug and post-drug I/O curves. The area under the experimental I/O curve was then determined and expressed as a percentage of the control value. Statistical significance was assessed by performing a 2-way ANOVA on the raw I/O data. CCK had no effect on spontaneous activity or I/O curves in either rat or guinea pig slices. In contrast, BZT caused spontaneous bursting in both rat (0.53 ± 0.14 Hz) and guinea pig (0.55 ± 0.31 Hz). In addition, BZT had opposite effects on I/O curves in these two species. In rat slices, BZT increased the area under the I/O curve by 47%, indicating enhanced synaptic transmission or pyramidal cell excitability. In guinea pig slices, BZT decreased the area under the I/O curve by 28%, indicating a net depressant effect. All effects of BZT reversed upon washout. These results imply that endogenous CCK has a primarily depressant action on area CA3 of the rat, but a primarily excitatory action in area CA3 of the guinea pig. This difference may be related to the different anatomical locations of CCK-IR in the two species.
(Supported by NIH grant 17771).

362.11 EFFECTS OF CCK-8 IN COMBINATION WITH OTHER GUT PEPTIDES ON FOOD INTAKE IN MICE. A.J. Silver,* J.F. Flood and J.E. Morley. GRECC, VA Medical Center, Sepulveda, CA 91343

A number of gastrointestinal peptides inhibit food intake. It has been suggested that a combination of these peptides may act physiologically to terminate a meal. This research tests the hypothesis that combinations of peptides have an additive effect on food intake. Further, it is postulated that somatostatin may attenuate bombesin's effect on food intake by reducing bombesin's ability to release CCK.

The effects of CCK-8, bombesin (BOM) and gastrin releasing peptide (GRP), inhibited food intake relative to saline treated mice in a dose response manner. Doses of CCK-8, BOM or GRP which inhibited feeding by about 20% were combined with doses of somatostatin (SOM, 80 ug/kg) or glucagon (GLU, 5 ug/kg) which also inhibited feeding about 20%. When BOM or GRP were combined with SOM the observed effect on feeding was less than one would predict assuming simple additivity. However, when GLU was combined with BOM or GRP the effect on food intake was only additive.

CCK-8, SOM and GLU were hypothesized to involve independent effects on food intake; we found that the effect of CCK-8, SOM and GLU given in combination was what would be expected based on additivity.

CCK-8 (1, 2, 3 ug/kg) and BOM (0.25, 0.50, 0.75 ug/kg) yielded 9% to 45% inhibition of food intake in a dose dependent manner. Combinations of CCK-8 and BOM at a fixed ratio of 4 to 1 (BOM to CCK-8) yielded less inhibition than would be expected based on additivity. Varying the ratio of BOM to CCK-8 from 4:1 to 6:1 yielded only slightly less than additive effects on food intake. The results confirm the hypothesis, in that, the observed amount eaten was less only in combinations involving BOM, GRP and SOM; BOM failed to add to CCK inhibition of feeding. This suggests that bombesin at low doses produces its main effect by releasing CCK. CCK, glucagon and somatostatin produced additive effects in keeping with the hypothesis that the release of a combination of GI hormones is responsible for the termination of a meal.

362.12 FURTHER STUDIES ON THE ALTERATION IN BLOOD PRESSURE FOLLOWING INTRATHECAL INJECTIONS OF NEUROPEPTIDE Y IN THE ANESTHETIZED RAT. X. Chen* and T.C. Westfall (SPON: M. Walz). Dept. of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

Previous studies from our laboratory have shown that the intrathecal injection of neuropeptide Y (NPY) has a depressor effect in normotensive Sprague-Dawley and Wistar Kyoto rats as well as DOCA hypertensive rats, but not in the Spontaneously Hypertensive Rat (Soc. Neurosci. Abs. 12:221, 1986). It has also been observed that the depressor effect of NPY involves alpha$_2$ and beta adrenoceptors. The purpose of the present study was twofold: first, to examine the effect of the intrathecal administration of NPY at a different spinal level on arterial blood pressure and heart rate in the anesthetized rat and second, to further examine the mechanism of the depressor effect of NPY by examining the response in animals treated with reserpine to deplete catecholamines.

Male Sprague-Dawley rats weighing 200-300 gms were used. Rats were anesthetized with urethane (1.6 g/kg, i.p.). For intrathecal injection the atlantooccipital membrane was exposed by a middle incision through the trapezoidal and rhomboid muscles, keeping the head flex at 35^{o} one polyethylene catheter (PE10) was inserted 4 or 6 cm down the spinal subarachnoid space (about T4 and T10, respectively) through a puncture of the atlantooccipital membrane. NPY was dissolved in saline and slowly injected at a volume of 10 µl. For reserpinized rats, reserpine (1 mg/kg, i.p.) was given at 48 hr and 24 hr before experiments. Arterial blood pressure was measured by a catheter placed on the left carotid artery. Heart rate was measured by EKG. For norepinephrine analysis, tissues (heart and a 4 cm segment of spinal cord) obtained at the end of the experiment were homogenized in 0.1 N perchloric acid, concentrated by alumina column chromatography, and norepinephrine measured by high performance liquid chromatography coupled to electrochemical detection. It was observed that there was a significant reduction of blood pressure and heart rate in Sprague Dawley rats following the intrathecal injection of 0.1 nmol of NPY into the spinal cord at the level of T4. The maximum reduction in blood pressure and heart rate was 16.20±1.39% ($P<0.01$) and 9.8±2.4% ($P<0.05$), respectively. Pretreatment with reserpine depleted norepinephrine levels in the heart and spinal cord by 94% and 53%, respectively. In these animals, the depressor effect of intrathecal injection of 0.1 nmol of NPY (T10) was significantly attenuated ($P<0.05$).

These results suggest that NPY produced similar depressor effects when injected to different spinal levels (T4 or T10). In addition, this effect is closely associated with spinal cord catecholamines.

(Supported by HL35202 and HL26319.)

362.13 **NEUROPEPTIDE Y: PRESYNAPTIC INHIBITION IN RAT HIPPOCAMPAL AREA CA1 MAY INVOLVE CALCIUM CHANNEL MODULATION.** W. F. Colmers, K. D. Lukowiak and Q. J. Pittman. Dept. of Medical Physiology, University of Calgary, Calgary, AB, T2N 4N1 CANADA

Neuropeptide Y (NPY), the most abundant peptide yet isolated from mammalian nervous tissue, has recently been shown to presynaptically inhibit transmission at the stratum radiatum-CA1 pyramidal cell excitatory synapse in the rat hippocampal slice, in vitro. The mechanism of NPY's action is not clear; however, two likely possible mechanisms are that NPY either turns on a potassium conductance, or turns off a calcium conductance at the presynaptic terminal. We tested these possibilities by manipulating potassium and calcium influxes.

Transverse, 400 µm thick slices were prepared from hippocampus of 125-300g male Sprague-Dawley rats, and maintained at 32° C submerged under constant perfusion of carbogenated medium, in which test substances were dissolved just prior to use. Standard techniques were used to record intracellularly (100-200 MΩ, K-acetate electrodes) and to record field potentials in area CA1 extracellularly as described previously (J. Physiol. 383:285).

Application of the K^+ channel blocker 4-aminopyridine (4AP) at the relatively low concentrations of 10-50 µM enhanced synaptic transmission evoked by a single, brief shock to stratum radiatum. Somatic input resistance of the CA1 pyramidal neurons was not significantly affected by these concentrations. NPY (1 µM), in the presence of 4AP, was without its usual potent inhibitory action on the extracellularly-recorded population spike (PS) or intracellularly measured EPSP. Because the delay in membrane repolarization caused by K^+ channel blockade could enhance terminal calcium influx through other calcium channels, so as to overwhelm a potential NPY blockade of some of the calcium channels, the calcium concentration in the medium was lowered to compensate. Application of 1 µM NPY in the presence of 10 µM 4AP and 0.7 mM Ca^{++} caused a reversible reduction in both PS and EPSP (49.3±14.31% and 66.1±3.5% of control, respectively; $P< 0.001$). While NPY was without effect in 50 µM 4AP and 0.7 mM calcium, a further reduction of calcium to 0.5 mM restored NPY's inhibition of PS and EPSP (50.1±10.9% and 51.4±5.1%; $P <0.001$).

The results indicate that NPY's effects are not due to activation of a 4AP sensitive conductance, as reduction of calcium influx restores the peptide's action. Because 4AP increases terminal input resistance, NPY-mediated activation of a different K^+ channel would actually have a greater hyperpolarizing effect, thereby enhancing inhibition. The data are consistent with an NPY-mediated decrease in calcium influx at the presynaptic terminals in CA1. Supported by MRC of Canada. WFC is an AHFMR Fellow: QJP is an AHFMR Scholar and MRC Scientist.

362.14 NEUROPEPTIDE Y AFFECTS THE RELEASE OF LHRH FROM THE MEDIAN EMINENCE IN VITRO. F.D. Sabatino and J.K. McDonald. Dept. of Anat. & Cell Bio., Emory Univ. Sch. Med., Atlanta, GA 30322.

Intracerebroventricular administration of neuropeptide Y (NPY) in female rats decreases or increases plasma levels of luteinizing hormone (LH) depending on plasma levels of gonadal steroids (Kalra & Crowley, Life Sci. 35: 1173, 1984; McDonald et al., Neurosci. Abstr. 10: 1214, 1984; PNAS 82: 561, 1985). We have investigated the hypothesis that these effects are mediated by direct effects on the release of LH-releasing hormone (LHRH) from the median eminence (ME). Recent evidence has also shown that NPY can stimulate the release of LHRH from medial basal hypothalamic fragments in vitro (Crowley et al., Neurosci. Abstr. 12: 1414, 1986). In the first set of experiments, individual ME fragments from ovariectomized (OVX) rats treated with estradiol (E_2), E_2+progesterone (P) or oil vehicle were incubated in Locke's medium containing low $CaCl_2$ (0.8mM) for 15 min followed by two 30 min periods. KCl (56mM), NPY (10^{-6}, 10^{-7}, and 10^{-8} M), or control medium was applied at the start of the second 30 min. The ratio of LHRH released post/pre stimulation was examined within and between steroid treatment groups. KCl significantly stimulated LHRH release although NPY had no effect on LHRH release from ME fragments obtained from OVX and OVX-E_2+P treated rats. However, NPY (10^{-6}M) significantly stimulated the release of LHRH in the OVX-E_2 group compared to both the OVX and OVX-E_2+P groups. In a second set of experiments, ME fragments were incubated in Krebs-Ringer medium containing higher $CaCl_2$ (2.5mM), and additional doses of NPY (10^{-5}, $5x10^{-6}$ and $5x10^{-7}$M). The ME concentration and basal release of LHRH was significantly greater in the OVX-E_2+P group than in both the OVX and OVX-E_2 groups. KCl significantly stimulated the release of LHRH in all groups examined although NPY had no effect on LHRH release from ME fragments from the OVX and OVX-E_2+P groups. However, NPY(10^{-5}M) significantly stimulated LHRH release from ME fragments of OVX+E_2 rats. The lower dose of NPY ($5x10^{-6}$M) produced an intermediate stimulation while 10^{-7}M significantly inhibited LHRH release in this group. These results suggest that NPY can stimulate or inhibit LHRH release from the isolated ME depending on the plasma level of gonadal steroids. Supported by NIH HD19731, HD00727, and March of Dimes 5-524.

362.15 MEASUREMENT OF THYROIDAL VIP CONTENT BY RIA UNDER HYPER- AND HYPOTHYROID CONDITIONS. M. Michalkiewicz, L.J. Huffman, J.M. Connors, and G.A. Hedge, Physiology, West Virginia University Medical Center, Morgantown, West Virginia 26506.

Vasoactive intestinal peptide (VIP)-containing nerve fibers impinge upon both follicle cells and blood vessels in the thyroid gland. Since the administration of exogenous VIP results in increased thyroid blood flow, we investigated changes in thyroidal VIP content under a variety of conditions which are known to change circulating thyroid hormone levels or thyroid blood flow. We first validated the use of a radioimmunoassay (RIA) for VIP for the measurement of tissue VIP content. The VIP antiserum, generated in the rabbit, did not cross-react with known homologs, e.g. PHI, secretin, and GRF or 16 other neuropeptides. The within and between assay precisions were 4.6 and 11.5%, respectively. Recovery of VIP from 22 samples of .67M acetic acid extracts of bovine and rat thyroid tissue was 83.3% and serial dilution curves of these extracts were parallel to standard curves using porcine VIP in the RIA. Thyroid VIP concentrations (ng/g) were found to vary among species: bovine (145.1); porcine (6.43); dog (34.12); and rat (19.65 + 2.57). Relative to concentrations of VIP in other tissues in the rat, thyroid VIP levels were lower than VIP levels in the gut, brain, anterior pituitary, and adrenal. We next assessed rat thyroidal VIP content under the following conditions known to change thyroid function: cold exposure (4°C, 4 hrs); propylthiouracil (PTU: 1 mg/100g, ip, 6 days); and T_4 (5 µg/100g, ip, 6 days) treatment. As expected, plasma TSH (.68 + .09 µg/dl) and T_3 (102.1 + 4.4 ng/dl) levels were elevated in rats undergoing cold exposure above values for control rats (.47 + .05 µg/dl and 59.5 + 4.8 ng/dl, respectively). Following administration of the thyroid inhibitor, PTU, plasma T_3/T_4 levels were suppressed while plasma TSH was increased two-fold. We have also found that this treatment is associated with marked increases in thyroid blood flow. In T_4-treated rats, plasma TSH levels were undetectable. In spite of these documented changes in thyroid function, thyroidal VIP content remained unchanged in cold-exposed, PTU, and T_4-treated rats. There was, however, a tendency for VIP concentrations to be lower in PTU-treated rats presumably due to the 78% increase in thyroid weight (16.5 + .5 mg in control rats vs. 29.3 + 2.5 mg, $p < .05$). Thus, our findings provide no evidence that the extensive VIP-ergic innervation of the thyroid is involved in modulating circulating thyroid hormone levels or thyroid blood flow. Supported by AM 35037.

362.16 CYCLIC AMP MEDIATES THE EFFECTS OF VASOACTIVE INTESTINAL PEPTIDE ON CHROMAFFIN CELL ENKEPHALIN LEVELS. Steven P. Wilson. Department of Pharmacology, University of South Carolina School of Medicine, Columbia, SC 29208

Vasoactive intestinal peptide (VIP) increases the levels of enkephalins and larger proenkephalin fragments containing enkephalin sequences (ECPs) in primary cultures of bovine adrenal medullary chromaffin cells (Wilson, S.P., Life Sci. 40:623, 1987). A maximally effective concentration of 5 µM VIP increased enkephalins and ECPs by an average of 72%; the half-maximally effective concentration was 1 to 2 µM. Maximum effects were observed after 48 hr of continuous treatment; however, only 8 hr of exposure to VIP was necessary to maximally elevate enkephalin and ECP levels when measured 3 days later. Secretin (10 µM) was approximately one-half as effective as VIP in increasing ECPs, whereas glucagon, neurotensin, somatostatin, and neuropeptide Y did not alter chromaffin cell ECPs.

VIP (5 µM) elevated cyclic AMP levels in the cultures by 40 to 150% in 15 min. Elevated cyclic AMP levels were also observed up to 24 hr after addition of VIP but were lower than the 15 min values. The effects of VIP on cyclic AMP levels were potentiated by cyclic nucleotide phosphodiesterase inhibitors including theophylline and 3-isobutyl-1-methylxanthine, which also increased chromaffin cell cyclic AMP levels, and Ro20-1724, which alone produced little or no change in cellular cyclic AMP. There was a general correspondence between the concentrations of VIP which elevated cyclic AMP and those which elevated enkephalin and ECP levels.

VIP caused some loss of cellular catecholamines; however, this effect was not blocked by hexamethonium or d-tubocurarine, nor did these drugs alter the effects of VIP on enkephalins and ECPs. In short-term experiments (up to 2 hr), VIP did not induce secretion but potentiated that in response to nicotine. Secretagogues, including nicotine and histamine, and other compounds such as substance P, angiotensin II, insulin, reserpine, and tetrabenazine failed to alter chromaffin cell cyclic AMP levels.

Hence, VIP appears to evelate chromaffin cell enkephalin and ECP stores via a cyclic AMP-dependent mechanism. Because VIP is found in nerve terminals innervating the adrenal medulla, this neuropeptide may be a trans-synaptic modulator of chromaffin cell function via adenylate cyclase. (Supported by National Science Foundation grant BNS 86-04295.)

362.17 EVIDENCE THAT VASOACTIVE INTESTINAL PEPTIDE ADMINISTERED DIRECTLY TO THE SPINAL CORD PRODUCES ANALGESIA IN RATS. Barry R. Komisaruk, Cynthia Banas*, Stephen B. Heller*, Beverly H. Whipple, Guy F. Barbato* and Frank Jordan*. Institute of Animal Behavior and Department of Chemistry, Rutgers-The State University of New Jersey, Newark, N.J. 07040.

Vaginocervical stimulation increases firing rate in the pelvic nerve (Science,178:1295,1972), whose terminal distribution in the spinal cord corresponds to that of Vasoactive Intestinal Peptide (VIP) (Somatosens. Res., 1:69,1983; Nature, 305:143,1983). We hypothesized that the powerful analgesia produced by vaginocervical stimulation would be mimicked by administration of VIP directly to the spinal cord. VIP (5ug in 5ul saline) injected intrathecally (i.t.) at the lower lumbar level via a chronically-implanted PE10 catheter significantly increased tail-flick latency (TFL) to radiant heat more than 120% over control pre-injection baseline levels within 3 min post-injection. VIP (500ng and 250ng, but not 50ng), also significantly increased TFL. TFL elevation persisted for 10 min only with 5ug VIP (n=10/group). In a second experiment (n=11/group), naloxone HCl was administered i.p.,(10 mg/kg), 30 min before i.t. injection of VIP (5ug or 2.5ug). Control groups received only saline, naloxone, or VIP alone at these doses. TFL in the VIP-alone groups was significantly greater (60-62% above baseline) than in the saline or naloxone-only groups (1-6% above baseline). TFL in the VIP + naloxone groups (18-49% above baseline) did not differ significantly from the VIP-only groups. TFL in the VIP (5ug, but not 2.5ug) + naloxone group was significantly greater than TFL in the saline group. Using a different behavioral assay, vocalization threshold to tail shock was significantly elevated by administration of VIP (5 ug) at 3-10 min and 20 min post i.t. injection. At 5 and 10 min the VIP 5ug group was also significantly elevated over the VIP (5 ug + naloxone) group. These data indicate that VIP produces analgesia via both opiate and non-opiate-modulated pain pathways, since naloxone was more effective in antagonizing the analgesic effect of VIP on the vocalization threshold test than on the TFL test. Suppported by NIH:NLS-2 1RO1NS22948-01A1 and the Charles and Johanna Busch Foundation.

362.18 SALMON CALCITONIN ANALOGUE WITH IMPAIRED HELICAL STRUCTURE INTERACTS SELECTIVELY WITH BRAIN RECEPTORS FOR CALCITONIN IN RAT. M. J. Twery, P. K. Seitz[s], G. A. Nickols[s], C. W. Cooper[s], R. Orlowski[1s], and J. P. Gallagher. Dept. of Pharmacology and Toxicology, Univ. of Texas Medical Branch, Galveston, TX 77550 and [1]Armour Pharmaceutical Co., Kankakee, IL 60901.

An amphipathic helical conformation in a region of the calcitonin (CT) molecule contributes to the high potency of the hormone in lowering serum calcium. Since CT also has potent effects on behavior and neuronal activity, the present investigation was carried out to compare the conformation-activity relationship of salmon CT (SCT) and gly[8]-des-leu[16]-D-arg[24]-SCT (CTA), an analogue with reduced helix forming ability, in brain and in a peripheral tissue possessing sensitivity to CT. Determinations of adenylate cyclase (AC) activity, ^{125}I-SCT binding and ^{125}I-calcitonin gene-related peptide (CGRP) binding were made using forebrain tissue and kidney cortical membranes taken from male rats (250-350g) and prepared by homogenization and differential centrifugation through sucrose. The specific binding of ^{125}I-SCT was displaced by CTA (0.1-10 uM) in brain, but not in kidney, membranes. Neither CTA (1 uM) nor SCT (0.3 uM) displaced the binding of ^{125}I-CGRP to brain membranes. In brain membranes basal AC activity (174 pmol/mg/min) was inhibited 24-50% by CTA (0.1 nM-1 uM) and 10-20% by SCT (0.1 nM-1 uM). In contrast, the AC in renal membranes activity was stimulated. SCT (0.1 nM-1 uM) increased basal AC activity (5.8 pmol/mg/min) two to five fold. CTA increased basal AC activity by only two fold at the highest concentration tested (1 uM). In renal membranes the guanine nucleotide-stimulated AC activity was also increased by SCT (0.1 uM) but not by CTA (0.1 uM).

Electrophysiological studies were performed in vitro using a submerged brain slice preparation from male rats (120-180g) containing the septal nuclei. Intracellular single electrode recordings made from septal neurons during superfusion of CTA and SCT revealed that both peptides were capable of producing either membrane hyperpolarization (2-10 mV) or depolarization (2-5 mV) accompanied by changes in membrane conductance. These results indicate that CTA has pharmacological activity in brain similar to that of SCT and that CTA lacks the high potency of SCT at CT receptors in peripheral tissues. The findings are consistent with the low potency of CTA (2.5 IU/mg) compared to SCT (4500 IU/mg) in lowering serum calcium peripherally. While the amphipathic helical conformation of the CT molecule appears important for the high potency of the hormone peripherally, we conclude that this conformation is not essential for the actions of CT at sites in the central nervous system.

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362.19 MULTIPLE ACTIONS OF CALCITONIN GENE-RELATED PEPTIDE ON RAT SPINAL DORSAL HORN NEURONS. P. D. Ryu* G. Gerber*, and M. Randic. Dept. of Vet. Physiol and Pharmacol, Iowa State University, Ames, IA 50011.

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide encoded in nervous tissue by alternate processing of the primary transcript of the calcitonin gene. In the dorsal horn of the spinal cord, CGRP-like immunoreactivity (CGRP-LI) is likely to be of primary afferent origin and is found in highest density in laminae I, II and V. In the dorsal root ganglia CGRP-LI was observed in most of the small and some of intermediate size cells. At the ultrastructural level, axonal varicosities containing CGRP-LI were presynaptic to vesicle-containing profiles. Asymmetrical synapses are formed on spines, small and large caliber dendritic shafts, and occasionally somata of dorsal horn neurons. In addition, the release of CGRP from dorsal root and trigeminal ganglia has been demonstrated in vitro. These data suggest that CGRP may play a functional role in the transmission of sensory information in the dorsal horn. Our experiments were designed to examine the actions of CGRP on membrane properties and fast and slow excitatory synaptic transmission in the spinal dorsal horn.

Rats 14-20 days old were used. Transverse or horizontal spinal cord slices, the latter with attached dorsal roots and ganglia were made. Intracellular recording from dorsal horn neurons using 3M K acetate microelectrodes was employed. Fast and slow excitatory synaptic potentials were evoked by electrical stimulation of a lumbar dorsal root or dorsal root ganglion neurons. Tested compounds were applied by bath perfusion in known concentrations.

CGRP (10^{-7} to 10^{-6} M for 1 to 15 min) produced a dose-dependent, prolonged depolarization, which is frequently preceded by hyperpolarization. Both membrane effects were present in a TTX-containing solution, and even enhanced. The depolarization was associated with increased synaptic activity and in some cells enhanced probability of action potential discharge in response to indirect stimulation. Neuronal input resistance during CGRP-induced depolarization appeared to decrease in some cells but increased in other cells. CGRP augmented depolarizations to L-glutamate and NMDA in about 30% of cells. In TTX and TEA solution, CGRP modified duration of Ca spikes of dorsal horn neurons. The reversible increase in Ca spike duration was also observed in dorsal root ganglion cells. In the presence of TTX, CGRP infrequently elicited rhythmic oscillations of the membrane potential which were blocked by cobalt ions. Augmentation of composite fast low and high threshold e.p.s.p.s., and slow e.p.s.p.s. was observed in more than half of tested dorsal horn neurons in which CGRP produced no depolarization or changes in input resistance. Our results are consistent with a neurotransmitter or neuromodulator role for CGRP in the rat spinal dorsal horn. Supported by NSF and USDA.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: VESTIBULAR SYSTEM III

363.1 HEAD MOVEMENTS OF THE LIZARD DURING OPTOKINETIC AND VESTIBULAR STIMULATION. H. B. Wang* and J. H. Anderson (SPON: J. Fohlmeister). Dept. Otolaryngology and Physiology, Univ. Minn., Mpls., Minn. 55455.

The contribution of optokinetic and vestibular inputs to the control of eye movements has been extensively studied in normal and sensory conflict situations. The latter has served to define some of the adaptive capabilities of the vestibulo-ocular reflex (VOR) in mammals. Also, the vestibulo-collic reflex (VCR), which contributes to the control of gaze together with the VOR, has been studied in some species under normal conditions. However, the adaptive behavior of the VCR has not been well defined and to more completely study adaptation of gaze it is necessary to do so. The present work is aimed at this.

The lizard, varanus exanthematicus, was used. Each animal was restrained but its head was free to move. Head position was measured with the magnetic search coil technique. Sinusoidal rotations from 0.02 to 0.8 Hz were applied during three stimulus conditions: rotation of the animal in the dark (vestibular), rotation of the visual surround (visual), and rotation of the animal together with the visual surround (visual/vestibular conflict).

During pure vestibular stimulation, the gain (head velocity/stimulus velocity) varied from 0.05 to 0.25 over the frequency range. At a given frequency the VCR gain increased as the maximum stimulus velocity increased from 10 to 50 deg/s. For frequencies from 0.02 to 0.1 Hz the phase lead of the VCR decreased from about 80 to 10 deg. During optokinetic stimulation the gain decreased from 0.35 to 0.05 and the phase changed from a lead of 20 deg to a lag of 30 deg over the frequency range. In the conflict situation there was a reduction in the amplitude of the head movement so that the gain was less than 0.05 for frequencies below 0.1 Hz. To test for possible adaptation of the VCR, continuous rotation at 0.08 Hz was used. After four hours in the conflict situation the gain of the VCR decreased from 0.15 to 0.025. If the animals were deprived of vision at this time, the gain remained low for one to two hours.

These results provide a quantitative description of the visual and vestibular evoked head movement responses in the lizard. The gain of the VCR can be reduced by a visual/vestibular conflict situation and this reduction can be maintained. (Supported by NIH NS-16567.)

363.2 A COMPARISON OF NECK REFLEXES IN ALERT AND DECEREBRATE CATS. J.M. Banovetz*, S.A. Rude*, S.I. Perlmutter*, B.W. Peterson, and J.F. Baker, spon. by S. Vanden Noven, Dept. of Physiol., Northwestern Univ. Med. Ctr., Chicago, IL 60611)

The two sensory systems involved in neck reflex control are organized in fundamentally different ways. Vestibular inputs are in a 3 dimensional canal frame and neck stretch inputs in a 30 dimensional muscle frame. To generate a neck movement, however, both must be projected to the muscle frame. Kinematic properties of the reflexes may provide clues as to how these transformations take place, and how the reflexes interact in head stabilization.

We have studied vestibulo-collic (VCR) and cervico-collic (CCR) reflexes in four cats, two in the alert state and two in both alert and decerebrate states. The reflexes were elicited by rotating either the head and body together (VCR) or rotating the body while the head remained fixed (CCR). Rotations were done in three dimensions about 22 axes passing through the C1-C2 neck joint. The cats rested in the prone position on the rotator with their heads held pitched 28 degrees below the stereotaxic plane. In alert cats we were able to elicit a well modulated VCR, but the CCR was inconsistent. Some muscles showed brief periods of CCR modulation, but all muscles remained at a tonic firing rate throughout most of the rotations. After decerebration, however, the CCR became much stronger, showing consistant modulation. The VCR remained strong after decerebration. 5 muscles were studied in detail, and each was characterized by a maximal activation direction (MAD), the direction of rotation which produced the maximum response in the muscle.

Biventer cervicis, Splenius, Occipitoscapularis, and Rectus Major had MAD's close to pitch, with significant yaw and roll components. Complexus had a MAD closest to roll, with a significant pitch component and a small yaw component. The following is a table of the normalized decerebrate VCR components for the 5 muscles, and the difference, in degrees, between the alert and decerebrate VCR's and the decerebrate CCR and VCR.

muscle	yaw	pitch	roll	aVCR-dVCR	dCCR-dVCR
biventer	0.221	0.962	0.160	4.6	3.5
complexus	0.272	0.478	0.831	4.2	19.0
occipito	0.347	0.776	0.526	16.0	17.6
splenius	0.472	0.704	0.512	16.7	3.9
rectus M.	0.356	0.880	0.284	2.7	10.0

Decerebration had little effect on the MAD's of the VCR in 3 muscles, and produced moderate shifts in 2. The CCR MAD's were also generally similar to those from the VCR experiments. These data suggest that neck muscles have one primary direction of activation for both the CCR and VCR reflexes, and that the CCR is often suppressed in the alert cat.

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363.3 HABITUATION OF THE EARLY VESTIBULO-OCULAR REFLEX (VOR) IN CAT. L.H. Snyder* and W.M. King. Dept. Physiology, Univ. Rochester, Rochester, NY 14642

Repeated steps of angular head velocity in cat cause habituation of vestibular nystagmus (VN). There appear to be two components of vestibular habituation: 1) a specific suppression of slow phase eye velocity (SPEV) during the first 4 s of nystagmus, and 2) a shortening of the duration of VN evoked by the velocity steps. Jeannerod, et al., (Biol. Cybern. 22: 39-48, 1976) assumed habituation to be an adaptive modification of the VOR, and modelled it as 2 parallel inhibitory mechanisms.

This report is concerned with the early suppression produced by habituation during the first 4 s of vertical VN. Further study of early habituation was prompted by evidence suggesting that the first seconds of the VOR are critical for gaze stabilization. To elicit the vertical VOR, cats were placed on their sides and rotated about their interaural axis. Eye velocity was recorded with high temporal resolution using a scleral search coil.

The following observations were made. 1) SPEV during the first beat of nystagmus was not altered by habituation. 2) SPEV was suppressed after the first nystagmic beat, but recovered within 4 s to the velocity attained in unhabituated VN. 3) SPEV appears to determine the frequency of fast phases (beat frequency) in unhabituated VN. However, when SPEV was suppressed by habituation, beat frequency was more appropriate to the unsuppressed SPEV of an unhabituated response. 4) Intense novel stimuli immediately extinguished early habituation.

Observation 2 suggests that although habituation strongly suppressed actual eye velocity, an internal representation of velocity was not effected. A velocity storage network has been posited to explain how VN outlasts peripheral nerve activity. If habituation reduced the charge received by the velocity storage network, SPEV could not have recovered to the level seen in unhabituated VN after the interval of early suppression. Therefore, velocity storage was uneffected by habituation. Observation 3 implies that beat frequency was dependent not on actual eye velocity but on an internal representation of eye velocity that also was unaffected by habituation.

Jeannerod, et al.'s model of habituation must be modified to account for these findings. First, the site of early habituation must be relocated to a site downstream of the velocity storage network. Second, the sparing of the first nystagmic beat must be incorporated into the model. Finally, evidence from this study and other data suggest that the early and late components of habituation may not be two aspects of a unitary process, but instead may originate from different neural networks possessing different connectivity and serving different functions.

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363.4 SUPPRESSION OF POST-ROTATORY NYSTAGMUS DEPENDS ON AMPLITUDE NOT FINAL POSITION OF ACTIVE HEAD MOVEMENTS. L. Michaud*, P. DiZio, and J.R. Lackner (SPON E. Sullivan). Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

Repositioning the head following a sudden stop from constant velocity vertical z-axis rotation shortens the time constant (Tc) of slow phase decay of the vestibuloocular reflex (VOR) (Guedry, 1965; Benson & Bodin, 1966). Larger amplitude head movements lead to more suppression (Schrader, Koenig & Dichgans, 1985). Our study was conducted to determine whether sensorimotor signals associated with head movements per se or final head position relative to gravity mediate suppression.

Subjects (n=17) were rotated in the dark for 60 s at 60°/s about a vertical z-axis with their heads upright (0°), ventriflexed (-20° and -40°) or dorsiflexed (+20° and +40°). Following a sudden stop, Tc was measured to assess the effects of head position alone. In other trials, 1 s after a sudden stop subjects made active, mechanically constrained 20° or 40° pitch back head movements ending at 0°, +20°, or +40°. All subjects were exposed to all conditions in balanced order on four test days within a one month period. We compared trials with equal amplitudes but different final positions and trials with identical final positions but different amplitudes.

The Mean Tc's (in seconds) and their standard deviations are presented below.

Amplitude	Final Position				
	-40°	-20°	0°	+20°	+40°
0°	15.3(6.2)	16.0(6.3)	15.1(5.7)	16.8(6.8)	14.7(2.9)
20°			10.7(2.9)	11.4(3.0)	10.1(2.2)
40°			10.0(2.9)	8.4(2.6)	9.8(3.1)

An analysis of variance showed no effect of head position on Tc following sudden stops without head movements. Tc was significantly ($p<.005$) shorter with 40° than with 20° amplitude head movements, and there was no effect of final head position.

Thus, suppression of post-rotatory nystagmus seems to depend on the amplitude of active pitch back head movements but to be independent of final head position relative to gravity. This suggests that efferent and cervical afferent activity as well as otolith and vertical canal activity during post-rotatory head tilts trigger VOR suppression.

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363.5 RETINAL IMAGE SLIP AND VESTIBULO-OCULAR REFLEX GAIN DURING LOCOMOTION AND VIGOROUS HEAD ROTATION. G.E. Grossman, D.J. Lanska*, W.P. Huebner*, S.M. Nazarian*, R.J. Leigh. Ocular Motility Lab, Cleveland VA Medical Center, Case Western Reserve University, Cleveland, Ohio 44106.

We measured retinal image stability in 5 subjects during walking, running, and vigorous active head rotations in yaw and pitch. While walking and running "in place" for 30 seconds, subjects attempted visual fixation of a target at a distance of approximately 100 meters. They also attempted visual fixation of a target (a cross) at a distance of 7.3 meters while rotating the head in yaw and then in pitch at a steady high frequency for 15 seconds. Gaze (eye position in space) and head rotations were measured using a magnetic search coil system. After filtering at 40 Hz, data were digitized at 100 Hz, differentiated and analyzed using an interactive program to yield values for velocity and power spectra. Result values below are medians (range).

All subjects experienced illusory movement of the visual target during large-amplitude vigorous head rotation; peak retinal error velocities (PREV) were 38°/sec (13-69) in yaw and 24°/sec (6.6-41) in pitch. PREV was greater during running than walking ($p<.05$). Running in place produced PREV in yaw of 2.0°/sec (1.4-4.1) and in pitch 7.3°/sec (5.6-10). During walking, PREV was 1.5°/sec (.8-1.7) in yaw and 1.2°/sec (.9-1.6) in pitch.

The gain of the vestibulo-ocular reflex (VOR), defined as peak eye-in-orbit velocity/peak head velocity, was less during vigorous head rotations, both in yaw and pitch, than during walking ($p<.05$). VOR gain during vigorous head rotation was .84 (.82-.93) in yaw and .91 (.83-.94) in pitch. VOR gain during walking was .93 (.92-.98) in yaw, and 1.0 (.91-1.1) in pitch. VOR gain of yaw rotations during jogging was .93 (.89-.98); this was not significantly different from active head rotations. However, pitch rotations during jogging had a higher VOR gain, 1.05 (.90-1.2) than voluntary pitch head rotations.

The lower VOR gain during voluntary head-shaking was not simply due to increased frequency. Pitch rotation frequencies during running, 5.6 Hz (2.4-7.6) were as high as or higher than those of active head rotations, 2.7 Hz (2.4-3.0), but VOR gain was lower during the latter. Similarly, we compared harmonic frequencies (pitch) during walking with fundamental frequencies of similar magnitude during active head rotation; VOR gain was lower for the latter. These data suggest that the higher retinal error velocities during vigorous active head rotation reflect (1) higher peak head velocities (2) a saturation of the VOR that may be velocity or acceleration dependent.

363.6 MEASURING VERTICAL CANAL FUNCTION THROUGH CALORIC INDUCED POSTURAL SWAY. R. N. Thakor*, T. C. Hain, R. Proctor* , Depts of Otolaryngology and Neurology, Johns Hopkins University, Baltimore, Maryland 21205.

Caloric testing does not detect unilateral lesions of the vertical semicircular canals primarily because their stimulation induces nystagmus that is vertical and torsional, which is not well measured using electro-oculography. However, vestibular stimuli that cause compensatory torsion of the eye, should induce lateral sway of the body. Lateral sway can be measured with a posture platform.

Accordingly, we performed calorics in 5 normal subjects, standing with the head pitched 30 deg down from upright, to make the lateral canals horizontal. Each ear was irrigated with water at 10° C for 40 seconds (flow rate 300 cc/min). Immediately thereafter, the position of the center of gravity was measured for 6 consecutive 20 second epochs on a servo-driven posture platform. In order to reduce proprioceptive cues, the lateral component of any change in position of center of gravity was fed back to the platform at 50% gain to partially null changes in the angle between the leg and the foot.

We found that caloric irrigation caused a shift of the center of gravity toward the side of irrigation. The mean maximum shift, occurred on average at 74 sec, (range 20-100) and was 0.53 ± .59° (mean ± sd, signs adjusted for side of irrigation). Peak-to-peak lateral sway, averaged over all 6 epochs, increased 0.43 ± 0.36° over the baseline. We also examined the effect of using other values of feedback gain. If no feedback was used, little shift of the center of gravity occurred. 100% feedback resulted in a fall in about half the trials.

Presumably, the ipsilateral shift in the center of gravity occurs to compensate for a sensation of roll to the opposite side. As the anterior vertical canal experiences a temperature differential during caloric irrigation (Dohlman, Acta Otol. Suppl. 5:1, 1925), our results could be due to its stimulation. While a direct thermally induced reduction in firing rate of the vestibular nerve is a second possible explanation, our results indicate that measurements of sway combined with caloric irrigation could be a powerful tool for evaluation of the function of the vertical semicircular canals.

363.7 THE EFFECTS OF AGING ON THE NYSTAGMIC RESPONSE TO IMPULSIVE CHANGES IN VESTIBULAR AND OPTOKINETIC STIMULI. P. DiZio and J. R. Lackner. Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

Falling-related injuries increase in frequency around age 65 (Fife, Barancik, & Chatterjee, Am. J. Pub. Hlth., 1984). This may be due in part to age-related structural changes in the vestibular system (Johnsson, Laryngoscope, 1971; Engstrom, Bergstrom, & Rosenhall, Arch. Otolaryng., 1974; Shuknecht, Igarashi, & Gacek, Acta Otolaryng., 1965). We have attempted to identify age-related changes in oculomotor responses attributable to specific vestibular changes.

We exposed 7 healthy elderly (mean age, 67.7) and young (mean age, 19.1) individuals to sudden stops after 60 s of 60°/s body rotation about a vertical axis in the dark. The step gain (Gvn) of nystagmic slow phase velocity was measured to assess the short time constant of the cupula-endolymph system and the integrity of the direct pathway of the vestibuloocular reflex (VOR); the time constant of slow phase velocity decay (Tvn) was determined to assess the long time constant and the combined activity of the direct and indirect pathways (Robinson, Exp. Br. Res., 1977; Cohen, Matsuo, & Raphan, J. Physiol., 1977).

There were no differences in Gvn across age groups, but Tvn was significantly ($p<.025$) longer in the elderly (20.1 s) than in the young (16.3 s). This pattern suggests that the long time constant and/or some aspect of the indirect VOR is more sluggish in elderly than in young individuals.

To test directly whether the velocity storage integrator of the indirect VOR (Raphan, Matsuo, & Cohen, Exp. Br. Res., 1979) was responsible for the longer time constant in the elderly we exposed 4 of the elderly and 4 of the young individuals to 60 s of 60°/s optokinetic stimulation and measured the decay constant of after-nystagmus (Tokan). We also measured the gain of the slow phase velocity of optokinetic nystagmus (Gokn).

In contrast to Tvn, Tokan was significantly shorter (3.60 s) in the elderly than in the young (8.97 s). For young subjects, Gokn was constant across the 60 s stimulation period, averaging 51.8°/s; however, for the elderly Gokn fell from 52.1°/s at 5-10 s to 35.9°/s at 55-60 s.

If the models of Robinson (1977) and Raphan et al. (1977) hold, the observed age-related increase in Tvn and decrease in Tokan may mean that there is an age-related increase in the cupula-endolymph long time constant and a decrease in the decay rate of velocity storage. These alterations in time constants and the unexpected decline in eye velocity during optokinetic stimulation in the elderly could diminish postural and visual stability during low frequency body movement.

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363.8 MONOCULAR AND BINOCULAR VISUAL SUPPRESSION OF VESTIBULAR NYSTAGMUS

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It is well known that visual vestibular interaction is an essential component of normal vestibular function. In a recent animal study, the elimination of visual input from the eye ipsilateral to an induced peripheral vestibular lesion, significantly retards central vestibular compensation.

The present study investigated the effect of monocular and binocular visual fixation suppression of caloric induced nystagmus in man. Patients with peripheral and central vestibular dysfunction (n: 80) underwent caloric testing using 0°C irrigation. Resulting nystagmus was recorded using D.C.-EOG with eyes closed. Fixation suppression was obtained for ten seconds under three different conditions: both eyes open, right eye fixating and left eye fixating. Nystagmus was analyzed by evaluating the maximum slow phase velocity in each fixation period. Fixation suppression (nystagmus velocity EO/EC) above 50% was regarded as abnormal.

Results showed that the eye ipsilateral to the irrigated ear suppressed nystagmus greater than the contralateral eye (factor of 2). The degree of fixation suppression was similar with both eyes as with the ipsilateral eye. Furthermore, the contralateral eye was not able to suppress nystagmus below 50%. It is concluded that ipsilateral visual input is essential in suppressing induced vestibular nystagmus and therefore, modifying visual vestibular interaction. Anatomical, physiological and clinical implications discussed.

363.9 HYPO AND HYPERGRAVITY IN PARABOLIC FLIGHT AFFECT OCULAR TORSION: HOW DO THESE CHANGES RELATE TO OCULAR COUNTERROLLING IN 1G? S.G. Diamond, C.H. Markham, K.E. Money*, N.M. Kirienko*, D.G. Watt, W.H. Johnson*. Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024, USA; DCIEM, Downsview, Canada; NRC Canada, Ottawa, Canada; St. Michael's Hospital, Toronto, Canada; McGill University, Montreal, Canada.

Ocular torsion is a reflex governed by gravity receptors in the otolith organs of the inner ear. Money et al. conducted a study during parabolic flight of a KC-135 aircraft to test the hypothesis that in 1G the eyes are normally held in the appropriate torsional position by central vestibular mechanisms that have adapted to otolith inputs which are not perfectly symmetric. Six healthy subjects were subjected to 0G and 1.8G alternately occurring in 6 to 10 parabolas. Two photographs of a single eye were taken in these hypo and hypergravity episodes, each of which lasted about 25 sec. Measurements of ocular torsion were later made using a twin projector system accurate to 0.1°.

Results showed that two subjects had a clear phasic response, with the eyes torting in one direction at 0G and in the other direction at 1.8G. The responses of these two persons were opposite in polarity, possibly reflecting their individual asymmetries. A third subject showed large phasic changes during the first 4 parabolas, with both the amplitude and phasic characteristics diminishing as that flight and a subsequent one progressed. Two other subjects showed moderate torsional changes without clear relation to gravitational changes. The sixth subject had consistent torsion in one direction with both hypo and hypergravity.

The wide range of responses suggested that an examination of ocular counterrolling (OCR) in the normal 1G environment might provide a baseline for better interpretation of the parabolic flight results. These studies are under way. Preflight examination of OCR consists of rolling the subject about the naso-occipital axis at 3°/sec constant velocity to 90° right ear down, reversed and rolled to 90° left ear down, and the procedure repeated without stopping. The same protocol is then followed in earth-horizontal long axis (BBQ) rotation. Postflight examination is a duplicate of preflight studies. Both eyes are photographed at each 10° of rotation. Well established norms of consistency, conjugateness, smoothness and symmetry permit evaluation of a given subject's OCR.

Inflight parabolic studies of subjects whose preflight and postflight OCR are known should enable a more definitive test of the otolith asymmetry hypothesis. Ideally, the preflight OCR will demonstrate the adapted response in the normal 1G environment, parabolic studies reveal inherent asymmetries, and sequential postflight studies show the return to the adapted state.

363.10 EVIDENCE FOR OTOLITH COUPLING TO VELOCITY STORAGE IN HUMAN OPTOKINETIC AFTERNYSTAGMUS. S.H. Lafortune, D.J. Ireland*, R.M. Jell. Depts of Physiology and Otolaryngology, University of Manitoba, Winnipeg, CANADA

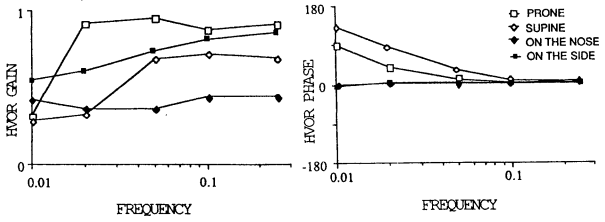
A common velocity storage mechanism is thought to be involved in the generation of both post-rotatory nystagmus (PRN) and optokinetic afternystagmus (OKAN). Schrader et al. (1985) have shown that active head tilts suppressed PRN in humans. In monkeys, OKAN can also be suppressed by tilts (Raphan & Cohen 1981; Waespe et al. 1985). This tilt induced suppression was attributed to activity arising in the otolith organs which had coupled to the velocity storage mechanism thereby "dumping" its contents. To test the hypothesis that otolith organ mediated activity can couple to the horizontal velocity storage in humans, we have examined the effects of active head movements about the pitch, roll, or yaw axes on human horizontal OKAN. Subjects (N=6) were exposed to randomized optokinetic sessions performed at weekly intervals: one session per head movement examined, one control session with no head movement. Each session consisted of one OKN/OKAN head movement trial in one direction followed by a head movement trial in the opposite direction, each trial preceded and followed by a control trial without head movement. One trial consisted of 60 seconds optokinetic stimulation at 40 deg/sec, followed by a "lights out" period of 60 seconds. In each head movement trial, subjects were instructed to briskly execute one 90 deg. active head movement on cue (4 seconds after lights out) and to maintain their head in the new position until the end of the trial. Head movements examined were: pitch forward, pitch back, roll left, roll right, yaw left, yaw right. The degree of tilt-induced OKAN suppression was measured as the area computed under the decay curve relative to the control curves. Active head tilts about the pitch axis, either forward or backward, were found to produce significant OKAN suppression i.e. "dumping" of the velocity storage ($p<0.01$) relative to control trials. Pitch forward tilts exerted the strongest effect ($p<0.005$). Active roll right tilts also produced significant dumping ($p<0.005$). Although not significant at $p=0.05$, a similar effect was observed with roll left tilts. No dumping was observed following a yaw movement. Head movement trials did not alter subsequent control trials as measured by the consistent OKAN double exponential decay coefficients obtained in control trials. We conclude that otolith organ mediated activity arising from pitch or roll tilts can couple to the horizontal velocity storage in humans, thereby suppressing ongoing OKAN. (Supported by the Medical Research Council of Canada.)

363.11 **Dynamic otolith stimulation improves the horizontal vestibulo-ocular reflex.** S. A. Rude* and J. F. Baker, Dept. of Physiology, Northwestern University Medical School, Chicago, Il. 60611

The horizontal vestibulo-ocular reflex (HVOR) is generally studied in an earth-horizontal plane during vertical-axis rotations. These rotations stimulate the semicircular canals to produce the HVOR without dynamically stimulating the otoliths. If the head and rotation axis together are tilted (e.g., ninety degrees from this vertical axis position to a horizontal axis position), the same rotation with respect to the head now presents the same stimulus to the semicircular canals but adds a dynamic stimulus to the otoliths. Does this added otolith stimulation contribute to the HVOR?

We compared the HVOR from horizontal canals to the HVOR from horizontal canals plus otoliths using electrooculographic measurements in 4 cats (13 experiments) during sinusoidal rotations in the dark at frequencies from 0.01 Hz to 2.5 Hz in 5 body orientations. For horizontal canal HVOR (vertical axis rotations) we placed the whole body in the normal (prone) position or on the back (supine). For horizontal canal plus otolith HVOR (horizontal axis rotations) we placed the whole body on the left side, right side, or with the nose down (as though descending a tree).

At 0.01 Hz, the horizontal canal plus otolith HVOR (filled symbols below) showed a higher gain and a more accurately compensatory phase than the HVOR produced by horizontal canal stimulation alone (open symbols). Canal plus otolith HVOR gain remained relatively constant across all frequencies, while the HVOR gain from canal only orientations fell dramatically during low frequency rotations. In addition, gain decreases and phase advances were seen in the supine position at frequencies higher than in the prone position. Supported by NIH grants EY05289, EY06485.



363.12 A BILATERAL MODEL FOR EYE VELOCITY STORAGE IN THREE DIMENSIONS. J. H. Anderson. Dept. of Otolaryngol. and Physiol., Univ. of Minn., Minneapolis, MN 55455.

Recently a three dimensional model for velocity storage has been proposed (Hain, Biol. Cybern., 54:337-350, 1986). It incorporates both spatial transformations and positive feedback loops for visual/vestibular interactions which give rise to the storage of an eye velocity signal. However, the dynamics of the storage network are first order and the model does not describe the reversal phases of optokinetic after-nystagmus (OKAN) and post-rotatory nystagmus (PRN). The present work proposes a generaliztion of a bilateral model for velocity storage in the horizontal plane (Anderson, Assoc. Res. Otolaryn., 1987). The dynamics are second order and the full time course of OKAN and PRN can be described.

The model has three bilateral networks for the canal planes, each of which has a coupling between the two vestibular nuclei (VN), a feedback pathway to each VN which includes a leaky integrator, and bilateral coupling of the optokinetic pathways. The model can be represented by a matrix equation:

$$\underline{o} = \underline{A}^{-1}(\underline{r} + \underline{H}^{-1}\underline{c})$$

$\underline{A}$ represents the bilateral coupling between the two leaky integrators for each canal plane and can be partitioned so that its diagonal elements are each 2x2 matrices, one for each canal pair ($\underline{A}_h^{-1}$, $\underline{A}_a^{-1}$, $\underline{A}_p^{-1}$). $\underline{H}^{-1}$ is diagonal and represents the leaky integrators. $\underline{c}$ and $\underline{r}$ are the vestibular and retinal slip vectors in canal coordinates. $\underline{o}$ is a vector representing the output of the storage network. Eye velocity is obtained with coordinate transformations of this vector.

Depending upon the relative strengths of the several gain elements, the system can be over- or underdamped. The latter can be the case if the coupling of the optokinetic pathways have gains which are non-linear functions of $\underline{r}$ and will give rise to the reversal phases of OKAN. If $\underline{A}_a = \underline{A}_p$, then the vertical component of OKAN can have a profile like that of the horizontal but the time constant of the eye velocity during the OKAN can have a value during upward stimulation which is different from that during downward stimulation.

The present model is a frequency domain representation of a network for the eye velocity storage mechanism. It can account for the reversal phases of OKAN and PRN and the asymmetry of the vertical component of OKAN. Also, deficits following unilateral vestibular dysfunction can be simulated.

(Supported by NIH NS-12125.)

LEARNING AND MEMORY: PHYSIOLOGY IV

364.1 PERSEVERATIVE RESPONDING IN MALE AND FEMALE WISTAR RATS: EFFECTS OF TESTOSTERONE. A. van Hest*, F. van Haaren and N.E. van de Poll*. Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, the Netherlands.

Behavioral differences between male and female rats in non-reproductive behavior have been observed in a number of different experimental procedures. When exposed to operant schedules of reinforcement, male-female differences have been shown to be determined both by the absence or presence of different gonadal hormones as well as by the specific requirements of the experimental procedures. It has been suggested that some of the differences between male and female rats may also be a function of the fact that males are less likely than females to adapt to changes in environmental conditions. Perseveration has been observed in autoshaping procedures in which male rats, but not female rats, continued to respond on a lever which was no longer correlated with the presentation of food (van Haaren, F., A. van Hest and N.E. van de Poll, Learn Motivat, in press). Perseverative responding in male rats has been attributed to high levels of circulating testosterone (Thompson, W.R. and J.S. Wright, Physiol Psychol, 7:291-294, 1979). The present experiment was designed to investigate the effects of gonadectomy and chronic testosterone suppletion on perseverative responding. Response perseveration was investigated in an experimental procedure which has previously been shown to be sensitive to pharmacologically-induced behavioral perseveration and stereotypy.

Different groups of intact, gonadectomized or gonadectomized plus chronically testosterone treated male and female Wistar rats were exposed to a procedure in which reinforcers were randomly assigned to one of two levers in the experimental chamber. One response on the lever to which the reinforcer was assigned was sufficient to produce a Bio-Serve 45 mg food pellet. Responding on the lever not selected for reinforcement had no programmed consequences. Sessions ended after 40 reinforcers had been presented.

Perseveration, defined as the percentage of trials on which more than one response on the lever not selected for food was made prior to switching to the selected lever was highest for testosterone treated subjects. Females made more responses on the lever which had been selected for food on the preceding trial, whereas males showed a preference for one of the two levers irrespective of its previous association with reinforcement. The results of the present experiment thus suggest that males might be less sensitive to the consequences of responding as compared to females. This behavioral difference between the sexes may at least be partly mediated by the male hormone testosterone.

364.2 EFFECT OF PHORBOL ESTERS ON INHIBITORY PROCESSES IN RAT HIPPOCAMPUS. J. Turnbull*, D. Muller*, M. Baudry, G. Lynch (SPON: D. Arst). Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

Activation of Protein Kinase C (PKC) has been suggested to be critically involved in the induction of long-term potentiation (LTP) in the hippocampus. This is based in part on the correlation of LTP with the phosphorylation of a PKC substrate (protein F1) and on the long-lasting increase in synaptic efficacy observed following treatment with phorbol esters which directly activate PKC. Moreover, it has been difficult to induce LTP after PKC activation with phorbol esters. However, the reported effects of these compounds are numerous, and include increased transmitter release, modification of receptors, and changes in the membrane resistance and channel characteristics of the post-synaptic cell. Thus it is not clear that the effects of the phorbol esters are specific and indicative of a selective role for PKC activation in LTP formation.

In the present study, we have investigated the effects of phorbol dibutyrate (PDBu) on inhibitory mechanisms in the rat hippocampus using a standard in-vitro slice preparation and a paired-pulse paradigm. Stimulating electrodes were placed in the stratum radiatum of hippocampal field CA1 and homosynaptic or heterosynaptic pulses were delivered, with a usual interpulse interval of 40 msec. Recording electrodes were positioned in the dendritic field and the cell body layer of field CA1. In the dendritic field, treatment with PDBu (2-10 μM) produced a marked enhancement of the first response as expected, while there was reduced facilitation of the second response to homosynaptic stimulation. In the cell body layer, the first response was markedly enhanced while the second response was profoundly inhibited in both the homosynaptic and heterosynaptic paired-pulse stimulation paradigm. This heightened inhibition was present over a wide range of stimulation intensities and was at least partially bicuculline sensitive. Moreover, significant LTP could still be obtained after PDBu treatment had produced a marked increase in the synaptic response.

These findings suggest that the known actions of the phorbol esters should be extended to include potentiation of inhibitory processes, and may explain in part the difficulty in producing LTP after phorbol ester administration. They do not provide any additional evidence supporting a central role of PKC in the formation of LTP.

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364.3 HIPPOCAMPAL PLACE CELLS AT WORK AND AT REST: A 2-DEOXYGLUCOSE AUTORADIOGRAPHIC STUDY OF HIPPOCAMPAL CIRCUIT FUNCTIONS. L.T. Thompson and P.J. Best, Depts. of Neuroscience and of Psychology, Univ. of Virginia, Charlottesville, VA 22903-2477.

Past work in our laboratory has examined chronic electrophysiological correlates of hippocampal pyramidal unit activity recorded in freely-moving rats. The rate of unit activity has been examined during sleep and during a number of behaviors, including cognitive mapping task performances on radial arm mazes. These neurons fire at high rates during slow-wave sleep (SWS) and are inhibited from firing during paradoxical (REM) sleep. Many of these neurons exhibit *place field* activity; i.e. their firing rate varies significantly as they pass through one or more places within a given environment while performing win-shift (WSH) or win-stay (WST) tasks. Work in other labs has suggested that EEG theta activity in waking rats reflects inhibitory modulation of neural activity within the hippocampus. Although much is known about the relationship of single-unit activity in such behavioral states, comparisons of regional metabolic activity have not been correlated with data from unit recordings. The present study examines regional glucose utilization in the hippocampus under waking and sleeping conditions relative to known electrophysiological properties of pyramidal neurons.

Six groups of young male Long-Evans rats were pretrained for experimentation. Two groups were selectively deprived of REM or of all sleep for 60 hr, and tested during REM or SWS rebound. One group was tested while running rapidly in a wheel for water reinforcement, a condition in which theta activity predominates in the hippocampal EEG. Two other groups performed radial maze tasks, with the place rewarded either changing each trial (WSH) or staying constant for each trial (WST). One group served as immobile waking controls. 250 µCi of [^{3}H] 2-deoxyglucose was administered, and the rats were sacrificed 45 min later. Autoradiographs were prepared from thaw-mounted chloroform-extracted sections using LKB Ultrofilm. The optical density of individual hippocampal laminæ in CA1, CA3, and dentate gyrus as well as subicular subfields was measured via densitometry, and ratios of grey / white matter glucose utilization were obtained.

Hippocampal unit activity reliably predicted glucose utilization measured in several laminæ in the waking groups. Under conditions in which pyramidal cells display *place field* related increases in firing, glucose utilization increased within dendritic and pyramidal cell body layers. Theta activity was associated with decreased glucose utilization in the same layers. Increased pyramidal cell activity was also associated with increased glucose utilization in subicular subfields. In contrast, although glucose utilization increased during SWS, the greatest increases were observed during REM, when pyramidal neurons are the least active. Clearly, in some of our conditions glucose utilization is predicted by single-unit data. Discrepancies indicate that similar patterns of unit firing can be generated by divergent patterns of hippocampal activation.

364.4 SELECTIVE CONTRIBUTION OF THE BASOLATERAL AMYGDALA AND THE PYRIFORM CORTEX TO THE ACQUISITION OF CONDITIONED TASTE- AND CONDITIONED ODOR-AVERSION LEARNING. N. Beaulieu*, S.Schwartzberg* and M. Petrides. Department of Psychology, McGill University, Montreal, Quebec H3A 1B1.

Various studies have implicated the amygdala, and more specifically the basolateral amygdala (BLA), in the control of flavor-aversion learning. Previous work conducted in our laboratory has also demonstrated that lesions of the BLA, that include the pyriform cortex, disrupt the acquisition of an aversion to an odor that is successively paired with Lithium Chloride (LiCl) toxicosis.

The present studies investigated the effect of bilateral electrolytic lesions restricted either to the BLA or to the pyriform cortex of rats on the acquisition of a conditioned taste aversion (CTA) and a conditioned odor aversion (COA). In the CTA experiment, the animals were tested for their ability to acquire an aversion to a sucrose solution (CS) that was paired with LiCl toxicosis (US). The animals were maintained on a water-deprivation schedule and trained to drink water from a Richter tube for 20 minutes a day for 5 consecutive days. On the day of conditioning, the animals were exposed to a sucrose solution (1.5%) which was followed by an intraperitoneal injection of LiCl (20ml/kg) to induce sickness. The acquired aversion was defined as a suppression of drinking when the animals were re-exposed to the sucrose solution, three days following the CS-US pairing day.

In the COA experiment, the rats were tested for their capacity to acquire an aversion to an odor (isoamyl acetate) that was paired with LiCl toxicosis. The animals were water-deprived and subjected to three CS-US pairings on 3 different drinking sessions. Three recovery days were allowed between each drinking session. The acquired aversion to the odor was again defined as a suppression of water-drinking in the presence of the odor after one, two and three conditioning sessions.

Lesions restricted to the BLA resulted in a clear attenuation of both the CTA and the COA, whereas damage to the pyriform cortex caused an impairment only in the acquisition of the COA. These results demonstrate a dissociation between the roles of the BLA and of the pyriform cortex in the acquisition of conditioned aversions to gustatory and olfactory stimuli. In light of this finding, it may be suggested that the BLA, which receives information from all sensory systems, is involved in the acquisition of aversions in various modalities, whereas the pyriform cortex, which receives information primarily from the olfactory system, may be involved more specifically in olfactorily-cued learning contingencies. Supported by NSERC grant A7466.

364.5 EXPERIENCE-INDUCED SPECIFIC CHANGES IN THE FREQUENCY RESPONSE FUNCTION OF UNIT CLUSTERS IN THE MEDIAL GENICULATE NUCLEUS OF THE GUINEA PIG. R.C. Lennartz*, J.F. Bourg*, K.T. Fan*, and N.M. Weinberger. Center for the Neurobio. of Learning & Memory and Dept. of Psychobio., University of California, Irvine, CA 92717.

Learning-induced changes in the responses of sensory neurons in the central nervous system have been reported by a number of investigators. Previous research has demonstrated changes in the evoked responses of cells in the medial geniculate complex (MG) and the auditory cortex during classical conditioning. Such effects could be due to learning-induced changes in general arousal level. However, CS-specific alterations in the frequency response function (FRF) of auditory cortex neurons were observed as a result of classical conditioning (Diamond and Weinberger, Brain Res., 1986, 372, 357-360), demonstrating that learning causes specific modifications in the processing of significant stimuli. The present study sought to determine if FRF changes develop in the MG consequent to auditory experience. FRFs were obtained while the animals were anesthetized to further control for arousal effects.

Unit clusters were recorded from the medial geniculate of guinea pigs with chronically implanted microelectrodes. The basic experimental procedure involved three days of recording. Day 1: the FRF (1 - 30 kHz) was determined with the animal under general anesthesia (Rompun, 20 mg/kg and ketamine, 100 mg/kg). Day 2: The animal underwent 20 classical conditioning trials (CS = tone, 6 seconds; US = shock, .3 sec, at US offset; ITI = 1 to 2 minutes). The subject was then anesthetized and an FRF determined. Day 3: A third FRF was obtained under anesthesia. This three day procedure was followed in most animals by either a second three day run (several days later) using a different tone as the CS or by a two-tone discrimination procedure (30 trials each of the CS+ and CS-).

The post-conditioning FRFs revealed changes that were specific to the tones used as the CSs; such specificity could be observed 24 hours after training. Therefore, CS-specific modification of FRFs are not restricted to auditory cortex. It is noteworthy that these data demonstrate for the first time that the anesthetized brain can show evidence of neural storage of information acquired while the animal is in the waking state.

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364.6 TIME-DEPENDENT MODIFICATION OF THE MEMORY TRACE EVIDENCED BY PRE-TEST CUING IN RATS: P. Gisquet-Verrier* and T. Alexinsky* (SPON: J. Hirsch). Dept de Psychophysiologie, LPN2, C.N.R.S., 91190 Gif-sur-Yvette, FRANCE.

Following a partial brightness discrimination avoidance training in a Y maze, various pretest cuing procedures can enhance the retention performance when delivered before the retention test. Presentation of the conditioned stimulus (CS) was the most effective treatment shortly after training while exposure to the experimental context was most effective at long term (Gisquet-Verrier and Alexinsky, 1987 and in Press). These results could be due to a spontaneous reorganization of the memory trace over time. They could also be due to effects of cuing on different aspects of the memory processes e.g. consolidation or retrieval. An effect on memory storage processes would be expected to be long-lasting while an effect on retrieval processes would be transient.

The 180 rats of the present experiment were trained to escape or to avoid a footshock in a Y maze with a brightness discrimination for 15 trials. Testing occurred either one hour or 21 days later. For the animals tested after one hour, presentation of the CS (5-2sec intermittent light flashes) occurred either 0, 5, 10 or 20 min prior to the retention test. For the animals tested after 21 days, presentation of the experimental context (exposure to the experimental room) occurred either 0, 5, 10, 20 min, 1 or 24 hr before the retention test. The results indicate that in both conditions, cuing significantly enhanced the retention performance when delivered 5 or 10 min before the retention test and had only slight effects when delivered 20 min prior to the retention test. Moreover, there was no effect from an exposure to the experimental context 1 or 24 hr before testing.

The transient effect of cuing obtained for both the retention intervals indicates that cuing does not induce an elaborative process that would maintain over time but rather induces a phasic effect that fits well with a retrieval interpretation. Our previous findings indicating that the nature of the initial information leading to the best retention performance changes as a function of time appears then to be due to a reorganization of the memory trace that occured spontaneously over time.

364.7 ELECTROPHYSIOLOGICAL INVESTIGATIONS OF HABITUATION AND PRE-PULSE INHIBITION USING A NOVEL TECHNIQUE TO RESTRAIN AWAKE RATS. J.Cassella and M.Davis, Dept Psychiatry, Yale Univ Sch Med, New Haven, CT 06508.

The need to relate single cell activity to behavior has necessitated the development of appropriate preparations. We have developed a technique that permits the study of single-unit activity using glass micropipettes and conventional microdrives in restrained, awake rats. A unique feature of this preparation is that rats are kept still during electrophysiological recording by infusing a small amount of local anesthetic through a catheter chronically implanted into the spinal epidural space, resulting in a temporary mid-body to hind-limb motor blockade. The animal is placed in a modified stereotaxic device by a bolt attached to its skull.

The acoustically-elicited pinna response, a component of startle, displays various forms of behavioral plasticity (Cassella & Davis, Beh. Neurosci., 100, 1986).The motor neurons innervating the pinna are located in the medial facial motor nucleus (MFMN). As an initial application of the above technique, single-unit activity was recorded from the MFMN in awake, restrained rats that received acoustic clicks under conditions known to produce behavioral habituation and pre-pulse inhibition. Each rat was infused epidurally with tetracaine HCl, placed in the modified stereotaxic, and given a brief adaptation period. While lowering the single barrel micropipette acoustic stimuli were presented every few seconds. When positioned near a cell that responded to these stimuli with a 5-10 ms latency the stimulation was terminated and 15 min elapsed before testing.

For habituation tests, an acoustic click was presented for 20 trials at a 2 sec interstimulus interval. For pre-pulse inhibition tests, the rats received 25 clicks presented alone or preceded 100 ms by a weak noise burst. During habituation testing cells in the MFMN fired less often and with increased first-spike latency as a result of repeated stimulus presentation. For pre-pulse testing, cells in the MFMN fired less often and/or at longer latency on the trials when the pre-pulse stimulus was presented. These data are consistent with behavioral observations found under similar conditions, supporting the validity of this technique for studying the cellular correlates of plasticity of the acoustically-elicited pinna response.

Previously we proposed that the pinna response pathway is composed of the ventral cochlear nucleus, paralemniscal zone of the ventral lateral lemniscus(PLZ), and MFMN (Cassella & Davis, Neurosci. Abs., 1986). We now find that cells in the PLZ respond to a click with a latency of approximately 3 ms. The pinna response pathway is currently under investigation using a variety of methods including orthodromic and antidromic stimulation. Once the structures in series with the MFMN are identified, the locus and nature of plasticity of the pinna response can be determined.

364.8 LOCALIZED ACETYLCHOLINE APPLICATION MODULATES SENSORY EVOKED RESPONSES AND PERFORANT PATH FIELD POTENTIALS IN BEHAVING RATS. T.C. Foster* and S.A. Deadwyler (SPON: J.H. Ryu) Dept. Phys. & Pharm, Bowman Gray Sch. of Med., Winston-Salem, NC 27103.

Past research from this lab indicates that two major pathways to the dentate gyrus are responsible for the two characteristic negative components of auditory evoked potential recorded in the outer molecular layer of the dentate gyrus (OM AEP). An early N1 component of the OM AEP reflects perforant path tone evoked synaptic activity in the dentate gyrus while the N2 component is modulated by septal input (Deadwyler et al., Science, 211:1181, 1981). The purpose of this study was to evaluate the effects of locally applied acetylcholine (ACh) on the two components of the OM AEP.

Saline and drug filled pipettes for recording and drug application were lowered by a microdrive assembly and localized in the OM via stimulation of the perforant path. Stable behavior was maintained in the animals by recording during criterion performance of a single tone discrimination task. Randomly on 1/2 of the trials in a 100 trial behavioral test session microdrops (70-100 picoL) of 500 uM ACh were pressure ejected 15 sec before tone onset. A single perforant path stimulus was also delivered 500 ms before tone onset. In other experiments pipettes lowered into the granule cell layer measured the effect of ACh on the amplitude of the perforant path evoked synaptic response (EPSP) as well as the population spike amplitude to determine the time course of the ACh effect. Results showed that ejection of ACh during performance of the tone discrimination task resulted in a significant decrease in the amplitude of the N1 component of the OM AEP ($p < 0.01$) and an increase in the amplitude of N2 which was marginally significant ($p < 0.05$). The amplitude of perforant path elicited field potentials recorded in the OM were also decreased slightly for ACh vs non ACh trials. ACh ejected in the granule cell layer resulted in a significant increase in the population spike amplitude ($p < 0.05$). The time course of peak population spike increase after ACh application was 15 sec. Control sessions utilizing saline ejection did not have a significant effect on any of the variables measured. Results suggest that cholinergic inputs to the dentate gyrus modify behaviorally sensitive sensory evoked potentials in the rat.

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364.9 LEARNING CAN AFFECT THE FACE-SELECTIVE RESPONSES OF NEURONS IN THE SUPERIOR TEMPORAL SULCUS OF THE MONKEY. Gordon C. Baylis, Michael E. Hasselmo* and Edmund T. Rolls. University of Oxford, Department of Experimental Psychology, Oxford, OX1 3UD, England.

There are neurons in the cortex of the superior temporal suclus of the macaque with responses selective for faces (Baylis et. al. Brain Res. 91, 91-102., 1985). These responses also show selectivity for the particular face stimulus. It has been suggested that this selectivity is based both on the identity and expression of the stimulus face (Hasselmo et. al. Neurosci Lett. 26: S571, 1986). The present series of experiments examines the extent to which learning is necessary for the selectivity between faces exhibited by these neurons.

In a first experiment the responses to a familiar set of faces was determined, then a totally novel face was introduced into the set. 32 cells were tested with a total of 43 novel faces. First, it was found that the mean response to novel faces in the first two presentations was significantly different from the mean response for all presentations of the novel faces, suggesting that learning was required to establish the final mean firing rate. Second, it was found that in 5 out of 43 cases a change occured in the pattern of responses to the familiar faces (as shown by a significant interaction of face and pre vs. post novel in an ANOVA), and 9 out of 43 cells showed a change in mean response after the addition of the novel image (as shown by a significant effect of pre vs. post novel).

In a second experiment a large set of novel faces were presented to the monkey whilst recording the activity of a cell with face-selective responses. 15 cells were tested on 19 different sets of faces. First, the responses to images in the first presentation varied from the subsequent baseline significantly more than the responses in other presentations, supporting the above data. Furthermore, in a subset of these cells the pattern of responses to the set of face stimuli changed across the early presentations, as measured by an ANOVA. This suggests that these cells differentiate faces in a way which is modified by experience.

These experiments show that changes in response to a face can occur as the face becomes familiar. In addition, for some cells there is a change of pattern of response to familiar faces as a novel face is incorporated with the familiar set. This pattern of data would be expected from a number of models of distributed memory. In particular, a competitive learning network model predicts both the change in response to novel faces and the alteration of response to familiar faces with the incorporation of new faces.

364.10 EFFECTS OF PHORBOL ESTER ON THE RELEASE OF EXCITATORY AMINO ACID IN THE CA1 HIPPOCAMPAL REGION OF THE ANAESTHETIZED RAT. By Y. BEN-ARI , L. ANIKZTEJN* and M.P. ROISIN.* U.29 INSERM. Hôpital de Port-Royal. 123 Bd de Port-Royal. 75674 Paris. Cedex 14.

In the hippocampal slice. application of phorbol ester produces a long term potentiation (LTP) of synaptic transmission which is reminiscent of the LTP produced by a high frequency train of stimulation (Malenka et al. Nature 1986. 31 - 175). The aim of the present study was to examine whether the LTP induced by phorbol ester is associated with a persistent enhanced release of endogenous excitatory amino acids.

Adult male wistar rats were anaesthetized with urethane and a push-pull cannula (0.5 mm O.D.) introduced in the stratum radiatum of CA1 : an electrode-glued to the cannula enabled to record the commisural-schaffer collateral response. ACSF was perfused at a flow rate of 10 ul/min and the concentrations of amino acid measured by HPLC in 50 ul (i.e. 5 minutes samples).

Application of phorbol 12-13 diacetate (PDAC - 500 uM) for 5 min produced an enhancement of the epsp (40 % over the control). The potentiated epsp persisted with little variation for prolonged periods (2 hrs or more ; n = 8). HPLC analysis of the perfusates revealed a highly significant increase in the concentration of endogenous glutamate and aspartate. This increase was maximal 5-10 min after application of the drug (560 % + 169 in comparison to the control pre-drug values ; n = 8). However, starting from the 3rd sample (15 min after PDAC application). the level of glutamate and aspartate returned to the control (pre-drug) values. Thus, 20 to 90 minutes after PDAC, the levels of endogenous glutamate and aspartate were not significantly different from the control values even though the field epsp was still potentiated.

These observations suggest that the long lasting enhancement of the epsp produced in CA1 by PDAC is not due to a sustained enhanced release of excitatory amino acids.

364.11 STUDIES OF CONDITIONING OF NEURONS IN LOCUS COERULEUS OF THE RAT. M. Segal* and S.J. Sara (SPON: M. David). Dept. of Neuroscience, Weizman Inst. of Science, Rehovot, ISRAEL and Lab. Physiologie Nerveuse 2, C. N. R. S., Gif-sur Yvette, FRANCE.

The noradrenergic nucleus locus coeruleus (LC) might play a unique role in learning and memory by modulating attention and providing the necessary tonal influence on target areas involved in specific information processing. If these neurons do perform such a function, they should respond early in conditioning to stimuli paired with positive as well as negative reinforcement. The context of the CSs might also be expected to elicit CRs in these neurons.

Pavlovian conditioning paradigms were used to study LC neuronal activity in parallel with the acquisition of behavioral responses to stimuli associated with shock or water reinforcement. Before training the rats were implanted with a movable microelectrode aimed at the LC; electrode placements were confirmed histologically.

One experiment paired a tone of a specific frequency with water reinforcement, while another tone (CS-) was never followed by water. In most cases there was a significant increase in neuronal response to the CS+ as conditioning progressed, with a decrease or total absence of response to CS-. When the significance of the CSs was reversed there was a rapid increase in response to the former CS-, usually on the first few trials, with no immediate decrease in response to the former CS+.

In an aversive paradigm, the CS+ was followed by ear shock. The presentation of each CS+ or CS- was announced by a flashing light which terminated with CS offset. At a behavioral level there was good differential responding to the CSs and little response to the light. At a cellular level, on the other hand, the neurons responded to both CS+ and CS- throught the habituation trials and the 60 conditioning trials. There was, however, a small but significant increase in the response to the CS+ early in conditioning. The response to the warning light habituated rapidly, but the cells began responding again to this stimulus on the first conditioning trials.

These results indicate that LC neurons are involved in learning in appetitive as well as aversive situations; in aversive learning there appears to be more generalization of the response to include CRs to the context which contains the nominal CSs (flashing light). That LC cells respond rapidly to changes in significance of the stimuli is consistent with the hypothesis that the noradrenergic system plays a role in learning by modulating selective attention.

364.12 STIMULATION OF THE PERFORANT PATH AS A CONDITIONED STIMULUS : LONG-TERM POTENTIATION IN THE DENTATE GYRUS AND BEHAVIORAL CONDITIONING. S. Laroche, V. Doyère* and V. Bloch*. Département de Psychophysiologie, C.N.R.S., 91190 Gif-sur-Yvette, France.

Learning a tone-shock association in rats is followed by increased long-term potentiation (LTP) a perforant path synapses (Laroche, Bergis, Bloch, Neurosci. Abstr., 9:645, 1983) and increased release of radiolabelled glutamate in the dentate gyrus (Laroche, Errington, Lynch, Bliss, Behav. Brain Res., 1987, in press). In the present experiment, high-frequency stimulation of the perforant path was used as a conditioned stimulus (CS) to allow direct estimation of the strength of a population of synapses necessarily involved in the learning task. Rats (n=40) were chronically implanted with a recording electrode in the dentate gyrus and a stimulating electrode in the ipsilateral perforant path. Nine animals also received a stimulating electrode in the contralateral hilus to activate the commissural fibers. Conditioned suppression of lever pressing for food reward was the behavioral index of conditioning. Rats were first trained to press a lever for food pellets (60-sec Variable Interval). Classical conditioning (C) or pseudoconditioning (PC) sessions with 6 trials a session were then given. The CS (400Hz-20msec perforant path trains at 1Hz during 6 sec) terminated with a footshock (0.5 sec) as US. The CS intensity was set to a value which gave a population spike of approximately 1mV in groups 1C (n=11) and 2PC (n=7), and at an intensity below spike threshold in group 3C (n=4). For groups 4C (n=5) and 5PC (n=4), each perforant path train, above spike threshold, was preceded by a high frequency stimulation of the contralateral hilus (400Hz-12msec) with 8 msec interval onset to onset. Nine control animals (group 6) received only high-frequency stimulation of the perforant path.

Learning the brain stimulation-shock association, as assessed by suppression of lever pressing for food during the CS period, was significant from session 2 in group 1C in which the CS induced LTP of the population EPSP and of the population spike. Animals in group 3C in which no LTP was produced did not learn the association even after 6 conditioning sessions. Unexpectedly, LTP in the dentate gyrus was masked rather than blocked by the commissural trains in groups 4C and 5PC: no change in the slope of the population EPSP occurred during session 1 but a significant increase in slope was observed 24h later, before session 2. Learning was retarded in group 4C as compared to group 1C in which LTP was evident on session 1.

These data desmonstrate that perforant path trains can be used as a CS in a conditioned suppression paradigm provided that stimulation parameters allow LTP to develop at the activated synapses. The parallel postponement in LTP and behavioral conditioning when nearly concomitant commissural and perforant path trains are used, further suggests a correlation between increased synaptic strength and learning the CS-US association.

364.13 ELECTRICALLY EVOKED STARTLE: REFRACTORY PERIODS AND TEMPORAL SUMMATION IN BRAINSTEM PATHWAYS. J.S. Yeomans, M. Davis, J.B. Rosen and J. Barbeau*. Dept. Psychology, Univ. Toronto, Toronto, Canada M5S 1A1, and Dept. Psychiatry, Yale University.

The acoustic startle reflex is thought to be mediated by a brainstem and spinal cord circuit consisting of the ventral cochlear nucleus, an area just medial to the ventral nucleus of the lateral lemniscus, nucleus reticularis pontis caudalis and spinal motoneurons (Davis et al., 1982). Startle-like responses can be elicited by electrical stimulation with a single pulse in each of these sites in rats. The present study used single pulse pairs to evaluate the refractory periods and temporal summation properties within this circuit. A stronger response occurred if a second pulse was delivered 0.5 to 10 msec after the first pulse. The added effect of this second pulse was measured by the increased startle amplitude, or by the decreased current required to produce a constant startle amplitude. In cochlear nucleus sites, the effect of the second pulse increased as the interval between the two pulses increased from 0.4 to 2.0 msec, consistent with recovery from refractoriness. In caudal pontine or medullary reticular formation sites, the effect of the second pulse increased sharply at interpulse intervals from 0.3 to 0.5 msec, suggesting that very short refractory period axons mediate the startle response in these sites. At these caudal sites, the effect of the second pulse declined exponentially at interpulse intervals from 2.0 to 50 msec, with a time constant of 4 msec. This time constant is almost identical to EPSPs recorded previously in cat spinal motoneurons driven monosynaptically by reticular formation stimulation (Grillner and Lund, 1968; Peterson et al., 1979). The fast exponential decline suggests that reticulospinal axons mediating startle activate spinal motoneurons monosynaptically. Temporal summation declined more slowly in cochlear nucleus sites, suggesting that these sites do not connect monosynaptically to motoneurons.

In reticular formation sites near the facial nerve, a second peak in the two-pulse curve was observed at interpulse intervals near 10 msec. The second peak was blocked by local anesthesia of the ipsilateral face, but not the contralateral face, suggesting that a single twitch of facial muscles facilitates startle.

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364.14 **BEHAVIORAL CORRELATES OF HIPPOCAMPAL PLACE CELLS.** C.A. Paul*, S.I. Wiener, and H. Eichenbaum. (SPON: A. Levey) Department of Biological Sciences, Wellesley College, Wellesley, MA 02181.

Several investigators have observed that the activity of hippocampal complex spike cells is determined by the animal's presence in specific locations in an open environment, called place-fields. In general, these experiments either ignored ongoing behavior, or arranged the task so that behavior was largely homogenous throughout the environment. In contrast, other investigators have observed that hippocampal units fire in relation to specific sensory or behavioral events in learning tasks. Often these relevant behaviors occur primarily when the animal is in a particular place, thus confounding the "behavioral" and "place" correlates.

We studied the behavioral correlates of hippocampal units in an open environment that is sensitive to place-related unit activity. Rats were trained to move to the center of a .4m-sq arena to initiate a 40sec trial, signalled by a pulsing tone. After trial initiation, the rat was given water-rewards on its first visit to each of four reward cups located near different corners of the arena. Return visits within a trial were not rewarded. Among complex spike cells, identified by average firing rates of about 1/sec and by localization within the CA1 pyramidal layer, over 80% had at least one clear place-field, consistent with other reports on place cells. However, the locations of place-fields were not uniformly distributed about the arena. Nearly every place-field included at least one of the reward-cups or the trial-initiation area. About half of the place cells fired selectively in more than one of these significant loci, with a preference for one place. Furthermore, unit firing was dependent on specific behavioral variables. The firing of place cells was usually time-locked to arrival at a reward cup. About half the place cells fired reliably according to the speed of the rat's movement through the field. Some units fired at higher rates the faster animal moved and other units fired maximally at a particular speed of movement. About half of the place cells varied firing rate reliably with the direction of movement. Most of these units preferred a single direction; a few had opposing bidirectional selectivity. Some cells fired selectively during particular goal-directed trajectories, e.g., during movement from one specific cup toward another. Other cells fired differentially on the initial versus return visits to a cup, or fired differentially depending on the order of cup visits.

Thus, consistent with anecdotal reports, we found that the activity of place units is modulated by ongoing behavior. Speed and directional tuning may be viewed as elaboration on the proposed spatial mapping functions of these cells. However, we interpret these findings as consistent with our other observations: that place-fields are found predominately at task goals and that most units fire during goal-directed movements. The combined results support the view that hippocampal units fire primarily during behavioral interactions with specific targets that are defined both by complex cues and by task-relevance, and their activity is not determined by place *per se*. Supported by PHS NS18744 and NSF BNS85-07677.

364.15 **HIPPOCAMPAL "DECISION" CELLS IN RATS IN A SIMULTANEOUS-CUE ODOR DISCRIMINATION TASK.** S.I. Wiener, H. Eichenbaum, C.A. Paul* and M. Kuperstein. Dept. of Biological Sciences, Wellesley College, Wellesley, MA 02181.

The purpose of this study was to determine the behavioral correlates of hippocampal unit firing during performance on an olfactory discrimination task. Previously, using a successive cue-odor discrimination paradigm, we[1] described: a) "goal-approach" cells that fire time-locked to movements toward task-relevant loci and b) "cue-sampling" cells that fire time-locked to the onset of odor sampling and during the discriminative response.

In order to better isolate the critical task events during odor sampling and response selection, and to examine spatial correlates of firing, we analyzed extracellular unit activity during performance on a simultaneous cue, go-left/go-right odor discrimination task. The rats initiated each trial by entering a small area where S+ and S- odors were presented from separate ports. To receive a reward, the rat was required to approach and poke its nose into the port emitting the S+ odor regardless of its location, which varied randomly across trials. In 200 to 300 trial sessions, rats were presented with two different odor-pair discriminations in random sequence.

Over 140 isolated units had firing increases time-locked with one or more specific task-related behaviors. Of these, 24 cells fired maximally as the rat paused before the odor ports, with a precipitous drop in firing at the onset of movement toward the odor port for the nose-poke response. These cells correspond to cue-sampling cells, however the firing occurred only during sampling prior to making a response. Since their firing correlates well with when the rat had to decide its response, they are termed "decision cells". Some decision cells fired maximally as the rat sampled a particular port, or sampled a particular pair of odors in a particular configuration. The activity of the decision cells did not seem to be determined by "place", since this firing increase did not occur as the rat passed through this same place while exiting the port. Other cells fired maximally at or just after the nose-poke (rather than prior to the nose-poke, as is the case for decision cells). Some of these cells also fired preferentially for a specific odor pair and/or spatial configuration.

In conclusion, a population of hippocampal neurons are selectively active during the decision-making phase of a discrimination task; a subgroup of these cells fired preferentially in relation to specific, relevant stimulus parameters. Each of these response patterns indicates that hippocampal units are sensitive to specific cognitive aspects of behavior.

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[1]H. Eichenbaum, M. Kuperstein, A. Fagan, J. Nagode. J. Neurosci. 7:716-732, 1987.

364.16 SIMILAR DEFICITS CAUSED BY FRONTAL AND SEPTAL LESIONS IN PROBLEM SOLVING MAY REFLECT DISRUPTION OF DIFFERENT MEMORY MECHANISMS. B. Poucet* & T. Herrmann+. (SPON : C. Thinus-Blanc). Lab. of Fonct. Neurosc., CNRS, 13402 Marseille, France and Dpt. of Psychol., U. of Guelph, N1G 2W1 Ontario.

Previous studies have shown profound deficits in rats with damage to the septo-hippocampal complex or to the medial frontal cortex on the Maier three-table problem. In this task, animals are required to integrate information concerning the spatial relations existing among the three tables with information concerning the daily locus of food. However, it is still unclear whether the deficit shown by lesioned animals is caused by an unability to form or to use a cognitive representation of the problem space, or by a more specific working memory impairment. In addition, it may be the case that septal and frontal lesions disrupt discrete and different psychological mechanisms involved in the rat's ability to use space to solve problems. An attempt to contrast the effects of lesions of the medial frontal cortex and of the septum was made in two experiments using the same basic procedure, i.e. a preliminary exploratory experience of the three tables followed by a feeding phase on one table and finally a test trial during which the subject had to return to the table on which it had just been fed. In Exp.I, the food was located on the same table throughout the 18 days of testing (spatial learning task) and the start tables were randomly varied from day to day. In Exp.II, the start was kept constant but the food was randomly distributed on one or the other of the two remaining tables (spatial memory task). In both experiments, frontal and septal animals were impaired relative to normals which performed successfully even at the start of testing. However, while performance of frontals and septals remained at a random level in Exp.II, it gradually improved with testing in Exp.I (reaching the level of normals on the last block of trials). Additionally, there was a mild but nevertheless consistent tendency of all lesioned animals to improve their performance over the successive trials of each daily session. Analysis of exploratory behavior revealed a selective deficit of septals both in terms of habituation and of efficiency. This suggests that septal animals unlike normal or frontal animals are unable to form a cognitive spatial representation providing flexible access to information although they can acquire some spatial information of constant and important value. In contrast frontal animals would be unable to maintain specific information in their spatial representation as a result of a storage deficit. It is concluded that frontal and septal deficits would be best accounted for by considering their common participation to a declarative memory system, as opposed to a procedural memory system.

364.17 SPATIAL PROBLEM SOLVING BY RATS WITH HIPPOCAMPAL LESIONS. N. Chapuis+and T. Herrmann+. (SPON : B.E. WILL)
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The aim of this study was to investigate the effects of hippocampal lesions on exploratory behavior and subsequent performances in a complex spatial problem solving task. An elevated "wheel-maze", made up with eight arms radiating from a central platform and a peripheral linking alley, was used. After a 15' exploration phase, the subjects were allowed for 1 min to eat food which was placed at the end of one of the eight arms. This phase was immediately followed by three test trials during which the rat was placed at the central platform and had to go to the food. The sequence "exploration-feeding phase-test trials" was repeated three times during eight days using the eight possible locations for food, pseudo-randomly distributed.

The results showed that hippocampals explored more than normals during each 15' session although both groups displayed within-session habituation.

In addition, during the tests, the intact rats chose more accurately the direct path leading to the goal and their performances improved both within and between the sessions. In contrast, the choice accuracy of the lesioned rats did not increase over trials either, within or between the daily test session. Since the location of the goal was the same over the three daily trials, the lack of within session improvement suggests that hippocampal animals would not even be able to use non spatial strategies. A detailed analysis of the exploratory patterns is currently conducted in order to find out differences in the exploratory strategies since the hippocampal rats habituate in this complex maze, what contrasts with the lack of habituation usually observed in such subjects.

365.1 IMIPRAMINE POTENTIATES COCAINE-INDUCED FACILITATION OF BRAIN STIMULATION REWARD. R.A. Frank, T. Pommering* and D. Nitz*. Dept. of Psychology and Psychiatry, Univ. of Cincinnati, Cincinnati, OH 45221-0376.

The tricyclic antidepressant imipramine has recently been used to treat cocaine dependence. It has been suggested that this drug reduces cravings for cocaine during abstinence and may even block cocaine euphoria (Kleber & Gawin, NIDA Monograph 50, 1984). The latter hypothesis was tested by examining the influence of imipramine and cocaine administered separately and together on self-stimulation train-duration response functions. Male Sprague-Dawley rats had electrodes implanted in the ventral tegmental area under sodium pentobarbital anesthesia. Following a 10 day post-operative recovery period, the animals were screened for self-stimulation and 20 rats were selected for further study. In the next phase, self-stimulation train-duration response functions were generated for each rat following 5 days of saline injections. Subsequently, the animals received 10, 20 or 30 mg/kg cocaine HCl (IP) 15 min prior to testing. Once the responses to cocaine alone had been determined, the rats in each dosage group were divided into cocaine and cocaine plus imipramine groups. Either 10 mg/kg imipramine or saline was injected (IP) 15 min prior to cocaine administration (which was given 15 min prior to testing and at the original dose). The double injection procedure was run for six consecutive days followed by a three day, postdrug baseline. Cocaine lowered self-stimulation thresholds in a dose-dependent manner, and imipramine accentuated this effect, doubling it at 30 mg/kg. Imipramine's potentiation of cocaine's effect was not due to the simple addition of cocaine and imipramine's effects because imipramine produced not change in thresholds when administered alone. The results of the present experiment indicate that acute imipramine enhances rather than blocks cocaine euphoria. This finding may limit the usefullness of tricyclic antidepressant treatment for cocaine abuse.

365.2 LACK OF TOLERANCE TO THE THRESHOLD RAISING EFFECTS OF MORPHINE ON THE DETECTION OF NON-REWARDING BRAIN STIMULATION. J. Williams and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Morphine sulfate (MS) lowers the threshold for rewarding brain stimulation to the medial forebrain bundle-lateral hypothalamic (MFB-LH) area and raises the threshold for escape from aversive brain stimulation to the mesencephalic reticular formation (MRF). Also, there is no tolerance to the threshold lowering effects of MS on rewarding brain stimulation, however, there is tolerance to the threshold raising effects of MS on aversive brain stimulation. Since the rewarding electrical stimulation is delivered to the MFB-LH and the aversive stimulation to the MRF, the possibility exists that the differences in the development of tolerance are a function of the site stimulated. In order to determine the role of the site of stimulation, detection thresholds for intracranial electrical stimulation to both MFB and MRF were measured after chronic administration of MS. The levels of stimulation used for determining detection thresholds are by themselves neither positively nor negatively reinforcing; however, these levels of stimulation can be used as discriminative stimuli in a simple instrumental task.

Bipolar stainless steel electrodes were stereotaxically implanted in male F-344 rats (Charles River Laboratories). Electrodes were located bilaterally into the MFB-LH and unilaterally into the MRF areas. Two groups of animals were trained to make an instrumental response to a 0.5 sec stimulation cue (S1) to the MFB or MRF, respectively. Responding to the cue was maintained by the delivery of a reinforcing stimulus (S2) to the MFB-LH area. Detection thresholds were determined by varying the current intensity of the brain stimulation cue (S1) according to a modification of the psychophysical method of constant stimuli. The reinforcing stimulus (S2) remained at a fixed highly rewarding intensity level. MS was infused for 7 days by means of an Alzet osmotic pump implanted subcutaneously between the scapulae of the animal. Chronic infusion of MS failed to cause tolerance to the threshold raising effects of a test dose of MS s.c. given on day 6 of the pump infusion period in either the MFB or MRF groups. MS infusion rates were sufficient to cause tolerance to the effect of the test dose of MS on the escape from aversive stimulation to the MRF or on the tail-flick analgesia procedure. These results suggest that the presence or absence of tolerance to MS effects on the threshold for rewarding, aversive, or detection of brain stimulation is not due to the specific brain site stimulated but is related to the functional nature of the task.
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365.3 EFFECTS OF CRF ON SEPARATION DISTRESS AND JUVENILE PLAY. J. Panksepp, L. Crepeau* & M. Clynes[1]. Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403 and [1]N.S.W. Conservatory of Music, Sydney, Australia.

Brain CRF systems are confluent with circuits which mediate distress vocalizations (DVs) resulting from social separation. Thus, in addition to mediating a generalized stress response, CRF neurons may have an executive function in elaborating distressful social emotions. To evaluate that possibility, we measured the effect of intracerebroventricular adminstration of rodent CRF on emission of separation induced DVs in young domestic chicks and rough-and-tumble play in juvenile rats.

Young domestic chicks (4 days of age; n=8-16 per group) were isolated from their flock and administered 0.04, 0.2, 1.0 or 5.0 µg CRF or 3 µl of acidified distilled water vehicle into the fourth ventricle region and frequency of DVs was automatically recorded for four 5 min blocks. Testing was conducted during the first and third test-periods in plain sound-attenuated isolation boxes (36 x 32 x 33 cm), and during the second and fourth periods in similar boxes in which the walls were coverd by mirrors. The average level of control DVs in the plain boxes was 280 per 5 min, and this was increased in dose dependent fashion, with the 5 µg dose producing a 41% increase. A more dramatic effect was observed in the mirrored condition (which reduced baseline vocalizations to 57 DVs/5 min). At CRF doses above 0.2 µg, the ability of mirrors to reduce vocalizations was essentially eliminated. This seemed to reflect a general inhibition of social comfort since animals continued to vocalize when returned to their flocks. The effect lasted up to six hours following the highest doses, and was present in birds which were tested up to 30 days of age. The ability of music to reduce DVs was also evaluated (Beethoven's Opus 106), and CRF also attenutated this inhibition.

If the increased DVs reflected emotional distress akin to that produced by social isolation, it was predicted that CRF would also diminish the vigor of social play. Play of juvenile Long-Evans rats (20-33 days of age; n=24) was measured by frequency of pinning, dorsal contacts, and overall rough and tumble activity on a motion detector platform. CRF, in doses of 0.5 and 1.0 µg administered to the 3rd ventricle region eliminated play 30 min following treatment (by all the indexes used). A 0.25 µg dose reduced play to about 50% of control levels, while a 0.125 µg was without reliable effect.

These results suggest that brain CRF systems may have a key role in the activation of emotional processes related to the perception of social isolation.

365.4 FUNCTIONAL MAPPING OF THE EFFECTS OF MIDBRAIN RETICULAR STIMULATION ON THE CEREBELLUM AND BRAINSTEM DEMONSTRATED WITH 2-DEOXYGLUCOSE. F. Gonzalez-Lima. Dept. of Anat., Coll. of Med., Texas A&M Univ., College Station, TX 77843.

Autoradiographic [^{14}C]2-deoxyglucose (2-DG) procedures were used to map the functional activity of the cerebellum and brainstem during electrical stimulation of the deep mesencephalic nucleus of the midbrain reticular formation (MRF) in behaving rats. Quantitative determinations of 2-DG uptake in 71 structures of MRF-stimulated rats were compared to those of control rats without stimulation. The major finding in the cerebellum was a large increase in 2-DG uptake observed in the flocculus of MRF-stimulated rats showing a "freezing" defensive response. The peak of labeling in the flocculus was greater than any other peak of labeling measured in the cerebellum of MRF-stimulated or control rats. Structures showing significant decreases in 2-DG uptake included the 3 deep cerebellar nuclei and the 3 vestibular nuclei. The most pronounced suppressive effects of MRF stimulation were on the medial and lateral vestibular nuclei. The structures activated in the midbrain, caudal to the stimulation site, are part of the reticular formation and the central gray. The changes in metabolic activity revealed by 2-DG provide a first anatomical demonstration of: (1) the activating effects of MRF stimulation on the flocculus, and (2) the suppressive effects of MRF stimulation on deep cerebellar and vestibular nuclei. The observed patterns of metabolic activation and suppression were correlated with the known electrophysiological properties of the structures affected by MRF stimulation. The findings are consistent with specific effects of MRF stimulation on floccular-vestibular-visual interactions that may be disruptive to learning functions such as adaptability of the vestibulo-ocular reflex. They also support the existence of arousal-dependent MRF mechanisms for the modulation of cerebellar function. In addition, structures activated during MRF-evoked bradycardia were located in the caudal medulla. The largest increase was observed in the caudal nucleus ambiguus. Significant increases were also found in the dorsal motor nucleus of vagus and in the nucleus of the solitary tract. It was concluded that bradycardia induced by MRF stimulation may be mediated by brainstem descending pathways between the activated regions of the midbrain and the medullary nuclei known to induce bradycardia upon electrical stimulation. The results suggest that the midbrain central gray and reticular formation may play a role as modulators of a functional cerebellar-medullary circuitry for emotional expression of somatomotor and autonomic responses to arousing stimuli.

365.5 LOCOMOTION AND ANALGESIA AFTER NICOTINE MICROINJECTIONS ALONG THE CHOLINERGIC PATHS ASCENDING FROM Ch5 AND Ch6 IN RATS. Edgar T. Iwamoto and Edwin C. Williamson*. Dept. of Pharmacology, University of Kentucky College of Medicine, Lexington, KY 40536.

The cholinergic projection arising from the pedunculopontine nucleus of the pontomesencephalic reticular formation (cell group Ch5, Mesulam et al. 1983) projects to the thalamus and the substantia nigra. The Ch6 neurons are within the laterodorsal tegmental nucleus in the periventricular gray. We now report the effects of microinjections of nicotine along these pathways in adult male Sprague-Dawley rats.

Nicotine base, 0.25 to 6 µg buffered in 0.5 µl of artificial CSF, was injected unilaterally into the pedunculopontine-nigral Ch5 path using a pressure injection method in freely-moving rats (Iwamoto et al. PBB 20:959, 1984). Animals were confined within a glass cylinder 20 cm in diameter and 20 cm high during the experiments. Nicotine induced dose-related, head-to-tail, turning movements immediately after injection which lasted less than 3 minutes. The direction of the whole-body turning was contralateral to the side of injection. A dose of 0.25 µg of nicotine induced 4 ± 1.2 (S.E.) complete turning movements within 3 min while the 6 µg dose induced 11 ± 0.9. Five µg of chlorisondamine injected into the Ch5 path 2 weeks before 6 or 12 µg of nicotine, and 10 µg of mecamylamine co-administered with 6 µg of nicotine, completely antagonized the nicotine-induced movements. Thirty min pretreatment with 30 µg/kg s.c. of spiperone completely blocked the turning induced by 12 µg of nicotine injected into the Ch5 path. Thirty min pretreatment with 0.1 but not 0.01 mg/kg s.c. of scopolamine blocked the turning induced by 6 µg of nicotine. Microinjections of 0.1 µg kainic acid at the same site caused a completely different syndrome: backward movements and convulsions. Injections of 0.5 to 6.0 µg of nicotine into the adjoining Ch6 sector in the periventricular gray induced (in addition to turning) antinociception for 15 min as assessed by the 50°C hotplate and tailflick methods; the analgesia was antagonized by coinjections of mecamylamine but not by 10 mg/kg s.c. of naloxone. The data suggest that the region of the pontomesencephalic reticular formation and periventricular gray is sensitive to low concentrations of nicotine. Our current hypothesis is that unilateral nicotine injections along the pedunculopontine-nigral Ch5 pathway in rats induces release of acetylcholine onto muscarinic receptors located on A9-A10 dopamine cell bodies causing the stimulation of ascending dopaminergic pathways which results in contralateral turning movements. In addition, the antinociceptive effects of nicotine injected into Ch6 may involve cholinergic mechanisms. (Supported by the KTRB).

365.6 EFFECTS OF INTRA-HIPPOCAMPAL ADMINISTRATION OF COLCHICINE ON INCENTIVE CONTRAST AND ON RADIAL MAZE PERFORMANCE. C.F. Flaherty, G.A. Rowan*, D.F. Emerich , T.J. Walsh. Psychology Department, Rutgers University, New Brunswick, NJ 08903

The consummatory behavior of rats shifted from a 32% to a 4% sucrose solution declines to a level substantially below that of unshifted animals that have experienced only the 4% solution (a negative contrast effect-NCE). This decrement in performance is attenuated by anxiolytics and by lesions of the amygdala (Becker & Flaherty, Psychopharm., 80:35, 1983; Becker, et al, Physiol. Behav., 22:903; 1979).

In three experiments we investigated the effects of intradentate administration of colchicine on successive negative contrast in consummatory behavior, anticipatory contrast and reversal, and radial-arm maze performance.

In Experiment 1 the colchicine treated rats were not different from CSF treated rats or untreated rats in the consumption of 32% and 4% sucrose solutions, nor in degree of negative contrast produced by a shift from 32% to 4% sucrose.

In Experiment 2 there was no difference in degree of anticipatory contrast nor in the reversal of anticipatory contrast in treated and untreated rats.

In Experiment 3 the colchicine treated rats were deficient in radial-arm maze performance--making fewer correct responses each day, and requiring more responses to attain eight correct arm entries. This decrement in maze performance is consistent with earlier reports of the effects of colchicine treatment (Walsh, et al, Brain Res., 398:23, 1986).

These data suggest hippocampal involvement in complex maze performance but not in consummatory behavior, and/or sensory memory.

365.7 SELECTIVE EFFECTS OF DIAZEPAM ON LATERAL HYPOTHALAMIC RESPONDING: EVIDENCE FOR SEPARATE NEURAL SUBSTRATES FOR REWARD AND ESCAPE. S. E. Carden and E. E. Coons*. Psychology Dept. New York Univ. New York, NY 10003

Lateral hypothalamic (LH) self-stimulation and escape responding was evaluated in 4 pure-reward and 4 reward-escape rats. Thresholds of response and barpressing rates across four stimulation levels were measured both for LH onset and offset.

With the administration of diazepam, self-stimulation thresholds decreased and barpressing rates for onset increased in a dose-dependent manner for both groups. Changes of this type are generally associated with the potentiation of reward.

Although Liebman (Neurosci. and Biobehav. Rev., 9:75-86, 1985) concluded that diazepam alters performance by diminishing an aversive component of LH stimulation, in the current study, escape thresholds and escape rates evidenced no drug-related modulation.

There is electrophysiological (Shizgal, P. & Matthews, G., Brain Res., 129:319-333, 1977) and pharmacological (Atrens, D., et.al., Psychophar., 71:97-99, 1980) evidence of separate neural substrates for reward and aversion in the LH. The results obtained here were consistent with the simultaneous stimulation of a diazepam-sensitive reward system and a diazepam-resistant aversion system, which is powerfully engaged in reward-escape animals only.

If this aversion is activated simultaneously with reward - but only in reward-escape animals - then there should be systematic differences between groups on self-stimulation trials. To test this possibility, in a second experiment, self-stimulation and escape rates for 6 pure-reward and 6 reward-escape rats were measured. Stimulation level and train length were manipulated, with and without diazepam. This allowed us to explore the summative effect of a longer train length in both groups.

All animals produced higher barpress rates for long trains than was predicted by short train performance, regardless of drug condition. However, the increase in reward value which accompanied longer stimuli was greater for pure-reward rats. The indication is that long trains are relatively more rewarding to pure-reward than to reward-escape rats. Therefore, even on self-stimulation trials, it is possible to distinguish pure-reward from reward-escape animals.

In addition, in the first experiment, self-stimulation thresholds were found to be higher and barpressing rates to be lower for reward-escape animals, across all conditions. This is consonant with a theory that sees in reward-escape rats a reward system which is being modulated by the simultaneous stimulation of an aversive substrate.

365.8 **THE NAPLES HIGH- AND LOW-EXCITABLE RATS: NEW GENETIC MODEL FOR WHAT?** **A.G. Sadile, A. Cerbone* and L.A. Cioffi*. Inst. Human Physiol. and Med. Phys., 1st Med. Sch., Univ. of Naples, I-80138, Naples, Italy.**

The Naples High- (NHE) and Low-Excitable (NLE) rat strains have been selectively bred for divergent locomotor reactivity in a modified Lat's box, from an outbred population of Sprague-Dawley rats by Sadile et al. ever since 1976 and now over 25 generations of continuous genetic pressure. Aim of this report is to outline their behavioral profile and to propose their usefulness in behavioral physiology. In order to verify whether the differences in exploratory activity were due to differences in "reactivity" or in "activity", NHE/NLE rats were tested in spatial novelty situations of increasing complexity, an open-field of the Animex type, an hole-board or a Lat's box for a 10min-exposure, or in an activity cage for 3 consecutive days. The circadian activity of NHE/NLE rats was strikingly similar in the activity cages, whereas their "reactivity" upon short exposures to "novelty" situations differed more as the level of complexity increased, i.e. by 20% in the Animex box, by 40% in the hole-board and by a factor of two in the Lat's maze. Moreover, this differential reactivity was not attibutable to different thresholds for nociceptive stimulation, since paw-lick latencies on hot-plate were also very similar.

Furthermore, indirect comparisons were made with other strains, which were based on two-way active avoidance in a shuttle-box, emotionality indices as defecation scores and corticosterone plasma level and arterial blood pressure. A) NHE/NLE rats are both learners in to-criterion acquisition of avoidance in a shuttle-box, in contrast with the Roman High- (RHA) and Low-Avoidance (RLA) or with the Syracuse strains (SHA/SLA). Upon retention tests on five consecutive days, their behavioral coping strategies shifted, since 50% of the NHE and NLE changed from avoidance to freezing responses. B) Corticosterone plasma level. Not different was the basal level, nor the level after exposure to an open-field (nor defecation scores), in contrast with Maudsley Reactive (MR) and non-reactive rats (MNR); C) Arterial blood pressure. NHE and NLE did not differ in arterial blood pressure, in acute anaesthesized preparations, in contrast with the Spontaneoulsy Hypertensive rats (SHR). Thus, the factors determining reactivity, avoidance learning and neurovegetative responses seem to be controlled by and located on different genes. For, the Naples lines offer the possibility to study reactivity to a spatial novelty independently from the others. In conclusion, since the NHE and NLE rats are hyper- and hypo-reactive in spatial novelty situations, with intact learning ability and neurovegetative responses, they could be used as animal model for the study of hippocampal function, since normal reactivity is thought to be dependent on its integrity, and of non associative behavioral processes.

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365.9 INTRAVENOUS SELF-ADMINISTRATION OF THE INDIRECT DOPAMINE AGONIST AMFONELIC ACID. N.L. Goodman* and L.J. Porrino (Spon: C. Kennedy). Neuropsychopharmacology Laboratory, Addiction Research Center, NIDA, Baltimore, MD 21224.

The effects of the psychostimulant, amfonelic acid (AFA), are mediated through its actions on central dopaminergic neurons. Behaviorally, AFA produces hypermotility and stereotypy in rats. These behavioral actions are similar to those produced by amphetamine and cocaine, psychostimulants which are known to be self-administered. Intravenous self-administration (IVSA) is a frequently used animal model that can provide information about the reinforcing efficacy as well as the abuse potential of a drug. We have assessed the reinforcing properties of AFA using the self-administration paradigm.

Four male Lewis (250-350 g) rats were prepared with chronic venous catheters under pentobarbital anesthesia. Following recovery from surgery, rats were trained to self-administer cocaine at a dose of 1.0 mg/kg/infusion on an FR10 schedule in daily 4 hour sessions. After stable rates were obtained, rats were tested with AFA in doses of .0625, 0.125, and 0.25 mg/kg/infusion in either 4 or 8 hour sessions.

AFA was self-administered in all rats tested. Increases in the unit dose of AFA produced dose-dependent decreases in injection rates. Response rates were consistent within animals for each dose, but there was a high degree of variability between animals. Rates of self-administration of AFA were generally lower than those obtained for cocaine even at the lowest AFA dose tested. This difference in overall rates may be a reflection of the longer duration of action and greater potency of AFA as compared with cocaine.

These data demonstrate that AFA is reliably self-administered in rats, and provide further support for the similarity of the behavioral effects of AFA and other psychostimulants shown in other paradigms (Schechter, PBB, 26:413-416, 1987; Aceto et al, Eur. J. Pharm. 10:344-354, 1970; Shore, J. Pharm. Pharmacol. 28: 855-857, 1976). Furthermore, these results strongly suggest that AFA may have significant potential for abuse.

365.10 IDENTIFICATION OF CANDIDATE REWARD PATHWAYS BY MEANS OF PSYCHOPHYSICAL INFERENCE AND COMPOUND ACTION POTENTIAL RECORDING. I. Kiss* and P. Shizgal. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, QUE., Canada H3G 1M8.

An important step toward identifying neurons responsible for brain stimulation reward is to demonstrate concordance between psychophysical and electrophysiological measures of neural properties such as the refractory period and conduction velocity. Of the electrophysiologically identified cells driven by rewarding stimulation, only those with characteristics within the range of the psychophysically-derived values can be considered as plausible candidates.

The present study complements previous work comparing electrophysiological and psychophysical estimates of refractory periods. To trace the fibers rather than the somata of the candidate reward neurons, macroelectrode recordings of compound action potentials were used in lieu of microelectrode recordings of antidromic, single-unit spikes.

Several problems were encountered in recording compound action potentials elicited by lateral hypothalamic (LH) stimulation. Despite the use of conventional methods for reducing the shock artifact, it sometimes obscured portions of the neural response. Also, paired-pulse records were sometimes difficult to interpret due to the overlap in time between components of the two responses. To remove the shock artifact from single pulse records, we used a double subtraction technique. A pair of pulses was delivered with the intra-pair interval set to a value close to the estimated absolute refractory period of the most excitable MFB neurons. A record obtained by delivering a single pulse was then subtracted from this paired-pulse record. If local potentials have decayed before recovery from refractoriness begins, if artifact and response components sum linearly, and if the artifact does not vary with the C-T interval, the result should be a record consisting only of the artifact. Moreover, subtracting this "artifact-only" record from a single-pulse record would yield a "pure", artifact-free record. To separate the contributions of the responses produced by conditioning and test pulses, we used an analogous triple subtraction technique.

Recovery from refractoriness in pathways directly activated by rewarding LH stimulation was estimated with the aid of these techniques. Recording sites were found in the region of the ventral tegmentum, the substantia nigra, and the ventral portion of the central grey in which the electrophysiological measure of recovery from refractoriness substantially overlapped psychophysical estimates obtained with the same stimulation electrodes, currents and pulse durations. These findings suggest that fibers coursing caudal to the ventral tegmental nucleus of Tsai may compose part of the directly activated substrate for the rewarding effect of LH stimulation.

365.11 REVERSAL OF MORPHINE INDUCED FACILITATION ON REWARDING ELECTRICAL BRAIN-STIMULATION BY NALOXONE: EVIDENCE FOR DIRECT PHARMACOLOGICAL ACTIVATION OF OPIATE RECEPTORS. M. Moolten* and C. Kornetsky. (SPON: L. Volicer). Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

Although morphine will increase the sensitivity of animals to rewarding brain stimulation and increase the rate of response for such stimulation, it has been argued that the effects are due to non-pharmacological actions of morphine, and thus not to opiate receptor activation. It was reported that the rate increasing effects of morphine during intracranial self-stimulation (ICSS) are not reliably reversed by naloxone once the morphine effect has been initiated (Hand and Franklin, 1986), suggesting that associative, non-pharmacological factors may be involved in morphine's reinforcing properties. In an attempt to further elucidate the mechanisms involved in morphine's facilitation of ICSS, we administered naloxone at the time of maximum effect of morphine on the threshold for rewarding brain-stimulation.

Bipolar stainless steel electrodes were stereotaxically implanted in the medial forebrain bundle (MFB) of male F-344 rats (Charles River Laboratories). Animals were trained to turn a wheel manipulandum to obtain rewarding electrical stimulation to the MFB. Brain-stimulation reward thresholds were determined by using a modification of the psychophysical method of limits. In all animals morphine (2 or 4 mg/kg s.c.) caused significant lowering of threshold which persisted throughout the 100 min testing session. When naloxone was given 40-60 minutes following morphine administration, it caused a dose-dependent antagonism of the threshold lowering effect. Doses as low as 0.125 mg/kg i.p. completely reversed the morphine induced facilitation in all animals at all morphine doses tested.

These results clearly demonstrate that morphine's facilitation of the reinforcing properties of rewarding brain-stimulation is mediated by opiate receptor activation.

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365.12 COCAINE EFFECTS ON REWARDING BRAIN STIMULATION AS ASSESSED BY THE QUANTITATIVE 2-DEOXYGLUCOSE METHOD. G.T. Bain*, L.J. Porrino† C. Caplan* and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118 and †Addiction Research Center, NIDA, Baltimore, MD and ‡Laboratory of Cerebral Metabolism, NIMH, Bethesda, Md.

Systemically administered cocaine has been shown to increase the sensitivity of the rat for rewarding electrical stimulation to the medial forebrain bundle (MFB) (Esposito, et al., Pharmacol. Biochem. Behav. 8:437, 1978). The specific structures which may be directly involved in this phenomenon have yet to be determined. In the present study the quantitative 2-(^{14}C) deoxyglucose (2-DG) method was used (Sokoloff, et al., J. Neurochem. 2:897, 1977) to determine those areas which are functionally involved in this interaction of cocaine and rewarding brain stimulation.

Bipolar stainless steel electrodes were stereotaxically implanted into the MFB of male F-344 rats (Charles River Laboratories). The animals were trained to press a lever to receive rewarding brain stimulation. Each response resulted in the delivery of a single 500 msec 160 Hz stimulation at an intensity adjusted for each subject to produce an 80% of maximal response rate. Animals were given 10 mg/kg of cocaine i.p. or saline immediately prior to the administration of 2-DG. The standard protocol (Sokoloff, et al., 1977) for determination of local cerebral glucose utilization (LCGU) was then followed. LCGU was measured both ipsilateral and contralateral to the site of electrical stimulation in the MFB and in selected portions of the forebrain previously implicated in brain stimulation reward and/or cocaine self-administration. Preliminary analysis of the data indicate that there was, for the most part, no difference in the anatomical distribution of rates of LCGU between the cocaine and the saline treated animals. However, a marked bilateral increase in LCGU was seen in the olfactory tubercle, and to a lesser extent in the nucleus accumbens in the cocaine treated animals as compared to the saline treated animals.

These effects of cocaine on LCGU during rewarding brain stimulation observed in the present study are similar to those reported previously with d-amphetamine (Seegar, et al., Neurosci. Abst. 307, 1984).

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365.13 EVIDENCE FOR DOPAMINERGIC AND NOT SEROTONERGIC MEDIATION OF THE THRESHOLD LOWERING EFFECTS OF MDMA ON REWARDING BRAIN STIMULATION. M.P. Bird*, †C.N. Svendsen*, C. Knapp*, †C.C. Hrbek*, †E.D. Bird and C. Kornetsky. (SPON: A. McCall) Laboratory of Behavioral Pharmacology, Boston Univ. School of Med., Boston, MA 02118 and McLean Hospital/Harvard Med. School, Belmont, MA 02178.†

We have previously shown that 3,4-Methylenedioxymethamphetamine (MDMA) lowers the threshold for rewarding brain stimulation, a model for drug induced euphoria. Recent reports have shown that MDMA produces long lasting depletion of brain serotonin (5-HT) with only transient changes in dopamine (DA) levels. Although there is evidence that both DA and 5-HT are involved in MDMA's behavioral effects the mechanism(s) underlying its reinforcing properties remain unknown. In order to determine the relative contribution of 5-HT and DA to MDMA's reinforcing actions, the effects on brain-stimulation reward of the 5-HT_2 antagonist Ly-53857 and the D_2 antagonist pimozide were determined alone and in combination with racemic MDMA.

Bipolar stainless steel electrodes were stereotaxically implanted in the medial forebrain bundle (MFB) of male F-344 rats (Charles River Laboratories). Animals were trained to turn a wheel manipulandum to obtain rewarding electrical brain stimulation to the MFB. Reward thresholds were determined using a modification of the psychophysical method of limits. MDMA (2.0 mg/kg s.c.) significantly lowered the reward threshold in each animal. By themselves pimozide (0.2 to 0.4 mg/kg i.p.) significantly increased the reward threshold while Ly-53857 (2.0 to 8.0 mg/kg i.p.) was without effect. In all but one subject pretreatment with 0.2 mg/kg of pimozide completely blocked the threshold lowering effect of 2.0 mg/kg of MDMA and raised the reward threshold of one animal, while a higher dose of pimozide (0.4 mg/kg) antagonized 2.0 mg/kg of MDMA in 2 animals and raised the reward threshold in 2 others. In contrast to pimozide, Ly-53857 had no effect on MDMA's threshold lowering action. To further assess the pharmacologic activity of MDMA brain monoamine concentrations were measured using HPLC with electrochemical detection (Clin. Chem. 30:1046, 1984). Preliminary data indicates that MDMA (2.0 mg/kg s.c.) significantly decreased 5-HT levels in striatal and hippocampal tissue but significantly increased concentrations of striatal dopamine 1 hour post injection. Taken together these results strongly suggest that the reinforcing effects of MDMA are mediated by D2 and not 5-HT_2 receptor stimulation.

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365.14 HALOPERIDOL BLOCKS CONDITIONED PLACE PREFERENCES INDUCED BY REWARDING LATERAL HYPOTHALAMIC STIMULATION IN RATS. C.L.Duvauchelle* and A. Ettenberg (SPON:H.J.Carlisle).Dept of Psychology, University of California, Santa Barbara, CA 93106

While dopamine antagonist neuroleptic drugs have long been known to interfere with reinforced operant responding, identification of the precise mechanism by which these drugs exert their behavioral effects has proved difficult. A major problem in this regard has been that both reward-attenuation and motor-impairment hypotheses make similar predictions about the behavioral deficits observed in drugged animals. To overcome this problem, we have employed a test procedure (the "conditioned place preference") in which neuroleptic induced changes in brain-stimulation reward can be identified at a time when the direct pharmacological effects of the drug are no longer present.

Male rats implanted with lateral hypothalamic electrodes were shaped to lever-press for rewarding 0.5s trains of 60Hz sine-wave stimulation. Current intensities were identified for each rat that produced steady responding during daily 15 min sessions. Once operant rates had stabilized, a 3-day preference procedure was begun. The test apparatus was a rectangular enclosure consisting of two distintive sides (ea 42x42x38cm) varying in color, floor texture an odor. On Day 1, a Baseline was determined by recording the amount o time a rat spent on each side of the apparatus. On Day 2, every rat received a single IP injection of either haloperidol (0.15 or 0.3 mg/kg HAL) or its lactic acid vehicle solution (VEH) 45 min prior t the first of five 5min exposures to each of the two distinct test environments. Rats were administered rewarding brain stimulation during the times they were in the "less preferred" environment (as determined on Baseline day). The stimulation rate and parameters were based upon each rat's self-stimulation performance during the final three days of training. No stimulation was delivered while rats were in the "preferred" side of the apparatus. These subjects had been pretreated with 0.0, 0.15 or 0.3 mg/kg HAL (n=6/group). Additional HAL and VEH control groups experienced the same condition (either rewarding brain stimulation or no stimulation) in both test environments. On Day 3 (24hrs after drug/veh treatments), a second place preference test was conducted in the same manner as on Day 1.

Rewarding brain stimulation produced a dramatic shift in place preferences towards the stimulation-paired side. This effect was prevented by pretreatment with the high, but not the low, dose of haloperidol. None of the control groups demonstrated changes in place preference and haloperidol did not in and of itself produce shifts from baseline preferences on test day (24 hrs post-injection) These data are consistent with the view that the reward produced by lateral hypothalamic stimulation can be attenuated by administration of dopamine receptor antagonist neurolpetic drugs.

365.15 EFFECTS OF CHLORDIAZEPOXIDE AND MORPHINE ON CER-ATTENUATED JUVENILE RAT PLAY. L. Crepeau* & J. Panksepp (SPONSOR: F. DeEskinazi) Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403

Juvenile play was evaluated during the acquisition of a conditioned emotional response (CER) paradigm where a tone was presented predicting inescapable footshock, as well as during extinction of the tone-shock contingency. The ability of chlordiazepoxide (CDP) and morphine sulfate (MS) to maintain and resurrect play behavior in the context of such threat was also evaluated.

Juvenile Long-Evans rats (21 days of age; n=40 pairs of same-sex littermates) were divided into 4 treatment groups. For 7 CER acquisition test days, group 1 (n= 5 pairs) controls received injections of vehicle and no footshock. Group 2 (n=10 pairs) received CDP. Group 3 (n=10 pairs) received morphine. Group 4 (n=15 pairs) received vehicle injections (1 cc/kg). 20-30 minutes after i.p. injections, animals were tested during three 2-minute trials each day During the middle 2 minute period a 1 KHz tone was presented, followed by a 1.5 second, .5 mA footshock. The two surrounding trials acted as pre- and post-shock measurement periods. For the 5 CER extinction test days, half the animals in groups 2 and 3 continued to receive CDP or MS, and half received vehicle. Group 4 was divided into three sets of 5 pairs. These animals received either vehicle, CDP or MS. Play was scored by measurement of pins, dorsal contacts and overall platform activity.

During acquisition testing, rates of play did not demonstrate a CER specific to the tone, but play, especially during the last two days of acquisition, was reliably reduced during all 3 test trials, suggesting the development of fear to contextual cues of the play situation.

During the last 2 days of CER acquisition testing, the overall mean frequency of pins per session was 7.8 and 8.4 for non-shocked control animals, 3.4 and 3.8 (55% lower than controls) for CDP and MS treatments, and essentially zero for the vehicle-treated group. Similarly, dorsal-contact solicitations averaged 11 and 11 in control group animals, 4 and 4 (64% lower) in the CDP and MS groups, and 2 and 1 (86% lower) in group 4. Activity counts among control group animals were 132 and 137, 44 and 45 (67% lower) in the CDP and MS groups, and 11 and 10 (92% lower) in group 4 (all reductions were reliably lower than control ($p < .01$), as well as CDP and MS levels ($p < .05$)).

CER extinction was faster in the CDP and MS groups, whether or not the drugs were still given during CER extinction. Play levels were comparable to non-shocked controls by the second extinction day in CDP-treated animals, and on day 3 in MS group animals. Animals in the vehicle group took 5 days before play levels had returned to normal. Both CDP and MS maintained play and facilitated recovery of play behaviors during CER training and extinction. The neural mechanisms which elaborate this effect need to be investigated further, since opioids and BZs appear to act on distinct neural structures.

365.16 THE HEXADYAD PRIMARY EMOTIONS AS A NEUROSCIENCE WORKING HYPOTHESIS. B. E. Morton, Dept. of Biochemistry and Biophysics
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Just as the infinity of perceived colors are produced from combinations of the three primary colors, so also the large variety of affects which can be experienced may be the summation product of a few primary emotions. Earlier lack of this concept would account for disagreement among authors about emotion classifications.

It is here proposed that in organisms with brains containing a limbic system, there are six independent, opposed pairs of primary emotions. Each primary emotion pair consists of a range between appetitive and aversive extremes. The output of a primary pair can reside only at one point within that range at a given time. The ongoing emotional experience of such an organism results from the continual summation of these six variable range points.

The proposed six primary emotion pairs (and their abbreviated biological significances) are:

1. CONFUSION-SURPRISE (ignorant) vs. CERTAINTY-EXPECTANCE (knows)
2. FEAR (less powerful than) vs. CONFIDENCE (more powerful)
3. DISGUST (rejects) vs. PLEASURE (accepts)
4. ANGER (harmed by other) vs. GRATITUDE (helped by other)
5. GRIEF (loss-failure) vs. ELATION (win-success)
6. DESIRE (deprived) vs. SATISFACTION (satiated)

Each primary emotion pair provides fundamental survival benefits to the organism and thus must have been selected and preserved by evolution. In humans if immediate emotional responses are prolonged for hours, they are moods; if relatively habitual, they become personality traits. The hexadyad primary emotions can be plotted in a circumpolar array which is useful in depicting such personality traits.

The output position within the range of an appetitive-aversive primary emotion pair appears to be controlled by the relative activity or balance of activities of specific brain elements which can also be manipulated by drugs. For example the Fear-Confidence primary emotion pair appears to be controlled by a norepinepherine output coming from the locus ceruleus. Thus, fear levels in humans can be manipulated by alpha2 and beta2-adrenergic ligands altering noradrenergic synaptic transmission. Such produce emotional manifestations ranging from foolhardiness to terror. Locus ceruleus activity itself can also be altered by benzodiazepine-GABA-chloride channel ligands to produce a like range of emotional responses.

Similarly, it appears that the Elation-Grief pair is produced by subelements of the dopaminergic-enkephalinergic systems separate from the locus ceruleus. The sites and pharmacologic identities of other primary emotion pairs can in principle also be determined. Thus, the hexadyad primary emotions hypothesis would seem to provide a useful framework within which to organize data and research on the production and significance of brain emotional output.

365.17 IBOTENIC ACID LESIONS AND LATERAL HYPOTHALAMIC SELF-STIMULATION
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Several recent studies (Velley, et al., Brain Res., 268: 79-86, 1983; Nassif, et al., Brain Res., 332: 247-257, 1985; Lestang, et al., Neurosci., 15: 379-388, 1985) support a role for endemic lateral hypothalamic neurons in self-stimulation in rats by using the excitotoxin ibotenic acid to destroy these neurons while leaving fibers of passage intact. Anatomical investigations (Schwarcz, et al., Brain Res., 37: 199-216, 1979; Hastings, et al., Brain Res., 360: 248-256, 1985) have confirmed this axon-sparing property by showing that forebrain monoamine concentrations are not significantly decreased following lateral hypothalamic ibotenic acid lesions. However, loss of myelin in intact axons following lesion could substantially alter stimulation's effectiveness in generation of action potentials.

We injected into the lateral hypothalamus of rats, over a 20 minute period, 1 ul of 3.5-4.0 ug/ul of ibotenic acid (Sigma) dissolved in phosphate buffer and titrated to a pH of 7.2-7.4 with 0.1 M NaOH, in accordance with the protocol used in studies cited above. After two weeks survival time, animals were sacrificed, brains were removed, and tissue was sectioned in the sagittal plane. Alternate sections were stained for Nissl substance (cresyl violet) and myelin (hematoxylin). Cresyl staining revealed the area of lesion as a region of cell loss and heavy glial proliferation. In the hematoxylin stained sections, the area of lesion appeared almost completely bleached, although fibers directly rostral and caudal were darkly stained. Behavioral testing showed that ibotenic acid lesions both rostral and caudal to the stimulating electrode can be effective in degrading reward effects, as measured by lateral shifts in rate-frequency functions relative to pre-lesion testing.

The histological results suggest that lateral hypothalamic injections of ibotenic acid may substantially demyelinate the local area. If so, then degradation of the effectiveness of stimulation in eliciting action potentials could account for the behavioral results of this and previous experiments. However, the fact that rostral ibotenic acid lesions can be more effective than similarly placed transections (Waraczynski, Neurosci. Abst., 11: 1173) argues for some role of endemic lateral hypothalamic neurons in stimulation reward effects. Resolution of this issue depends on histological studies of the demyelinating effects of ibotenic acid in and out of the lateral hypothalamus. This work is in progress.

365.18 REFRACTORY PERIODS FOR HYPOTHALAMIC SELF-STIMULATION ARE SHORTENED BY ALPHA-FLUPENTHIXOL INJECTIONS. P. Krevs*, J. Yeomans and K. Buckenham* (SPON: J. Nobrega). Dept. of Psychology, Univ. Toronto, Toronto, Canada M5S 1A1.

Refractory periods for medial forebrain bundle (MFB) axons mediating self-stimulation in rats are mainly in the 0.4 to 1.2 msec range when measured with standard frequency threshold methods (Yeomans, 1979). MFB axons of midbrain dopamine cells have absolute refractory periods from 1.2 to 2.5 msec (Yeomans, Maidment & Bunney, 1986), and so cannot be the primary substrate for MFB self-stimulation. When high currents, small electrode tips or long pulse durations are used, however, the range of refractory periods for self-stimulation widens to 0.4 to 5.0 msec (Yeomans, Mercouris & Ellard, 1985; Bielajew, Jurgens & Fouriezos, 1985). These stimulation conditions allow high-threshold axons, such as dopamine axons, to be directly excited (Yeomans, Maidment & Bunney, 1986). Are the long-refractory period, high-threshold axons added by high currents, small tips and long pulse durations dopamine axons?

We tested this idea that dopamine axons can be a second directly activated substrate for MFB self-stimulation by injecting the dopamine receptor blocker alpha-flupenthixol into self-stimulating rats. In a narrow dose range (0.1 to 0.4 mg/kg i.p.), the refractory period curves shifted to the left. These doses shifted the baseline frequency threshold only 10 to 30%. A dose of 0.05 mg/kg had no effect, while doses above 0.4 mg/kg prevented reliable threshold testing. Since small tip electrodes were used, the baseline refractory periods were 0.4 to 5.0 msec, but alpha-flupenthixol reduced the range to 0.4 to 1.5 or 2.0 msec. The biggest effects were at the intrapair intervals of 1.0 and 1.5 msec. The shift was similar to the leftward shift observed by using large electrode tips (Yeomans, Mercouris & Ellard, 1985). This suggests that dopamine axons can be a second directly activated substrate for MFB self-stimulation.

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AGING AND DEMENTIA: MOLECULAR BIOLOGY II

366.1 REARRANGEMENTS OF CHROMOSOME 21 IN KARYOTYPICALLY NORMAL DOWN'S SYNDROME AND ALZHEIMER'S DISEASE. J.M. Delabar, N. Créau-Goldberg, D. Goldgaber, A. Nicole, I. Ceballos, Z. Rahmani, J.L. Blouin, Y. Lamour, M. Roudier, P. Davous, A. Gegonne, P. Amouyel, D. Stehelin, D.C. Gajduzek and P.M. Sinet, CNRS, Laboratoire Biochimie-Génétique, Hôpital Necker, 149 rue de Sèvres, 75743, Paris, France. (SPON: P. Dutar)

The copy numbers of the Cu-Zn superoxide dismutase(SOD1)gene, of the proto-oncogene ets2(ETS2), of the gene encoding the amyloid polypeptide of Alzheimer's disease(AD-AP gene), of the estrogen-inducible sequence expressed in breast cancer(BCEI) and of an anonymous DNA sequence(D21S11) were assessed in leukocyte DNA from karyotypically normal Down's syndrome patients and from sporadic cases of Alzheimer's disease.

Human cDNAs for the pro α 1(I) collagen gene localized on chromosome 17 and for the proα2(I) collagen gene localized on chromosome 7 were used as reference probes.

Karyotypically normal Down's syndrome patients have a duplication of SOD1, ETS2 and AD-AP. Alzheimer patients have a duplication of ETS2 and AD-AP. D21S11 and BCEI show a normal copy number. DNA analysis by pulse field gel electrophoresis of this rearranged "critical" region of chromosome 21 is presented.

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366.2 PYRAMIDAL NEURON RNA IN PICK'S VS ALZHEIMER'S DISEASE. J.A. Doebler, R.E. Rhoads*, A. Anthony* and W.R. Markesbery*. Dept. of Biochemistry and Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536 and Dept. of Biology, Pennsylvania State University, University Park, PA 16802.

Although disturbances in nucleic acid metabolism/protein synthesis are characteristic of Alzheimer's disease (AD), other dementing disorders, such as Pick's disease (PD) remain unstudied. We conducted comparative analyses of RNA alterations in frontal (area 9) and occipital (areas 18,19) cortex of patients with autopsy proven PD and AD. Azure B-RNA staining and scanning-integrating microdensitometry were used to determine total RNA contents of pyramidal neurons in layers III and V. Significant (15-45%) RNA loss was evidenced in neurons of both cortical areas and layers in both PD and AD, relative to those of age-matched, non-demented controls (Table). This RNA loss was generally more marked in PD than in AD. In both PD and AD the severity of the RNA loss was not greatly enhanced in frontal cortex, which is more prone to morphological alterations than the relatively resistant occipital cortex. AD-associated neuronal RNA loss was substantially greater in layer V than in layer III in both cortical areas, but this was apparent only in the atrophic frontal area in PD. Overall, the data support the existence of a major disorder in neuronal RNA metabolism in PD as well as AD. However, this RNA loss does not appear to be related to the abundance of classical neuropathological alterations in either disease. Whether the augmented RNA loss in layer V signifies selective or primary damage to the lower cortical layers remains to be ascertained. (Supported by NIH grants 1PO1-AG05119 and 1P50-AG05144).

Table. Pyramidal Neuron RNA (Absorbency units ± SEM)

	Area 9		Areas 18, 19	
	Layer III	Layer V	Layer III	Layer V
Control (N=12)	105.9±5.1	107.3±4.6	85.8±3.9	82.2±1.8
PD (N=6)	71.2±6.2*	59.0±4.9*	60.4±4.9*	56.0±4.5*
AD (N=11)	89.7±4.9*	69.1±3.2*	69.7±3.0*	55.0±2.8*

*Significantly different from the control value, $P<0.05$, 2-sided t-test.

366.3 CLONING OF POLY(A)RNA DIFFERENTIALLY REGULATED IN ALZHEIMER'S DISEASE FROM A HIPPOCAMPAL cDNA LIBRARY. P.C. May, S.A. Johnson*, J.N. Masters, M. Lampert-Etchells* and C.E. Finch. Andrus Gerontology Center, USC, Los Angeles, CA 90089.

We examined the effects of Alzheimer's disease (AD) upon gene expression in the hippocampus. A hippocampal cDNA lambda gt10 library was prepared with poly(A)RNA extracted from normal and AD hippocampus. Total RNA was isolated from frozen brain tissue by the guanidinium isothiocyanate/cesium chloride procedure and poly(A)RNA enriched by two passes over oligo d(T) cellulose. In contrast to a recent report (Boyes et al., 1986, Soc. Neurosci. Abs. 12:944), comparable yields of total and poly(A)RNA were obtained from AD (382±24 ug RNA/g tissue, 1.1% poly(A); n=9) and control (334±32 ug RNA/g tissue, 1.1% poly(A); n=11) hippocampus. Poly(A)RNA from AD (n=4) and control (n=6) were separately pooled (ca. 2-3 ug/individual) and aliquots used as a template for cDNA synthesis. Similar sized cDNA was synthesized from AD and control poly(A)RNA and ranged from approximately 0.5 Kb to 5 Kb (average size 1.5 Kb). The cDNA library was prepared by pooling equal amounts of AD and control cDNA, cloning the combined cDNA into a lambda gt10 vector and packaged in vitro to give a library of 2 X 10^6 recombinant lambda gt10 phage. Of 50,000 recombinants screened using differential hybridization with cDNA probes made from AD or control poly(A)RNA, 61 gave differential signals in AD hippocampus (either increased or decreased in prevalence). Nine clones of 14 clones picked for further study have been confirmed by 3 independent slot blot hybridization experiments to be differentially regulated in AD. These clones range in size from 0.4 to 4.4 Kb and represent poly(A)RNA sequences in AD hippocampus that are increased in prevalence from 2 to 5 fold relative to their levels in normal hippocampus. Clones selected as non-changing controls expressed differential signals averaging 0.9 ± 0.1 (mean ± SEM, n=5). These AD-specific clones will be further characterized by hybridization to individual AD and control RNA blots to ascertain the range of prevalence changes between individual AD samples and analyzed by in situ hybridization techniques to identify the cell types containing the cloned sequences. These AD-specific changes in RNA prevalence may reflect genomic responses to neurodegeneration and deafferentation. For example, degeneration of the entorhinal cortex/hippocampal circuit represents an early and major lesion in AD which results in a functional deafferentation of the hippocampus. This study may also identify a subset of genes whose increased expression in AD hippocampus predisposes select neurons to neurodegenerative insults. Supported by ADRC Grant # AG05142 and the MacArthur Foundation Research Program On Successful Aging (CEF)and the Samuel A. Blank Research Grant from the ADRDA (SAJ).

366.4 GLIAL FIBRILLARY ACIDIC PROTEIN mRNA INCREASES DURING A WASTING AGONAL STATE IN OLD MICE. IMPLICATIONS FOR THE INCREASED GFAP mRNA IN ALZHEIMER'S DISEASE. J.R. Goss*, D.G. Morgan, and C.E. Finch. (SPON: M.N. Gordon). Andrus Gerontol. Ctr. & Dept. of Biol. Sci., Univ. of Southern Calif., Los Angeles, CA 90089-0191.

The examination of postmortem human brain tissue forces biological researchers to consider extraneous variables such as postmortem interval, medication history, and the agonal state of the tissue donor. In previous studies we found no major influence of postmortem interval on the quantity or quality of brain RNA up to 48 hours in young rats (Johnson, Morgan, and Finch, J.Neurosci. Res. 16:267, 1986). In an attempt to model the human agonal condition in Alzheimer's disease (AD) which often includes a prolonged wasting phase of cachexia, hypoxia, and acidosis, we examined the brains of a few mice which were slowly approaching death from natural causes during aging.

Wasting mice were identified by daily observation. They typically failed to ambulate when gently prodded, exhibited slight resting tremors, and felt slightly cold to the touch. Necropsies revealed a gross intestinal tumor in one animal, enlarged spleens in two mice, and empty stomachs in two mice. Wasting mice consisted of 3 female C57BL/6NNia (27mo.) and 1 male C57BL/6J (31mo.). Healthy mice (confirmed observationally and by necropsy) of the same cohort served as controls. Brains were removed and frozen at necropsy. RNA was prepared from frozen brains by the guanidinium thiocyanate/CsCl centrifugation method. RNA blot hybridization was performed using 8ug total RNA and single-stranded [^{32}P] labled antisense RNA probes.

The yield of total RNA was similar for the wasting and control mice (290 v. 330 ug/gm tissue). Ethidium bromide stains of electrophoresed total RNA indicated intact RNA. RNA blot hybridization autoradiograms showed a 3-fold increase in glial fibrillary acidic protein (GFAP) mRNA signal, but no change in the signals for beta-tubulin, Thy-1 antigen, somatostatin, or glutamic acid decarboxylase. Degradation of RNA assessed by RNA blot hybridization was minimal. In a parallel study of mouse cortical samples, freezer storage up to 4 years had no effects on the quantity or quality of RNA.

In AD, we found a similar 3 fold increase in GFAP mRNA in frontal and temporal cortex compared to age-matched control samples (n=8 per group). We originally interpreted this increase in GFAP message as a glial reaction to the neurodegenerative events in AD. However, we now consider it possible that this increase in GFAP mRNA is a response to the premorbid conditions associated with AD, rather than a direct effect of the disease process. Supported by the John Douglas French Foundation (DGM) and the A.D.R.C. of Southern California (AG-05142 to CEF).

366.5 LEVELS OF CYTOSKELETAL mRNAs IN ALZHEIMER CORTEX. A.W. Clark, C.A. Krekoski*, I.M. Parhad, D.I. Hoar*, E.A. Swedberg* Depts of Pathology and Medical Biochemistry, Univ. of Calgary, Calgary, Alta, Canada, T2N 4N1.

Abnormal accumulations of cytoskeletal elements are present in cortical neurons of brains with Alzheimer disease (AD). These accumulations may reflect an abnormality in metabolism of the cytoskeleton. In this study we evaluated mRNA expression of specific cytoskeletal components in AD brain tissue. Postmortem brain tissue was studied in 6 cases (age: 74±2 yrs; brain weight: 953±33 gms) with a history of dementia and with neuropathological features of AD, and 6 age matched controls (age: 70±3 yrs; brain weight: 1306±108 gms). Counts of neurofibrillary tangles (Nft) and neuritic plaques (Np) in a $1mm^2$ section of parietal cortex in the AD cases were Nft:17±3; Np:25±5; in controls no Nft or Np were detected except in an 82 year old with Np but no Nft. Immunohistochemical studies with GFAP showed increased gliosis in the AD cases. At autopsy the parietal cortex was removed and rapidly frozen (AD: 8.2±2.9; Control: 9.8±2.6 hrs). Total RNA was isolated, and 20 µg samples from each brain resolved on 1.2% agarose/2.2 M formaldehyde gels, transferred to nylon membranes and hybridized with ^{32}P radiolabeled cDNA probes. The following probes were used: a 300 bp Pst I fragment of murine Nf-L (Lewis & Cowan, J Cell Biol, 1985) for the 68 Kd neurofilament protein, human bα1 tubulin (Cowan et al.,Mol Cell Biol 1983), and GFAP (Lewis et al., Proc Nat Acad Sci, 1984). Recovery of total RNA was decreased by 20% in the AD group as compared to controls (AD: 399±29; Control: 500±17 µg/gm tissue; Mann Whitney U p=0.01). Gel profiles were similar in AD and control cases with intact ribosomal bands. Northern blots showed bands at approximately 1.8Kb for bα1 tubulin, 3.0Kb for GFAP, and 2.5Kb for Nf-L. Some degradation of the mRNA was evident. Some nonspecific binding to ribosomal RNA was seen especially with the Pst I fragment of NF-L. No qualitative differences in the bands for mRNA of the cytoskeletal components studied were seen in AD as compared to control cases. Cytoplasmic dot hybridization was performed and quantitated using a densitometer. This system is sensitive enough to detect >= 30% change in RNA levels. Using this system no differences were seen between AD and control cases for any of the 3 probes. These results indicate 1) decreased recovery of total RNA from brains of AD cases as compared to age matched controls. 2)Based on Northern analysis and staining patterns on Northern gels, however, there is no evidence for differences in generalized degradation of RNA in AD as compared to controls, 3) and no marked change in the mRNAs of these 3 cytoskeletal components. (Supported by the Alberta Mental Health Council and the Alberta Heritage Foundation)

366.6 CLONING OF RNA SEQUENCES WHOSE PREVALENCE IS INCREASED IN ALZHEIMER CORTEX. S.A.Johnson* and C.E.Finch. Andrus Gerontology Ctr, USC. L.A. Ca. 90089.

To better understand the molecular basis for the selective cellular changes which occur in the Alzheimer Disease (AD) brain, we are analyzing AD and control (CTL) cortical poly (A) RNA for changes in RNA prevalence. We have prepared an AD cortex cDNA library in lambda gt10 and differentially screened it with cDNA probes from single individual AD and control(CTL) cortex poly(A)+RNA(Pair# 1 in Table). From a primary screen of ca. 30,000 clones, 50 clones showed detectable differences between AD and CTL on duplicate filters. Seventeen clones continued to show differential signals after two rescreenings at low plaque density (100/plate). To better assess the differential prevalence of these cDNA's between AD and CTL, recombinant phage DNA was bound to nylon membrane in a slot blot apparatus and hybridized to AD or CTL high specific activity cDNA probes. Five clones showed 1.5-2 fold greater signal in AD cDNA after 3 rounds of hybridization.

Five identical RNA gel blots each containing four pairs of age matched AD/CTL total RNA samples were each hybridized to one of the five AD differential cDNA clones (gel purified cDNA inserts were labeled by the random prime/hexamer technique).Signal intensities on the blots were quantitated by computerized image analysis. These data are shown in the Table.

	Insert		Prevalence Increase on RNA Blot (AD/CTL)			
Clone	Size	Gene Copy #	Pair 1	Pair 2	Pair 3	Pair 4
6-4	1.2kb	repeat	6.6	2.1	1.6	1.1
8-4	0.8kb	single copy	4.1	0.4	1.4	0.5
9-1	0.9kb	single copy	2.1	0.9	0.8	1.0
9-3	0.7kb	repeat	2.2	2.4	1.3	1.3
10-2	0.4kb	repeat	2.0	1.9	0.9	0.7

Clone 9-1 hybridizes to single RNA species of 1.5kb, while 8-4 hybridizes to two species of 1.2 and 2.0kb. Clones 6-4, 9-3 and 10-2 each give a smear upon hybridization to identical RNA gel blots, strongly suggesting the presence of repeated sequences in these clones . Southern genome blot analysis with human AD and CTL cerebellar DNA and rat liver DNA shows the presence of a high copy number repeated sequence in all three clones after high stringency hybridization . These three clones are only very weakly reactive with each other, suggesting they are divergent members of the same repeated sequence family. Similar analysis of clones 8-4, and 9-1 indicates these are each distinct single copy elements in the genome. This suggests that 8-4, which gives two bands on a RNA blot, is differentially spliced. However only the larger 8-4 transcript shows changes in RNA prevalence.

At present, we do not understand the variability in differential signal between individuals . These blots were done with our very best (undegraded) age-matched Total RNA samples to avoid differential poly(A)RNA yield. Further work is underway to see if large polymorphic differences are normal.

These data show that very few (3-5/10,000 detectable plaques) of the prevalent and/or moderately prevalent human cortical transcripts have modulated prevalence in AD vs CTL. Furthermore, we have found a family of human specific repeated sequences, members of which may be transcribed differentially in the AD cortex. SAJ was supported by the Samuel A Blank Research Grant through the ADRDA. Frozen cortex was obtained from Dr. Allen Roses at Duke U. and from the ADRC of SO. Cal.(AG-05142)

366.7 RADIOACTIVE URIDINE INCORPORATION INTO RNA BY POSTMORTEM HUMAN BRAIN TISSUE. EVIDENCE FOR POSTMORTEM TRANSCRIPTION IN THE ALZHEIMER BRAIN. E.M. Sajdel-Sulkowska and C.A. Marotta. Dept. of Psychiatry, Harvard Med. Sch.; Mailman Res. Ctr., McLean Hosp., Belmont, MA 02178.

The incorporation of [^{3}H]uridine into RNA has been studied in human postmortem brain incubated under standard tissue culture conditions. Brain tissues from 11 different control and Alzheimer's disease (AD) cases, 57-91 years of age, with postmortem intervals of 2.5 to 18 hours were examined. The incorporation of [^{3}H]uridine into RNA was observed in all cases and the reaction proceeded linearly for 60-90 minutes in both control and AD cultures. The extent of incorporation of the radioisotope in postmortem brain from a control case (73 years old, 10 hours postmortem) was compared with that of fresh brain and liver tissue from a 6 month old C57 mouse. Expressed per mg of DNA, the incorporation observed in the human case represented 39% of the fresh mouse brain and 5% of the fresh mouse liver. The specific activity of the human postmortem RNA was 1.0 x 10^4 dpm/mg RNA. The incorporation of [^{3}H]uridine into RNA was sensitive to inhibition by actinomycin or α amanitin. In a typical experiment the radioactive RNA could be extracted by the hot phenol method, and the specific activity recovered after extraction was 0.76 x 10^4 dpm/mg RNA. These results are consistent with the possibility that the transcriptional machinery of postmortem brain remains active for short periods of time. We previously established that postmortem control and AD brains contain structurally and functionally intact RNA that could be used for both in vitro protein synthesis studies and for the preparation of recombinant cDNA libraries (these Proceedings and reviewed in Prog. Brain Res. 70:303, 1986). Our findings indicated an altered RNA metabolism in AD and this may be contributed to by transcriptional as well as degradative factors. Since the present studies provide evidence for active transcription in the postmortem human brain, the factors that affect this process in AD may ultimately be amenable to direct investigation. Supported by AG02126, AG04522, AG05134, AHAF and McKnight Foundation.

366.8 INABILITY OF BRAIN MICROTUBULES IN ALZHEIMERS DISEASE TO COLD-DEPOLYMERIZE. S.R. Campbell*, S. Khatoon*, B.E. Haley* and J.T. Slevin (SPON: K. W. Barron). Depts. of Neurology, Biochemistry and Medicinal Chemistry, Sanders-Brown Center on Aging, Lucille Parker Markey Cancer Center, VA and Univ. Kentucky Med. Ctrs., Lexington, KY 40536-0084.

GTP is a requirement for tubulin polymerization. The nucleotide photoaffinity probe 8-azidoguanosine 5'-triphosphate (8N$_3$GTP) has been shown to be a biological mimic in this system and an effective active site probe of the exchangeable GTP binding site of β-tubulin. We have previously shown a decrease (>90%) of γ and α [^{32}P]8N$_3$GTP photoinsertion into a cytosolic protein (55 K Mr) of Alzheimer's (AD) brain, identified as the β-subunit of tubulin. Of 5 AD brains tested, total protein comigrating with tubulin protein was similar in AD and age-matched controls. Therefore, AD brain tubulin, while present, has its exchangeable GTP binding site blocked/modified such that it can not interact with [^{32}P]8N$_3$GTP.

Recent experiments in which AD and control brain homogenates were photolabeled at 4°C with γ[^{32}P]8N$_3$GTP and fractionated by centrifugation (100 xg for 30 minutes, 4° C) showed that essentially all of the protein corresponding to the α and β subunits of tubulin of AD homogenates was recovered in the pelleted fraction and was completely unlabeled. In contrast, control brains showed less than 50% of the tubulin protein in the pellet. Photolabeling of the β-subunit in the control supernatant was 6-fold higher than that seen in the corresponding pellet. These results indicate that, whereas microtubules cold depolymerize in control brains, microtubules in AD brain do not depolymerize at 4°C. We hypothesize that lack of photoinsertion of γ [^{32}P]8N$_3$GTP into the β-subunit of tubulin in AD brain is a consequence of AD tubulin protein being totally in the polymerized microtubular form. In this state, its exchangeable GTP site is unavailable to added 8N$_3$GTP.

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366.9 EXPRESSION OF "NEURONAL" AND "ALZHEIMER" ANTIGENS IN CULTURED SKIN CELLS. A.C.Baker* and J.P.Blass.
Dementia Research, Burke Rehab. Center, Cornell University Medical College, White Plains, NY 10605.

Abnormalities in cultured skin "fibroblasts" from patients with Alzheimer's disease have been reported from several laboratories, but their relevance to the clinically important changes in neurons remains unsettled.

Skin cell lines from Alzheimer and matched control subjects were obtained from the Coriell Institute. When grown under a combination of conditions described by others to favor the differentiation of neurons, the morphology of skin cells from both Alzheimer and control subjects became less spindle shaped and more "neuron-like"- rounder cells with larger nuclei and some processes.

Antisera whose specificity has been established were kindly donated by the indicated individuals. Under "differentiation conditions", both six Alzheimer and six control cell lines stained with a polyclonal antibody to "neuron-specific enolase" (commercial) and a polyclonal antibody to neurofilaments (C. Marotta). Three Alzheimer, but not three control cell lines stained with a monoclonal antibody to paired helical filaments (I. Iqbal), with the Alz-50 monoclonal antibody to A-68 protein (P. Davies), and with a polyclonal antibody recognizing phosphorylated and nonphosphorylated tau (I. Iqbal). Preliminary experiments suggest that manipulations of cellular metabolism can accentuate or ameliorate the expression of the abnormalities.

These data suggest that cells cultured from skin can be useful models for studying cellular mechanisms associated with neurons in vivo in a neurological disease.

366.10 CYTOSKELETAL PROTEIN PHOSPHORYLATION IS ALTERED IN THE BRAINS OF ALUMINUM-TREATED RATS. G.V.W. Johnson and R.S. Jope. Dept. of Pharmacology and the Neuropsychiatry Program, University of Alabama at Birmingham, Birmingham, AL 35294.

Cytoskeletal abnormalities are a major pathological characteristic of several neurodegenerative diseases, including Alzheimer's disease. There is increasing evidence that an association exists between these abnormalities and protein phosphorylation. Also, brain Al^{3+} levels are elevated in Alzheimer's disease and Al^{3+} has been shown to be associated with NFTS. Previously we have demonstrated that cAMP levels are significantly elevated in the brains of rats maintained on 0.3% Al^{3+} for one month (Brain Res. 403:1, 1987). This and other evidence has led us to formulate the following hypothesis for the mechanism of Al^{3+} neurotoxicity. We postulate that when brain Al^{3+} levels are elevated there is increased activation of adenylate cyclase, possibly via interactions of Al^{3+} with G proteins, which leads to increased cAMP-dependent protein kinase activity, resulting in aberrant protein phosphorylation.

To examine the effects of Al^{3+} on protein phosphorylation we measured total in vivo protein phosphorylation and in vivo phosphorylation of 2 cytoskeletal proteins (NF 200kD & MAP2) using immunoprecipitation techniques with monoclonal antibodies in control or Al^{3+}-treated rats (maintained on 0.3% dietary Al^{3+} for 1 month). Four hours after 1mCi of ^{32}Pi was injected icv the rats were anesthesized and perfused with cold buffer. A homogenate was prepared from the cerebral cortex and total in vivo protein phosphorylation and phosphorylation of MAP2 and NF 200kD were determined. Overall ^{32}Pi incorporation into brain phosphoproteins was significantly elevated in the Al^{3+}-treated rats compared to controls, however, not all proteins showed an increase in phosphorylation with Al^{3+}. The phosphorylation of proteins with apparent molecular weights of 90kD, 200kD, and 300kD was significantly increased in the aluminum-treated rats, whereas that of a protein of an apparent molecular weight of 150,000kD was significantly decreased. To further examine the identity of the 200kD and 300kD phosphoproteins, MAP2 and NF200kd were immunoprecipitated with monoclonal antibodies and subjected to SDS-PAGE. Both MAP2 and NF200kD from the Al^{3+}-treated rats were phosphorylated to a significantly greater extent when compared to controls. These studies demonstrate that certain cytoskeletal proteins are abnormally phosphorylated in Al^{3+}-treated rats and since these proteins can be phosphorylated by cAMP-dependent protein kinases we hypothesize that the Al^{3+}-induced increases in cAMP may be responsible in part for this increased protein phosphorylation.
Supported by AG04719 from the NIA.

366.11 **DECREASED GROWTH FACTOR RESPONSE AND PROTEIN PHOSPHORYLATION IN FIBROBLASTS FROM AGED AND ALZHEIMER DONORS.** C. Peterson and C.W. Cotman, Dept. Psychobiology, University of California, Irvine, CA 92717

Cytosolic free calcium is depressed in cultured skin fibroblasts from aged and Alzheimer donors (PNAS **83**:7999). The present study was designed to determine whether calcium mediated processes, such as the response to growth factors and protein phosphorylation, are altered in these same cells.

Cultured skin fibroblasts from young (23±1 yr), aged (61±2 yr) and Alzheimer (62±1 yr) donors were obtained from the Camden Cell Repository. Alzheimer cell lines were age- and sex-matched and only donors with confirmed Alzheimer's disease or those that had first degree relatives with confirmed Alzheimer's disease were used in these studies. To avoid complications due to *in vitro* aging all cells were studied at early passages (5-10).

For the growth factor studies fibroblasts were plated on day 0 into 24 multiwell plates (10,000 cells/well) in Dulbecco's modified Eagles medium with 10% fetal calf serum. On day 3 the medium was removed and replaced with serum-free. On day 4 the cells were fed serum-free medium that contained ^{3}H-thymidine (0.5 uCi/well) and a growth factor (e.g., platelet derived growth factor, epidermal growth factor, fibroblast growth factor or serum) at concentrations that increase cytosolic free calcium (JBC **258**:8066). Twenty-four hours later the cells were harvested and thymidine incorporation was quantitated by liquid scintillation counting. Protein was determined in parallel non-radioactive cultures. Basal thymidine incorporation was depressed similarly by aging and Alzheimer's disease. Growth factor stimulated DNA synthesis was lower in cells from aged donors and even less in cells from Alzheimer's donors when compared to young donors.

For protein phosphorylation experiments fibroblasts were plated in 75 cm^2 flasks on day 0. Six days later the cells were harvested by scraping, sonicated in buffer and aliquots (100 ug protein) were incubated for 2 min in the presence of ^{32}P-ATP and either calcium, calcium+calmodulin or cyclic AMP+isobutyl-methylxanthine. Proteins (50 ug) were separated by electrophoresis on a 10% acrylamide gel and the phosphorylated proteins were visualized by autoradiography. Cyclic AMP dependent protein phosphorylation was depressed by aging and further reduced by Alzheimer's disease. In the presence of either calcium or calcium+calmodulin only high molecular weight protein phosphorylation declined in cells from aged donors and was reduced even further by cells from Alzheimer's donors.

Thus, growth factor stimulated DNA synthesis and protein phosphorylation are depressed in cultured skin fibroblasts from aged and Alzheimer's donors and these may be related to decreased cytosolic free calcium.

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366.12 SHORT AND LONG SCRAPIE INCUBATION TIME MICE HAVE DISTINCT PRION PROTEIN GENE ALLELES. D. Westaway*, P. Goodman*, C. Mirenda*, G. Carlson* and S. B. Prusiner. Dept. of Neurology, University of California, San Francisco, CA 94143-0518; Jackson Laboratory, Bar Harbor, ME 04609.

Biochemical evidence implicates a protein, PrP^{Sc}, as the major and possibly sole component of the scrapie prion. PrP^{Sc} is related to a benign cellular protein, PrP^{C}; both PrP isoforms are encoded by the same cellular murine gene, *Prn-p*. Mendelian analysis of prototypic short (NZW: 113 days), and long incubation period mice (I/Ln: 200-385 days) has defined a single autosomal dominant gene, *Prn-i*, as a major determinant of the length of scrapie incubation periods. A *Prn-p* Xba 1 restriction fragment length polymorphism (RFLP) cosegregates with the incubation period phenotype, establishing linkage between *Prn-p* and *Prn-i* (Carlson et al., *Cell* 46:503, 1986). We have isolated molecular clones of chromosomal *Prn-p* genes from NZW and I/Ln mice, and have defined a restriction/modification phenomenon which has interfered with previous attempts to clone PrP genes in *E. coli*. Mapping of these clones reiterates that the gross structure of *Prn-p* genes is the same in NZW and I/Ln mice (Westaway and Prusiner, *Nucleic Acids Res.* 14:2035, 1986) consisting of a spliced 5' leader sequence separated from the coding sequences. However, the NZW and I/Ln alleles are distinguished by Eco Rl, Pst 1, Sst 1 and Taq 1 polymorphisms in addition to the Xba 1 polymorphism described above. Using these enzymes, inbred mouse strains can be divided into at least six *Prn-p* RFLP haplotypes. Interestingly, three long scrapie prion incubation period mice, I/Ln, P/J and IM, share a distinctive RFLP haplotype, emphasizing the linkage between *Prn-p* and *Prn-i* and implying that these mice carry the same allele of *Prn-p*. [IM mice are homozygous for the p7 allele of the *Sinc* gene (Bruce and Dickinson, in *Slow Transmissible Diseases of the Nervous System* 2:71-86, Academic Press, NY, 1979)]. We have sequenced the coding regions of the NZW and I/Ln *Prn-p* genomic clones. The I/Ln allele differs from the NZW sequence in at least two amino acid codons: one of the nucleotide variations corresponds to a replacement in a codon conserved in mouse, hamster and human PrP genes. These sequence variations have been assessed in other inbred mouse strains. Our findings suggest that changes in the properties of scrapie prions ("strain instability") passaged in long incubation period mice may be due, at least in part, to a novel PrP gene sequence. These data also contend that some instabilities of scrapie "strains" observed by other investigators (Bruce and Dickinson, op. cit.) may be epigenetic, rather than mutational in origin.

366.13 HYBRIDIZATION ANALYSES OF ALZHEIMER'S BRAINS FOR HERPESVIRUS DNA. D. Walker*, J. R. O'Kusky, P.L. McGeer and E.G. McGeer. Kinsmen Lab. of Neurol. Res., Dept. of Psychiatry, and Div. Medical Microbiology, Dept. of Pathology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The role of a neurotropic virus in Alzheimer's pathology (accumulation of neurofibrillary tangles, senile plaques and loss of cholinergic neurons) has been suggested. The association of Herpes simplex virus type 1 (HSV-1) with the pathogenesis of Senile dementia of the Alzheimer's type (SDAT) was examined using a sensitive in situ hybridization method, with negative results. Various sections of brains from SDAT cases, along with appropriate controls, were examined. In addition, to confirm that the analyses were sufficiently sensitive to detect latent HSV-1 DNA, positive sections of trigeminal ganglia from SDAT and normal cases were obtained.

Sections from paraffin embedded samples were processed for in situ hybridization using the procedure of Brigati et al. (Virology 126:32-50, 1983), with minor modifications. Two cloned HSV-1 DNA restriction enzyme fragments from HSV-1 strain F (Eco R1 fragments D and G), representing 22% of the complete virus genome, were used as probe. These fragments did not hybridize with normal human DNA under the conditions used. The cloned HSV-1 DNA was separated from the plasmid vector and labeled by random priming with ^{35}S dCTP to a specific activity of approximately 10^{9} cpm/ug. Slides were coated in Kodak liquid film emulsion and exposed for 4-8 weeks. Four sections from each sample of frontal cortex, temporal cortex, hippocampus and substantia innominata from SDAT cases were examined. In every sample, sections adjacent to those used for hybridization showed evidence of typical SDAT pathology. The presence of cells with numbers of grains above background levels was not detected in any of the Alzheimer's brain sections. Positive hybridization was also not detected in regions of brains from nondemented controls. However, the presence of HSV-1 DNA in trigeminal ganglia of normal and SDAT cases was detected, after 4 weeks exposure. The percentage of positive cells, calculated using computer-assisted grain counting, was less than 6%. The ability to detect HSV-1 DNA in trigeminal ganglia leads us to conclude that the methodology should have been sufficiently sensitive to detect Herpes simplex virus in brain tissue at levels capable of producing neuronal pathology.

(Supported by grants from the ADRDA and MRC Canada).

366.14 UBIQUITIN RESPONSE IN CULTURED NERVOUS TISSUE AFTER HEAT SHOCK AND ALUMINUM INTOXICATION.
A. Morandi*, V. Fried*, H. Smith*, G. Perry and P. Gambetti. (SPON: PJ Whitehouse)
Institute of Pathology, Case Western University, Cleveland, OH 44106 and St. Jude Children's Research Hospital, Memphis, TN 38101.

Ubiquitin (Ub), a highly conserved protein, plays a central role in an ATP dependent non-lysosomal proteolytic system. In this system Ub-conjugated proteins are targeted for proteolysis and rapidly broken down by ATP dependent proteases. Recently, it has been shown that the Ub system becomes especially active during heat shock or stress conditions in which altered proteins accumulate in cells. Although studies on Ub-conjugates in heat shock have been carried out in various cell type, little is known about the role of Ub dependent proteolytic system in neurons. Interest in the Ub conjugates in the nervous system has been highlighted by the recent discoveries that they are increased in Alzheimer disease and in other degenerative diseases of the nervous system. We have investigated the presence and the distribution of Ub-conjugates in human and rabbit dorsal root ganglia explants exposed to stress and in normal conditions, using four monoclonal and two polyclonal antibodies to Ub conjugates. The cultures were let grown on polylysine coated plastic dishes and maintained in serum free defined medium. Two stress conditions were produced: 1) Heat shock at 45^{0} C for 20 or 5 minutes; 2) Intoxication with aluminum lactate for eight days. Two monoclonal and both polyclonal antibodies reacted with the cultures.

Both stress conditions result in a different distribution of Ub-conjugates and an overall increase in the intensity of the immunostaining compared to untreated cultures. In control cultures Ub-conjugates were demonstrated in the nuclei and perikaria with variable intensity according to the Ub-antibodies used, whereas immunostaining of neurites was weak or absent. Following both stress conditions, immunostaining of nucleus and perikarion remained variable although generally it increased in intensity. On the contrary, a marked increase of immunostaining was consistently observed in neurites.

These findings indicate that the ubiquitin system is present in human and animal neurons and that is activated under different types of stress conditions. The presence of a large amount of Ub-conjugates epitopes suggests that, under stress conditions, the Ub system is especially active in neurites where it may play a protective role.

Supported by NIH Grants NS14503 and AG00795.

366.15 EXPRESSION OF HLA-DR AND INTERLEUKIN-2 RECEPTOR ON REACTIVE MICROGLIA IN SENILE DEMENTIA OF THE ALZHEIMER TYPE. S. Itagaki*, P.L. McGeer, H. Tago* and E.G. McGeer (SPON: D. D. Greenwood). Kinsmen Lab. of Neurol. Res., Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

HLA-DR is a class II cell surface glycoprotein of the human histocompatibility complex usually expressed on the surface of cells that are simultaneously presenting foreign antigen to T-lymphocytes. Interleukin-2 (IL-2) is a lymphokine with many functions including growth induction of certain T- and B-cell lines. Using immunohistochemical procedures with multiple specific monoclonal antibodies to HLA-DR and the IL-2 receptor, we have found microglia reactive to these membrane surface proteins throughout the cortical gray matter of postmortem brains of patients with senile dementia of the Alzheimer type (SDAT). They were particularly concentrated in areas of senile plaque formation and were also seen suurounding degenerating neurons. Double immunostaining with antibodies to glial fibrillary acidic protein (GFAP) showed that HLA-DR and IL-2 receptor positive cells were different from the reactive astrocytes which form scar tissue in brain following neuronal death. However, occasional positively staining giant astrocytes were seen. Small numbers of resting microglia were HLA-DR positive in white matter of both normal and SDAT brains. Double immunostaining for choline acetyltransferase (ChAT) and HLA-DR in the substantia innominata of SDAT cases demonstrated degenerating ChAT neurons being phagocytosed by HLA-DR positive reactive microglia. The SDAT cases all had reduced cortical choline acetyltransferase levels as measured biochemically. In the 11 brains studied, the number of hippocampal HLA-DR positive cells was positively correlated with the number of plaques and negatively correlated with average cortical ChAT. (Supported by grants from the Medical Research Council of Canada and the Alzheimer's Association of B.C.)

366.16 BRAIN GLIAL CELLS EXPRESS MARKERS OF IMMUNE COMPETENCE IN AGING AND DEMENTIA. J. Rogers and S. D. Styren* (SPON: Steven F. Zornetzer). Institute for Biogerontology Research, 13220 N. 105th Avenue, Sun City, AZ 85351.

Glial proliferation in aging and Alzheimer's Disease (AD) has been documented, but the context in which this occurs remains unclear. An immune or autoimmune context would explain numerous aspects of AD pathogenesis, as well as the age-related increase in neural autoimmune disorders. Unfortunately, the usual markers of immune function such as HLA-DR (an MHC antigen necessary for T-cell recognition and antigen presentation) have been difficult to detect in brain, leading to the concept of brain immunologic privilege.

Using micro- and ultrastructural immunohistochemical techniques, we have tested for HLA-DR, HLA-DQ, HLA-DP, IL-2, T4, and T8 and can now show profuse brain reactivity for several of these immune system antigens. The cells expressing such markers include microglia and macrophages. The presence of immune-related antigens on these cells strongly suggests that their normal phagocytic role may, in some cases, be carried out in the context of an immune response. To argue that these cells may be of hematologic origin is to miss the point: the point is that phagocytic cells expressing classic immune-related antigens can now be observed profusely in human elderly and AD brain, and that this finding provides a potential mechanism for immune involvement in age-related neurologic disorders.

366.17 RETINAL GANGLION CELL DEGENERATION IN ALZHEIMER'S DISEASE. C.J. Bassi*§, J.C. Blanks§, D.R. Hinton*†, A.A. Sadun§ and C.A. Miller*†. §Doheny Eye Foundation, Dept. of Ophthalmology and †Dept. of Pathology, USC School of Medicine, Los Angeles, CA 90033.

Our group had previously demonstrated degeneration of the optic nerves and retinal ganglion cell layer in patients with Alzheimer's disease (AD) (N. Engl. J. Med. 315:485-487, 1986). The present study further characterizes degeneration in AD retinas. Donor eyes from AD patients and age-matched controls were either prepared as whole-mounts or processed for routine transmission EM.

In retinal whole-mounts, loss of ganglion cells (ranging from 20 to 80%) was observed in AD patients relative to age-matched controls. The loss was not confined to any retinal area. Many of the remaining ganglion cells had evidence of degeneration, such as darkly stained or vacuolated cytoplasm.

Similar observations were found in radial sections. Damage to the retina was observed in the ganglion cell layer (GCL), while the outer and inner nuclear layers appeared normal. Affected cells in the GCL of AD cases were either swollen or shrunken, had a vacuolated appearance, and some had evidence of nuclear disintegration. At the ultrastructural level, swelling of the mitochondria and endoplasmic reticulum, pale cytoplasmic density, and dispersed nuclear chromatin were observed. There was no evidence of neurofibrillary tangles within the ganglion cells, and no neuritic plaques or amyloid angiopathy were found in the retina. Extensive glial processes were sometimes seen adjacent to the vacuolated ganglion cells.

These findings, together with our previous paper, provide compelling evidence for degeneration of the primary visual pathway in AD. This degeneration may be the anatomical substrate for visual deficits found in AD.

366.18 GLIAL NUMBERS CORRELATE POSITIVELY WITH DENDRITIC NEUROPIL IN NORMAL AGING HUMAN CORTEX AND IN ALZHEIMER'S DISEASE. P. D. Coleman and D. G. Flood. Depts. of Neurobiology and Anatomy and Neurology, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

Cerebral cortex was sampled from middle frontal gyrus and superior temporal gyrus of clinically, neuropsychiatrically, and neuropathologically defined normal and Alzheimer's disease (AD) human brains. The interval between death and obtaining the tissue averaged 6.4 hours with a minimum of 2.5 hours and a maximum of 10.5 hours. Blocks of tissue from homologous regions of each hemisphere were embedded in celloidin, sectioned at 200 or 30 μm and processed, respectively, for Golgi-Cox staining for quantitative study of dendritic extent of single neurons or for Nissl staining with cresyl violet for counting of numbers of neurons and glia. The data presented here were obtained by making computer-aided measurements of the dendritic extent of layer II pyramidal neurons in both regions and by computer-aided counts of layer II neurons and layer I/II glia. All cell profiles were entered into Apple II+ or IIe computers manually via a graphics tablet. Average dendritic extent per layer II pyramidal neuron was multiplied by the number of layer II neurons in a strip of cortex to obtain an estimate of dendritic neuropil.

Examination of the measure of estimated dendritic neuropil as a function of numbers of glia reveals a strong positive correlation of +0.87 for middle frontal gyrus and +0.75 for superior temporal gyrus. AD cases and normal cases appear to show a similar relationship between dendritic neuropil and glia, with the exception of one case of early onset (long duration of 15 years) AD with a large number of glia but low dendritic neuropil.

These data are consistent with an interpretation of glia as sources of neurotrophic factors. Furthermore, the data suggest that in AD the glia continue to be normal sources of trophic factors, with the possible exception of early onset, long duration AD, when glia may play a more traditional neuropathological role in formation of scar tissue.

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366.19 EXPRESSION OF $pp60^{c-src}$ IN BRAIN OF AGING RAT. M. F. Matocha*, S. W. Fitzpatrick*, J. R. Atack*, and S. I. Rapoport. (SPON: C. Grady). Laboratory of Neurosciences, NIA, NIH, Bethesda, MD 20892.

There is considerable evidence that abnormal phosphorylation is associated with cytoskeletal disorders in the aging brain, and it has been suggested that phosphorylation of tyrosine residues is involved in the neuropathology. Of the several protein-tyrosine kinases that have been identified, the $pp60^{c-src}$ kinase has been extensively characterized and found to be highly expressed in brain relative to other organs. In the present study, we quantitated $pp60^{c-src}$ kinase activity and determined the relative levels of this protein in brains of rats of different ages as a first step in assessing whether it has a role in abnormal states.

A 100,000g supernatant and pellet fraction was prepared from whole brain of individual male Fischer-344 rats, aged 4 (n = 7), 14-16 (n = 7), and 22-23 (n = 8) mo. An immune complex kinase assay was carried out by reacting supernatant or pellet protein with a monoclonal antibody against RSV $pp60^{src}$, followed by incubation of the immunoprecipitated protein with [γ-^{32}P]ATP, unlabeled ATP, and casein as the exogenous substrate. The reaction mixture was subjected to polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and the radiolabeled proteins were detected by autoradiography. Appropriate bands were excised from the gel and quantitated by liquid scintillation counting. Samples from individual animals and corresponding controls that had not been reacted with antibody were analyzed in triplicate.

Casein phosphorylation increased linearly with the amount of protein and time of incubation, and kinase activity was found exclusively in the supernatant fraction. The specific activities (mean ± SD, fmol phosphate transferred to casein / min X mg of protein) of brain $pp60^{c-src}$ detected in animals ages 4, 14-16, and 22-23 mo were 251 ± 37, 211 ± 31, and 214 ± 47, respectively, and did not differ significantly by 1-way ANOVA ($p > 0.05$). Brain $pp60^{c-src}$ also was immunoprecipitated from the same supernatant fractions and detected by immunoblot analysis. There was no substantial change in the level of $pp60^{c-src}$ protein among the various-aged rats.

These data correlate with our previous findings showing that levels of c-src-like mRNAs in rat brain are age-invariant (Matocha et al, J. Cell Biol., 103, 425A (1986). The persistence of activity and protein levels of the $pp60^{c-src}$ kinase in the mature brain provides further support for its role in maintaining normal brain structure and function.

HIPPOCAMPUS

367.1 RECIPROCAL CONNECTIONS BETWEEN THE INTERPEDUNCULAR NUCLEUS AND THE HIPPOCAMPAL FORMATION. B. Fass[1] and G.S. Hamill[2] (SPON: R.B. Hamilton). [1]Dept. of Anatomy, Univ. Louisville Med. Sch., Louisville, KY 40292; [2]Dept. of Anatomy, M.S. Hershey Med. Sch., Hershey, PA 17033

The interpeduncular nucleus (IPN) is a brainstem component of the limbic system. Recent studies have shown that the IPN is directly connected to basal forebrain structures; e.g., the medial septum and diagonal band nucleus (Hamill & Fass, 1984; Groenewegen et al., 1986). It also has been shown that the IPN projects to the hippocampus (HPC) (Groenewegen et al., 1986; Wyss et al., 1979). The cells-of-origin of the IPN's projection to the HPC are concentrated within a specific subnucleus; i.e., apical. We now report that there is a reciprocal pathway from the HPC to the IPN which terminates primarily in the central subnucleus.

Adult male albino rats were injected with WGA-HRP (10% in DMSO) and allowed to survive 48 hrs. The injection was placed in the anterodorsal (n=6) or posteroventral (n=5) HPC. Frozen sections were processed using tetramethylbenzidine as the chromogen (after Gibson et al., 1984).

The anterodorsal injection-site included CA3 and the dentate gyrus of the HPC's septal pole. The posteroventral injection-site included CA1 and CA3 of the HPC's temporal pole, plus the subiculum. WGA-HRP did not spread into the habenula, and there was no anterograde labeling in fasciculus retroflexus.

Numerous retrogradely-labeled cells were present in the apical subnucleus. There were qualitatively more of these labeled cells after posteroventral HPC injections than after anterodorsal HPC injections. In addition, retrogradely-labeled cells were observed in the lateral subnucleus bilaterally. No labeled cells were found in the central or rostral subnuclei. These observations are consistent with previous reports (Groenewegen et al., 1986; Wyss et al., 1979).

An unexpected finding was the presence of anterograde labeling in the IPN. The densest labeling was in the central subnucleus. Qualitatively, the density of this labeling was comparable to that in the molecular layer of the contralateral dentate gyrus. There was little or no anterograde labeling in the apical subnucleus.

The present findings indicate that the IPN shares reciprocal connections with the HPC. These connections appear to be highly organized; a specific subnucleus of the IPN (apical) projects to the HPC, while a different subnucleus (central) receives inputs from the HPC. Studies are in progress to identify the cells-of-origin of the HPC's projection to the IPN.

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367.2 SEROTONERGIC AND NONSEROTONERGIC PROJECTIONS FROM THE RAT INTERPEDUNCULAR NUCLEUS TO THE SEPTUM, HIPPOCAMPAL FORMATION AND RAPHE: A COMBINED IMMUNOCYTOCHEMICAL AND FLUORESCENT RETROGRADE LABELLING STUDY OF NEURONS IN THE APICAL SUBNUCLEUS. K.T.Montone*,B.Fass[1] and G.S.Hamill[2].Departments of Anatomy, Penn State University College of Medicine, Hershey, PA 17033, and [1]University of Louisville, Louisville, KY 40292.

The apical subnucleus can be clearly identified in the caudal end of the interpeduncular nucleus in rats by its population of large, multipolar neurons containing serotonin. In this study, the subnuclear distribution and immunocytochemistry of apical neurons projecting to the diagonal band/septum, hippocampal formation and/or raphe was determined using a technique combining retrograde fluorescent labelling and immunocytochemistry on the same tissue section.

Sprague-Dawley adult rats were anesthetized with Nembutal (40mg/kg), and 100-500 nl of both (0.05%) fast blue and (50%) rhodamine-conjugated microspheres stereotaxically injected into either the diagonal band or dorsal hippocampal formation respectively, or into the raphe. Following 4-8 days survival and an intraventricular injection of colchicine to inhibit axoplasmic transport (200ug/25ul H2O; 24 hours prior to perfusion), the rats were perfused through the heart with 10% buffered formalin. Frozen sections were cut coronally through the injection sites and IPN, coverslipped (n-propyl gallate) or processed for 5HT immunocytochemistry.

After injection of fast blue into the diagonal band/septum and rhodamine into the dorsal hippocampal formation, or vice versa, retrogradely-labelled cells were found throughout apical subnucleus. Analysis of sections incubated with 5HT antiserum showed a small number of labelled cells contained serotonin. Occasional apical cells contained both fast blue and rhodamine, indicating a dual projection to each area. Injection of rhodamine into the raphe also labelled apical cells that did not contain serotonin.

This study revealed that some apical neurons containing serotonin, and others an unidentified transmitter/s innervated the diagonal band/septum, dorsal hippocampal formation and raphe through ascending and descending projections. Occasional apical cells were found that projected to both septum and dorsal hippocampal formation via collaterals. Since the IPN receives reciprocal projections from each of these regions, one of its functions may be the integration of subcortical limbic activity.

This study was supported by grants from [2]Penn State University, and the [1]Medical Research Committee, University of Louisville Medical School.

367.3 MODULATION OF PERFORANT PATH-DENTATE FUNCTIONAL PROPERTIES BY COMMISSURAL SYSTEM: ACUTE EFFECTS OF CONTRALATERAL HIPPOCAMPAL ABLATION. R.L. Port, R.J. Sclabassi and T.W. Berger, Departments of Behavioral Neuroscience, Psychiatry and Neurological Surgery, University of Pittsburgh, Pittsburgh, PA 15260.

We have previously described long-term changes in the functional properties of the perforant path-dentate (PP-DG) synapse in unilaterally hippocampectomized rabbits (Port, et al, Soc Neurosci Abstr, 12, 1986). The system response to a pair of impulses was profoundly altered. At four weeks post-lesion, peak facilitation was reduced from a control mean of 47.5% to 9.3%; the duration of inhibition increased from 62.5 ms to 175 ms. The present experiment quantifies the immediate effect of commissural denervation on DG reactivity to perforant path input.

Adult, male albino rabbits were acutely prepared under halothane anesthesia. Bipolar stimulating electrodes were placed in the PP and a recording electrode positioned in the granule cell layer of the DG. Random impulse trains (1004 impulses, Poisson distribution with a mean frequency of 2.0 Hz) of electrical stimulation were delivered to the PP and evoked field potentials were recorded from the DG. Stimulation intensity was adjusted to elicit a population spike of 10-20% maximum amplitude to a single impulse. The contralateral dorsal hippocampus was aspirated and a second impulse train delivered. Kernels were computed by cross-correlating interval duration with population spike amplitude.

Pre-lesion data revealed significant second order nonlinearity at intervals less than 250 ms. Short intervals (<50 ms) resulted in a suppression of population spike amplitude whereas longer (60-250 ms) intervals produced facilitation. Peak facilitation (90-160%) occurred around 100 ms. Ablation of the contralateral hippocampus produced a modest decrease in response amplitudes at short intervals and a robust increase in the degree of facilitation found at longer intervals. These data characterize the manner in which commissural input influences the functional characteristics of the DG. It is possible that the decreased responsivity to short intervals reflects a loss of direct excitatory input and the increased responsivity to longer intervals may be due to the loss of indirect input via inhibitory interneurons (Douglas, et al, J Comp Neurol, 219, 1983). Supported by The Whitaker Foundation, Office of Naval Research, and NIMH (09433).

367.4 DIFFERENCES IN NONLINEAR PROPERTIES OF THE MEDIAL AND LATERAL PERFORANT PATH. T.W. Berger, C.L. Weikart* and R.J. Sclabassi. Departments of Behavioral Neuroscience and Neurological Surgery, Univ. of Pittsburgh, Pgh., PA 15260.

We have been applying nonlinear systems analytic methods to investigate functional properties of the perforant path-dentate system in the rabbit. For this analysis, trains of 4064 electrical impulses were applied to the perforant path. Intervals between impulses varied randomly according to a Poisson distribution having a mean interval of 500 ms, with intervals ranging from 1 ms to 5000 ms. Amplitude of the population spike evoked by each impulse was measured, and cross-correlation techniques were used to compute first, second, and third order kernels of a power series expansion. First order kernels reflect the average population spike amplitude to all impulses in the train. Second order kernels represent the modulatory influence of the preceding impulse on the system response to the present impulse. Third order kernels represent the modulatory influence of the two preceding impulses.

We previously have reported that second order nonlinear properties of the medial perforant path include almost complete inhibition of the population spike to inter-impulse intervals of 10-20 ms, and facilitation of approximately 100% to intervals in the range of 50-300 ms. For intervals longer than 300 ms, the system functions linearly. Significant third order interactions that are primarily inhibitory occur in response to a wide range of stimulus combinations.

In the present experiments, we examined the nonlinear properties of the lateral perforant path and found them to be markedly different. Second order kernels for the lateral perforant path reveal inhibition for almost all inter-impulse intervals within the range of 10-1000 ms. In some preparations, a slight facilitation (10-20%) of population spike amplitude is seen in response to intervals of 50-100 ms. In further contrast to the medial perforant path, third order kernel values are almost exlusively positive, indicating facilitative interactions.

These data provide further evidence that the perforant path-dentate projection is not homogeneous, and incorporates at least two functionally distinct subsystems.

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367.5 NONLINEAR RESPONSE CHARACTERISTICS OF THE PERFORANT PATH-DENTATE GYRUS SYSTEM IN THE IN VITRO RABBIT HIPPOCAMPUS. T.P. Harty, T.W. Berger, R.J. Sclabassi and G. Barrionuevo. Departments of Behavioral Neuroscience, Neurological Surgery and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

Nonlinear systems analytic procedures were used to study network properties of the in vitro rabbit hippocampus. The procedures involve stimulation of the perforant path using trains of 4064 impulses with randomly distributed inter-impulse intervals (mean frequency = 2.0 Hz). A cross-correlational analysis determined the relationship between inter-impulse interval and amplitude of the evoked population spike recorded from the dentate granule cell layer.

We previously have shown that second order kernels of granule cell responses from in vivo preparations are characterized by almost complete (80-90%) inhibition of spike amplitude in response to inter-impulse intervals of 10-20 ms, which returns to baseline (no interaction) at 65 ms. Inhibition is followed by spike facilitation which reaches a maximum of 70-80% at about 100 ms and returns to baseline at intervals of 250-350 ms. In the present studies, we examined granule cell response in the in vitro hippocampal slice. Initial results from slices at a recording temperature of 34° C are characterized by maximum facilitation of 300-400% at the shortest intervals, which decays to baseline at intervals of 100-200 ms. When the recording temperature is 37° C, second order kernels are similar to kernels at 34° C, but with greater facilitation at the shortest intervals. At 31° C, trains tend to show less facilitation than 37° or 34° at the shortest intervals, which falls to baseline more quickly (100-150 ms). Facilitation is followed immediately by 20-30% inhibition for a period of 200-600 ms.

In general, in vitro second order kernels do not exhibit the inhibition at short intervals characteristic of in vivo second order kernels. Instead, the early inhibition is replaced by a peak of facilitation that is 80-90 ms earlier and 4-5 times greater than the facilitory peak of the in vivo preparation. In addition, the in vitro facilitatory peak decays to baseline 100-200 ms earlier than the in vivo peak. One potential basis for these differences in nonlinearities is that several feed-forward and feedback connections of the dentate gyrus and hippocampus are reduced (inhibitory basket cells) or eliminated (commissural fibers and return fibers to entorhinal cortex) in the slice preparation. Supported by The Whitaker Foundation; NIMH (MH 30915); NIH (NS 24288); Office of Naval Research; BRSG (RR 07084); and an RCDA (NS 01196) to G.B.

367.6 STATIONARY CURRENTS LOCALIZED TO CA1 STRATUM RADIATUM IN ADDITION TO SYNAPTIC CURRENT IN S. LACUNOSUM-MOLECULARE FOLLOWING ANGULAR BUNDLE STIMULATION IN THE RAT: CURRENT SOURCE-DENSITY EVIDENCE. M. A. King. Laboratory for Systems Neurodynamics, Department of Neurosurgery, University of Virginia, Charlottesville, VA, 22908.

The current source-density (CSD) method was used to calculate the position, relative magnitude, and time course of currents generated in hippocampal region CA1 following electrical stimulation of entorhinal-hippocampal afferents in the angular bundle. Averaged (4-8) extracellular recordings of population evoked potentials were obtained at 30-50 micron intervals across the apical dendrites, in urethane-anesthetized adult male albino rats. High and low intensity stimulation was alternately delivered at 1/30 sec.

In addition to a reliable current sink in the stratum lacunosum-moleculare (SLM) synaptic terminal field, one or more regions of inward current flow were observed in stratum radiatum (SR). Twenty-six CSDs from 14 rats showed an SR sink between 208 + 16 and 274 + 17 (s.e.m.) microns dorsal to the hippocampal fissure, corresponding to 36.3 + 2.5 - 48.6 + 3.2 % of the distance from the fissure to stratum pyramidale. A second, more proximal sink was observed in 13 CSDs (343 + 23 - 413 + 30 microns, 58.8 + 3.9 -61.2 + 6.6 %), and a third observed in 4 CSDs (366 + 25 - 425 + 38 microns, 62.7 + 5.2 - 69.8 + 8.4 %). These sinks were virtually constant in width (65 + 5, 58 + 8, and 59 + 14 microns, 11.8 + 1.1, 9.7 + 1.5, and 9.8 + 2.1 %, respectively), and magnitude (112 + 27, 99 + 22, and 41 + 20 % of SLM, respectively), over the time course of the synaptic current in SLM. They were not present prior to the onset of pEPSPs, indicating that they do not represent regions of passive conductivity differences, (i.e., artifactual sinks or sources). There are at least 3 possible explanations for these standing sinks: they may be related to dendritic "spikes", they may represent a hitherto unrecognized synaptic input (feed-forward excitation) from a population of afferents relayed through the angular bundle, or they may in fact be current sources for (feed-forward) inhibitory synapses. A videotape exhibiting these currents over time will be presented.

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367.7 PHASES OF FIRING OF MEDIAL AND LATERAL SEPTAL NEURONS AND THE IMPORTANCE OF HIPPOCAMPAL FEEDBACK. M. Stewart and S. E. Fox, Department of Physiology, SUNY Health Science Center, Brooklyn, New York 11203.

The septal nuclei are intimately connected with the hippocampus. In fact, it has been suggested that the theta rhythm is the result of oscillations in a loop consisting of connections from the medial septal nuclei (MSN) to hippocampus to lateral septal nuclei to MSN. Rhythmically bursting neurons of the MSN, and hippocampal pyramidal cells and interneurons all fire phasically with the theta rhythm. The firing of lateral septal neurons relative to the theta rhythm has not been studied previously, so the significance of a septo-hippocampal loop remains unclear.

Systemic atropine, sufficient to eliminate the urethane theta rhythm, eliminates the phase-locked firing of hippocampal pyramidal and interneurons as well as the rhythmic firing of one group of MSN cells. These atropine-sensitive MSN cells may derive their rhythmicity from a septo-hippocampal loop or from another input such as a local interaction with atropine-resistant MSN cells.

Single cells were recorded extracellularly from medial and lateral septum of urethanized rats. EEG was recorded near the hippocampal fissure (dentate) and above the alveus (CA1). A cooling probe was placed in the ventral hippo-campal commissure (VHC) to reversibly eliminate the theta rhythm.

The distribution of mean firing phases of rhythmic MSN neurons (n=73) is extremely broad. The population mean is near the positive peak of the dentate theta rhythm (negative peak of the CA1 theta rhythm), but every phase is well represented. The distributions for confirmed atropine-resistant (n=11) and atropine-sensitive (n=7) cells are equally broad. Of the lateral septal neurons analysed, 11/20 were significantly phase-locked. The distribution of these cells is much less broad, with most cells firing a single action potential on the positive-going phase of the dentate theta cycle.

Cooling the VHC produced no effect in 18/20 rhythmic medial septal neurons. (The two that were apparently cooling-sensitive were atropine-resistant.) Cooling eliminated the phasic firing of lateral septal cells by suppressing their spontaneous firing altogether.

We conclude that the rhythmic neurons of the medial septum do not in general require hippocampal feedback for their rhythmic behavior, while those lateral septal cells which are phasic seem to depend upon an extremely powerful input from the hippocampus for their firing during the theta rhythm.

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367.8 CURRENT SOURCES FOR THE ALTERNATING AND SUSTAINED POTENTIALS OF THE HIPPOCAMPAL THETA RHYTHMS OF THE RAT. J. Brankačk* and S.E. Fox. Dept. Physiol., SUNY Health Sci. Ctr. Brooklyn, NY 11203.

The neuronal circuitry underlying the generation of the extracellularly recorded hippocampal theta rhythm is poorly understood despite thirty years of research. Current source density (CSD) analysis is a powerful method for location of sources and sinks of transmembrane current, and in combination with other data can identify active synaptic sites. Since hippocampal inputs are highly laminated, this leads to straightforward predictions of the anatomical projections involved in theta rhythm generation.

Rats were prepared with a fixed electrode above CA1, a reference electrode on the skull and a microdrive for advancing an electrode through the hippocampus. Stimulating electrodes were implanted in the ventral commissure and in entorhinal cortex. The rats were trained to run in a wheel for water reward to generate periods of hippocampal theta separated by non-theta EEG. Profiles of EEG and evoked potentials were recorded from the fixed and moveable electrodes at 80 um intervals. CSD profiles were computed by taking a weighted second derivative of the voltage profiles as a function of depth. Recordings from the fixed electrode provided a control for consistency, and a phase reference for averaging theta cycles. CSD profiles of the evoked potentials provided precise depth data for unknown EEG sources.

Amplifier time constants were greater than 6 s to allow recording of the sustained shift in potential associated with the presence of theta rhythm. For the EEG recordings the average non-theta voltage at each depth was taken as a baseline for the average theta wave. Measurement of sustained components is necessary for CSD analysis of the EEG, since it is not simply a transient event. Failure to do so will cause a sustained source or sink to be missed completely, or worse, a diminution in a sink to appear as a source (or vice versa).

The most prominent phasic generator of theta rhythm is a sink near the hippocampal fissure whose peak occurs at the phase of the peak negativity at that site (positive peak of CA1 theta). This may represent a phasic excitatory input from the entorhinal cortex. A weaker phasic sink appears in stratum radiatum of CA1, peaking on the positive-going phase of the CA1 theta. Other CA1 sources and sinks are relatively small. A large phasic sink at the level of the basal dendrites of CA1 pyramids in urethanized rats (peaking on the CA1 negativity) was conspicuously absent during walking. The most prominent sustained generator is a sink in the inner one-third of the dentate molecular layer that is flanked above and below by sustained sources. This dentate pattern was identical to that found in urethanized rats, and may reflect tonically increased activity in septal afferents.

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367.9 IMMUNOHISTOCHEMICAL AND ELECTROPHYSIOLOGICAL EVIDENCE FOR DISTINCT POPULATIONS OF RAT HIPPOCAMPAL CA1 PYRAMIDAL CELLS. K.G. Baimbridge, H.McLennan*, J.J.Miller and M.J.Peet*. Dept. of Physiology, University of British Columbia, Vancouver, B.C. Canada V6T 1W5

The observations of Cajal and Lorente de Nó suggested that CA1 pyramidal cells could be classified into two subtypes, referred to as "large deep" and "small superficial" and that the relative proportion of these cells was somewhat species dependent. Bayer has also noted a clear difference in the neurogenesis of the deep and superficial cells in the rat. Recent reports have for the most part simply referred to "pyramidal cells" and have not suggested that there may in fact be more than one population perhaps with different biochemical and physiological characteristics.

Using immunohistochemical methods to study the distribution of two calcium binding proteins, calbindin D28K (CaBP) and parvalbumin (PV), it became apparent that CaBP immunoreactivity was restricted to the closely packed superficial layer of rat CA1 pyramidal cells. Furthermore, PV immunoreactivity, which is localized within the somata and axonal arborizations of a subpopulation of GABAergic interneurons in the CA1 region, forms an intense band of staining around the deep (non-CaBP) pyramidal cells which would suggest, according to Lorente de Nó, that the interneurons are of the polygonal basket cell type. Thus, on the basis of PV and CaBP-immunoreactivity, the two distinct layers of cells are clearly distinguishable. Of additional interest is our previous observation that the CA1 pyramidal cells of the guinea pig (alone among the many species examined) are completely devoid of CaBP immunoreactivity and are anatomically arranged in an almost completely uniform layer some 3-4 cells in width.

We are presently combining electrophysiological recording from rat hippocampal slices maintained in vitro with lucifer yellow (LY) injection and subsequent CaBP immunohistochemistry. Preliminary results have confirmed two populations of LY injected CA1 pyramidal cells by the presence or absence of CaBP immunoreactivity. Our results also suggest a difference in their electrophysiological responses to depolarizing current pulses. The identified cells were not interneurons on the basis of their lack of axonal arborization in the pyramidal cell layer, the absence of PV-immunoreactivity and their electrophysiological properties. We suggest that the clear distinction of two populations of pyramidal cells of the rat hippocampus should be considered when interpreting the functions of the CA1 region.

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367.10 HIPPOCAMPAL AND ENTORHINAL PLACE CELLS: WHAT HAPPENED WHEN THE LIGHTS WENT OUT. G.J. Quirk, R.U. Muller*, J.L. Kubie, and J.B. Ranck, Jr., Dept. of Physiology, SUNY Health Sciences Center at Brooklyn, N.Y. 11203.

The location-specific firing of hippocampal place cells is controlled by enviromental cues. Physical rotation of the cue set produces a similar rotation of the firing fields of place cells. Cue removal studies have shown that the location-specific firing is not dependent on any single cue modality (O'Keefe and Conway, Exp. Brain Res., 31:573, 1978). O'Keefe, in his early characterization of place cells (Exp. Neurol., 51:78, 1976), observed that spatial firing persisted in the dark for the majority of cells. This study was qualitative in nature, and did not take into account possible changes in firing rate, field size, field shape, etc. We have reexamined the question of place cell firing in the dark using infrared tracking, and a high resolution mapping system for describing location-specific firing (Muller, Kubie, and Ranck, J. Neurosci., 1987).

Experiments were performed in a cylindrical enviroment (76 cm in diameter and 51 cm high) uniformly gray in color except for a white card affixed to the wall. The rat chases food pellets in the cylinder while spike data and the rat's position are sampled by computer. An infrared emitter was attached to the rat's headstage and a video camera, sensitive in the infrared range, was used. The spectral sensitivity of the rat's visual system falls off sharply at 620 nm (Birch and Jacobs, Vision Res., 15:687, 1975), which is well below the infared range. Lights were turned off midway into a 16 minute session and the spatial firing patterns in the two 8 minute halves were compared.

For hippocampal place cells, spatial firing persisted in the dark, confirming the findings of O'Keefe. The location of the firing fields in the two halves of the session were identical. In some instances, the in-field firing rates were higher in the lighted half. Thus, turning out the lights had only a small effect, even though the white card exerts stimulus control over place cell firing (Muller and Kubie, J. Neurosci., 1987). The persistence of spatial firing in the absence of visual cues may be due to the use of non-visual cues and/or a mnemonic representation of the enviroment. In this context, it is interesting to ask if the recently reported location-specific firing of cells in the entorhinal cortex persists in the dark (Quirk and Ranck, Soc. Neurosci. Abst., 12:1524, 1986).

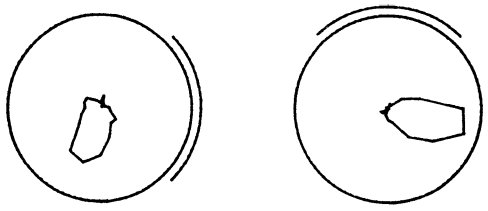
Supported by NIH grants NS14497, NS07117, and NS20686.

367.11 A QUANTITATIVE ANALYSIS OF HEAD-DIRECTION CELLS IN THE POSTSUBICULUM. J.S. Taube, R.U. Muller*, J.B. Ranck, Jr. Dept. of Physiology, SUNY Health Sciences Center, Brooklyn, NY 11203.

Many neurons in the deep cell layer of the postsubiculum discharge as a function of a rat's head direction in the horizontal plane, independent of the rat's behavior and place in the environment (Ranck, Neurosci. Abstr. 10:599). We report here results from using a quantitative method for analyzing the firing rate of "head-direction" cells as the rats move freely in a simple environment.

Single cell recordings were made from head-direction cells in the post-subiculum while rats moved freely throughout a 76 cm diameter, 51 cm high cylinder. The cylinder was gray except for a large white cue card taped to the inside wall; a curtain surrounded the cylinder. Two light-emitting diodes (LEDs), spaced 8 cm apart, were secured horizontally 2 cm above the rat's head; a red LED was positioned above the rat's nose and a green LED positioned over the rat's back. An automatic video/computer system digitized the position of each LED at 60 Hz. Horizontal head direction was calculated from the relative positions of the two LEDs and sorted into twenty 18° bins. The number of spikes fired during each 1/60th sec was counted, so that firing rate as a function of head-direction could be computed.

An angular firing specificity score was computed by taking the ratio of the firing rate in the bin with maximal firing, to the average firing rate in the three diametrically opposite bins. The mean ratio for 8 cells was 31.9 (range: 4.65-83.8). Following a standard session, the cue card was rotated 90° and another session was performed. In 5 cells, the head direction of maximal firing rotated with rotation of the cue card; this result was reversible upon returning the cue card to its original position. Below are the results from one cell.



Standard 90° Counter-clockwise Rotation

The vector from the center of the circle to a point on the polygon indicates the cell firing rate as a function of the rat's head direction. The arcs outside the 15 spikes/sec circle represent the cue card position.

These results indicate that some postsubicular cells encode information concerning the animal's head direction and that selective visual cues in the environment can exert control over the firing rate of head-directional cells.

(Supported by N.I.H. grants NS 07117, NS 14497, and NS 20686)

367.12 SOME PROPERTIES OF EARLY-FIRING CELLS IN THE RAT DENTATE GYRUS. E. W. Kairiss and G. V. Goddard. Department of Psychology and The Neuroscience Centre, University of Otago, Dunedin, New Zealand.

The dentate gyrus of the rat contains a diversity of neuronal morphologies including granule cells and numerous non-granule cell types. The latter may influence the response of granule cells to synaptic excitation from the perforant pathway (PP) by feed-forward inhibition. In this study, we have used extra-cellular unit recording techniques to examine the responses of cells which may be involved in feed-forward inhibition.

Experiments were performed on urethane-anesthetized Sprague-Dawley rats. The ipsilateral PP and the contralateral hilus (CH) were stimulated using monopolar electrodes. Field and unit potentials were recorded with glass micropipettes, positioned near the cell body layer. Since we were interested in the particular class of neurones which may mediate feed-forward inhibition, cells were selected for study which discharged before the onset of the population spike.

A total of 10 units from 38 experiments met our acceptance criteria. The unfiltered spike width at base ranged from 0.2-0.5 ms, and the spontaneous activity of these cells never exceeded 5/s. The responses to PP stimulation at low intensities generally consisted of single action potentials, but higher stimulus strengths could elicit 2 or 3 spikes. The latency of discharge decreased rapidly with increasing stimulus strength, and often reached asymptote at stimulus levels subthreshold for the generation of a population spike. Cells were not strongly influenced by recurrent inhibition as measured by paired-pulse tests. Stimuli applied to the CH, effective in inhibiting the PP-evoked population spike, had variable effects on unit firing. CH stimulation alone fired units in only 3/10 cases (latencies from 3.6-11.9ms). Unit responses to PP stimulation were also affected by preceding CH stimulation, displaying either a change in the latent period, or complete inhibition.

The effects of high frequency stimulation (four 100 Hz trains, each 30ms long, separated by 1s) applied to the PP were studied in 4 cases. Potentiation of the population spike was seen to persist for up to several hours following the trains. Although the general pattern of unit discharge remained unaltered, the apparent threshold for activation was decreased in all 4 cells.

These observations (1) support the existence of a heterogenous subpopulation of low-threshold neurones, with properties distinct from granule cells, and which may be involved in local-circuit interactions; and (2) underscore the need for a more extensive examination of the electrophysiology of these cells coupled with morphological identification.

(Supported by Canadian MRC and New Zealand MRC)

367.13 PHYSIOLOGY OF STRATUM LACUNOSUM-MOLECULARE INTERNEURONS IN CA1 REGION OF GUINEA PIG HIPPOCAMPUS. J.-C. Lacaille and P.A. Schwartzkroin. Dept. Neurological Surgery, Univ. of Wash., Seattle, WA 98195.

To further elucidate the role of local circuit neurons (interneurons) in hippocampal CA1 circuitry, we have characterized with intracellular recordings 1) the membrane properties, 2) the synaptic responses and 3) the synaptic interactions with other hippocampal cells, of interneurons in str. lacunosum-moleculare (L-M) in CA1 region of hippocampal slices.

The intracellular response characteristics of L-M interneurons (n=43) were distinctly different from responses of pyramidal cells and of other interneurons (basket cells and oriens-alveus interneurons). L-M interneurons had a high RMP (-58 mV), a high R_{in} (64 megohms), a time constant of 8.6 ms and a large amplitude (60 mV), relatively long duration (2 ms) action potential. A large afterhyperpolarization (11 mV, 34 ms) followed a single action potential. A late afterhyperpolarization (5.4 mV, 242 ms) usually developed after a train of action potentials (31/43 cells). Most L-M interneurons did not display any spontaneous firing.

Excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) were evoked in L-M interneurons from stimulation of major hippocampal pathways. EPSPs were most effectively elicited by stimulation of fiber pathways in transverse slices. The efficacy of major pathways in eliciting EPSPs was (in descending order): str. L-M > rad. > oriens > alveus > dentate str. mol. > CA3 str. lucidum. In contrast when major pathways were stimulated in longitudinal slices, IPSPs were predominantly evoked. These IPSPs were followed by depolarization-hyperpolarization oscillations of the membrane.

Local synaptic interactions were examined with simultaneous intracellular recordings between L-M interneurons and a) intrasomatically impaled (49 pairs), or b) intradendritically impaled (46 pairs) pyramidal cells, and c) str. pyramidale interneurons (4 pairs). In 9 of 42 (21%) intrasomatic recordings from pyramidal cells, a hyperpolarization developed (mean peak amplitude 0.91 mV, peak latency 86 ms, time to decay 93 ms) during L-M interneuron current-evoked (0.5 nA, 100 ms) firing. In 11 of 46 (24%) intradendritic recordings from pyramidal cells, a hyperpolarization was also produced (mean peak amplitude 0.67 mV, peak latency 74 ms, time to decay 76 ms) during current-evoked firing of L-M interneurons. Following intrasomatic (49 pairs) or intradendritic (46 pairs) stimulation-induced (0.5 nA, 100 ms) firing of pyramidal cells, correlated changes in membrane potential were not observed in L-M interneurons. In 2 of 4 str. pyramidale interneurons, hyperpolarizing potentials (mean peak amplitude 0.74 mV, peak latency 58 ms, time to decay 70 ms) were seen during current-evoked firing of L-M interneurons.

These physiological results show that L-M interneurons have neuronal and synaptic properties distinct from other hippocampal cell types. Functionally, L-M interneurons may mediate feedforward, but probably not feedback, inhibition of pyramidal cells.

Supported by NIH, NINCDS grants NS 15317 and NS 18897, by NSERC of Canada, and by FRSQ of Quebec.

367.14 MORPHOLOGY OF STRATUM LACUNOSUM-MOLECULARE INTERNEURONS IN THE CA1 REGION OF GUINEA PIG HIPPOCAMPUS. D.D. Kunkel, J.-C. Lacaille and P.A. Schwartzkroin. Department of Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

Previous morphological and physiological studies have shown numerous types of nonpyramidal neurons (interneurons) in the hippocampus. We have physiologically identified an interneuron type in the CA1 region of lacunosum-moleculare (L-M) in guinea pig hippocampus (Lacaille and Schwartzkroin, accompanying abstract). These interneurons were intracellularly injected with Lucifer Yellow or HRP and examined morphologically using light and electron microscopic techniques.

This interneuron population was clearly nonpyramidal, and forms a relatively homogeneous group. The somata were situated in str. L-M near the str. radiatum border. Most of the identified cells were fusiform and multipolar, with cell bodies approximately 20 x 25 um in size. At the EM level, the somata were similar to other interneurons with a small convoluted nucleus and a high density of cytoplasmic organelles. Dendrites of these interneurons radiated from the soma along str. L-M; after running a short distance, the dendrites turned primarily into str. radiatum where they branched. In a few instances, dendrites crossed the pyramidal cell layer and continued into str. oriens. Some dendritic branches could be observed ascending in str. L-M, crossing the hippocampal fissure, and projecting into the dentate gyrus molecular layer. Dendrites were smooth and somewhat varicose, especially distally. Numerous synaptic contacts were seen onto these aspinous dendrites.

The axon usually originated from a primary dendrite in str. L-M. It projected along str. L-M toward the CA1/CA3 border and/or toward the subicular regions. The axon branched shortly after emerging from the dendrite and the collaterals continued into strata radiatum and pyramidale, occasionally entering str. oriens. In some cases collateral branches ascended toward the hippocampal fissure and crossed into the outer molecular layer of the dentate gyrus.

Axonal processes formed synaptic contacts with spiny dendrites of CA1 (presumably pyramidal cells) in both strata L-M and radiatum. Synaptic contacts were made onto dendrites of granule cells in the outer molecular layer of the dentate gyrus. Occasional synaptic contacts were seen on other interneuron-like dendrites in strata L-M/radiatum and in the outer molecular layer of the dentate gyrus. Serial section analysis of these synaptic contacts showed them to be of the symmetric type.

Afferent pathways have been lesioned to determine afferent synaptic input onto these str. L-M interneurons.

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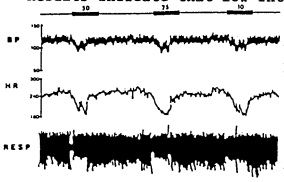
367.15 CARDIOVASCULAR RESPONSES TO ELECTRICAL STIMULATION OF HIPPOCAMPUS IN RAT. K.G. Ruit and E.J. Neafsey. Department of Anatomy, Loyola University Medical Center, Maywood, IL 60153.

Our laboratory has previously reported a projection from the infralimbic and prelimbic regions of the rat medial frontal cortex (MFC) to the vagal solitary nucleus (NTS) (Terreberry and Neafsey, 1983), and electrical stimulation of these regions of the MFC elicits changes in heart rate (HR), blood pressure (BP), respiration (RESP), and gastric motility. A major afferent projection to these regions of the MFC arises in the CA1 and subicular regions of the hippocampal formation (Swanson, 1981). The present study examined the effects of electrical stimulation of the hippocampus on HR, BP, and RESP.

Rats were anesthetized with Ketamine HCl (100mg/kg, IP), a tracheal cannula inserted for artificial ventilation and to monitor RESP, the femoral artery cannulated for recording BP, and EKG leads placed in the chest and back to monitor HR. The hippocampus was stimulated using a glass insulated tungsten microelectrode (tip exposure 100 microns). Stimulation parameters were 60sec trains of negative 0.25msec pulses at 25Hz beginning at a current intensity of 50uamps and gradually lowering the intensity until a threshold current was determined for a physiological response. Physiological effects were recorded on a physiograph and small marking lesions were made along each electrode track for histological reconstruction. In some experiments, methyl atropine (0.4mg/kg) was administered in order to determine if the response was vagally mediated. In other experiments, a lesion was placed in the ipsilateral MFC in order to determine the pathway underlying the response since it is possible that the hippocampus may exert its cardiovascular effects via a HIPP=>MFC=>NTS circuit.

Results indicate that low intensity (<50uamps) electrical stimulation of all fields of the hippocampus produces marked decreases in HR, BP, and slower, more regular RESP (see figure). The decreases in HR and BP are also seen in artificially ventilated animals indicating that these effects are not due to respiratory changes. Administration of methyl atropine blocks the bradycardia completely, indicating that these responses are largely mediated by the vagus. Ablation of the MFC by aspiration markedly attenuates responses to stimulation of the ventral but not the dorsal hippocampus. This observation is consistent with the fact that it is only the ventral CA1 and subicular regions which project to the MFC (Swanson, 1981). In conclusion, MacLean's concept of the hippocampus as part of the limbic "visceral brain" appears to be valid. (Support: Loyola Pott's Fund)



367.16 RADIATION-INDUCED HIPPOCAMPAL DAMAGE PRODUCES LOCOMOTOR HYPERACTIVITY AND SPONTANEOUS PERSEVERATIVE TURNING BUT NOT ENHANCED TURNING SPEED.
J.L. Ferguson*, M.A. Mulvihill, T.J. Nemeth and G.A. Mickley*. Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

X irradiation of the neonatal rat hippocampus causes a selective reduction in the population of granular cells of the dentate gyrus. This brain damage is correlated with locomotor hyperactivity that can be measured by square crossings in an open field (Bayer, S.A. et al., Nature New Biol. 242: 222-224, 1973) as well as infrared beam breaks (Mickley, et al., Soc. Neurosci. Abstr. 12/2: 1531, 1986). Radiation-induced hippocampal damage also causes perseverative spontaneous turning in an isolated bowl (Mickley, et al., Soc. Neurosci. Abstr.12/2: 1531, 1986). Previous measures of stimulated activity have relied predominantly on metrics of distance traversed rather than speed of motion. In the present experiment we measured the rate of spontaneous bowl turning in rats with radiogenic hippocampal damage.

Neonatal rat's cerebral hemispheres received fractionated exposure to 1300 rads of X rays during the first 15 days post partum (Bayer, S.A. and Peters, P.J., Brain Res. Bull. 2:153-156, 1977). This procedure depleted 86% of the hippocampal granular cells while sparing other brain areas. Control animals were sham irradiated. Ten- to 25-week-old subjects were placed in large bowls and spontaneous turning was recorded over 5 or more 30-minute sessions. We measured the length of turning bouts as well as the duration of individual quarter turns. Through the use of the Omnitech Digiscan system we also recorded locomotor activity by counting infrared beam breaks.

In the brain-damaged rats we observed statistically significant ($p<0.05$, ANOVA) enhancement of locomotion as measured by infrared beam breaks in the horizontal plane. Further, irradiated rats exhibited significantly longer bouts of bowl turning ($p< 0.05$, ANOVA) than did sham-irradiated animals. The speed of turning was not enhanced, however, in the rats with hippocampal damage. Speed of movements during the long bouts of irradiated animals was not different from the rate of turning observed during the shorter bouts of the sham-irradiated rats.

These data highlight the need for further specificity in the reporting of locomotor hyperactivity. Metrics that reveal both area traversed as well as speed of motion are relevant. As was observed in the case of rats with depleted hippocampal granular cell populations, these two measures of hyperactivity are not necessarily positively correlated.

367.17 A VASOPRESSIN-LIKE SUBSTANCE (VP-LS) IS RELEASED FROM THE RAT DORSAL HIPPOCAMPUS (DH): IN VITRO EFFECT OF NOREPINEPHRINE (NE). A. D. Ramirez* and V. D. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

We report for the first time the release of a VP-LS from the DH using in vitro superfusion and in vivo push-pull perfusion methods.

Bilateral DH slices from four decapitated rats were randomly distributed into four superfusion chambers ($\bar{X}$=54.61 mg DH wet weight/chamber). In a control chamber superfused for 4.2 h (starting about 11:00 h) the mean release rate of VP-LS from the DH as measured by RIA with a 2% cross-reactivity with oxytocin was 0.352±.08 pg/min with 5 distinct episodic pulses. At interval 27, 30 mM K^+ was infused for 10 min. A marked mean increase in VP-LS of 6.97±4.7 pg/min (7 intervals) was noticed. In two chambers with low spontaneous mean release of VP-LS (0.56±.32; 6 intervals) a first infusion of 10^{-5} M NE increased the mean release rate of VP-LS close to 3 fold whereas a second infusion using an identical dose of NE increased the release of VP-LS over 15.6 fold over control values. Lastly, a depolarizing dose of 30 mM K^+ further augmented the release of VP-LS to 15.2±5.4 (7 intervals). In 4 other chambers in which the spontaneous mean release rate of VP-LS was 3.57±0.4 (6 intervals) NE 10^{-5} M slightly increased its release to 4.04±0.82 (6 intervals); however, the second dose of 10^{-5} M NE was clearly effective since the mean release rate rose to 6.03±.78 (7 intervals); the K^+ challenge rose even further the release rate of VP-LS to 7±0.96 (4 intervals). In three chambers in which the mean control release rate was very high (15.05±1.6; 9 intervals) a slight increase to the first (18.6±1.4; 6 intervals) but a significant increase to the second infusion of NE (22.5±3.3; 7 intervals) was observed. A robust response was seen after K (47.8±4.0; 4 intervals).

In three rats bearing PPC in the DH and perfused for as long as 3 h, clear pulses of VP-LS were detected in the 10 min perfusate collections (5 pulses/rat) with a mean release rate of 1.7±.41, 2.8±.51, and 3.3±.76 pg/min, respectively.

The data indicate that the DH of freely behaving rats releases a putative peptide similar to vasopressin in a pulsatile manner with a remarkable consistency among these rats. In addition, in vitro slices of the DH release a similar VP-LS though a marked variation in its release was noticed under these conditions. Notably, all preparations responded with a consistent significant increase in VP-LS release to a second dose of 10^{-5} M NE but not to a first identical dose of NE, indicating clearly that this neurotransmitter can potentiate the release of this substance under in vitro conditions.

367.18 EFFECTS OF FORNIX TRANSECTION ON MATERNAL BEHAVIOR IN THE RAT. B. Osborne, B.C. Woodside* and J.E. Jans*. Dept. of Psychology, Concordia University, Montreal, Quebec, Canada H4B 1R6.

Previous investigators have reported that dorsal hippocampal lesions result in gross deficits in maternal behavior (Kimble, Rogers & Hendrickson, J. Comp. Physiol. Psychol., 63, 401-407, 1965) while fimbria damage although resulting in some deficits in retrieval and nest building did not affect survival rate of the offspring (Terlecki & Sainsbury, 1978). In this study the effects of fornix transection performed prior to mating on the subsequent performance of maternal behavior was investigated.

Fornix transections were performed on nulliparous female rats under sodium pentobarbital anaesthesia. A second group of females were sham-operated. Fornix transction which also caused extensive fimbria damage did not apparently affect the probability of females becoming impregnated, the progress of pregnancy or of parturition.

On both Days 2 and 7 postpartum lesioned animals showed marked deficits in retrieval and nestbuilding. A difference in the pattern of mother-litter contact between the groups was also observed. Lesioned animals had more frequent contacts with their offspring throughout the observation period. Typically these contacts were very short and resulted in an overall increase in nest time only during the light phase in the second week postpartum.

These data suggest that fornix transection produces deficits in maternal behavior similar to effects seen in other species-typic behaviors; that is a disruption in the frequency and sequencing of behavioral components.

367.19 CALCIUM BINDING PROTEIN (CALBINDIN) IMMUNOCYTOCHEMISTRY IN NORMAL RAT HIPPOCAMPAL GRANULE CELLS: HIGHEST DENDRITIC CONCENTRATION PRECISELY WHERE HILAR MOSSY CELLS INNERVATE R.S. Sloviter Neurology Res. Ctr., Helen Hayes Hospital, NY State Dept. Hlth., W. Haverstraw, NY 10993

Calbindin-D28K (Calcium Binding Protein; CaBP) is widely distributed in brain and has been suggested to influence calcium dependent processes (1). Like others (1-3), we have mapped CaBP in the rat hippocampus immunocytochemically using DAB as a chromagen, with similar results. CaBP is present in many, but not all, hippocampal interneurons, dentate granule cells, CA2 and CA1 pyramidal cells. CA3 pyramidal cells and nearly all hilar neurons, cells highly susceptible to seizure-induced damage (4), are CaBP-negative. Using benzidine dihydrochloride (BDHC) as the chromagen, a previously unreported finding was made. Use of BDHC gives discrete stain particles with little background, allowing differentiation between lightly and darkly stained elements. This characteristic of BDHC made visible a darkly stained lamina in the exact region innervated by the hilar mossy cells that form the ipsilateral associational/commissural (IA/C) input to the inner dentate molecular layer. This stained lamina could be the CaBP-positive terminals of this pathway or an increased concentration of CaBP in granule cell dendrites exactly where this pathway innervates. To address the question of localization, rats received bilateral perforant path stimulation that selectively destroys hilar mossy cells and somatostatin-positive cells (4). 3 days later, silver staining showed degeneration of the dentate IA/C input. CaBP staining at 3 to 14 days survival showed that removal of this pathway did not affect the dense CaBP staining in the inner molecular layer. These data suggest that CaBP is concentrated in the exact portion of the granule cell dendrite where the IA/C pathway, which apparently activates kainate-displaceable glutamate binding sites (5), innervates area dentata. The possibility exists that the presence of CaBP in granule cells may protect them from kainate receptor-mediated seizure-induced damage (4) and that the vulnerability of CA3 and hilar neurons to kainate receptor-mediated mossy fiber discharge may, in some way, be related to the absence of CaBP in these cells.

(1) Baimbridge and Miller; Brain Res. 245:223,1982.
(2) Jande et al; Nature 294:765,1981.
(3) Feldman and Christakos; Endocrinology 112:290,1983.
(4) Sloviter; Science 235:73,1987.
(5) Monaghan et al; Nature 306:176,1983.

CATECHOLAMINES: MPTP

368.1 DESTRUCTION OF DOPAMINE (DA) NEURONS WITH MPTP DECREASES DA AUTORECEPTOR SENSITIVITY IN MICE, L.M. Hryhorczuk* and M.P. Galloway, (SPON: R. Lisak), Lafayette Clinic and Wayne State Univ. Sch. Med., DETROIT, MI 48207

Although Parkinson's Disease (PD) is marked by a substantial loss of DA-containing neurons, a subset of DA neurons remains intact and is presumably implicated in the conversion of l-DOPA to DA and the subsequent release of DA. As such, adaptations of the regulatory mechanisms of the remaining DA neurons takes on added significance. We have utilized the GBL model for studying synthesis modulating DA autoreceptors (ARs) in vivo in C57/BL mice pretreated with the neurotoxin MPTP.

Mice treated with MPTP (51 mg/kg, ip, x 6 days) and sacrificed 10 days later had a 90% depletion of striatal DA. Enhanced activity of the remaining DA neurons was evidenced by increases in the DOPAC/DA, HVA/DA, and DOPA/DA ratios of 2.3, 5.3, and 2.5 fold, respectively. Administration of GBL to mice pretreated with MPTP increased striatal DA synthesis (i.e., DOPA accumulation after NSD-1015) suggesting that terminal ARs exert tonic inhibition of DA synthesis in remaining neurons. Reversal of the GBL induced increase in DOPA synthesis was measured after administration of different doses of apomorphine (30, 100, 500 ug/kg,sc) and the results expressed as percent of the GBL control. Under these conditions, there was a decreased responsiveness to a given dose of apomorphine in mice lesioned with MPTP. Similar, but less robust results were obtained in animals with a less severe DA depletion suggesting that the degree of AR desensitization may be related to the extent of DA cell loss.

Overall, the results suggest that compensation of DA neurons that remain after generalized DA cell death may arise as a consequence of decreased functional sensitivity of inhibitory terminal DA AR's. Chronic treatment with a DA agonist, such as in PD, would be predicted to accelerate AR desensitization and lead to an unpredictable, relatively unregulated release of DA. Alternatively, use of an AR-selective agonist as an adjunct to l-DOPA therapy in PD, as suggested originally by Carlsson, may achieve proper DA tone by manipulating AR function. Support (MPG) from the United Parkinson Foundation, DA-04120, MH-41227, and the State of Michigan-DMH

368.2 MPTP AND 6-HYDROXYDOPAMINE EFFECTS ON TETRAHYDROBIOPTERIN (BH_4) AND BIOGENIC AMINE METABOLISM IN RAT AND MOUSE BRAIN. Robert A. Levine, Sandy K. Demetriou*, Sandy Tait*, and [1]Sheldon Milstien*, Laboratory of Molecular Neurobiology, Lafayette Clinic and Wayne State Univ. Sch. of Med., Detroit, MI 48207, and [1]Laboratory of Neurochemistry, NIH, Bethesda, MD, 20892

l-methyl-4-phenyl-l,2,3,6-tetrahydropyridine (MPTP) was used in mice and 6-hydroxydopamine (6-OHDA) in rats to lesion nigrostriatal dopamine neurons and investigate the cellular localization in striatum of enzymes involved in the synthesis of BH_4. BH_4, the cofactor which is rate-limiting for the activity of tyrosine (TH) and tryptophan hydroxylases, is an important regulator of dopamine and serotonin synthesis. BH_4 levels in CSF and postmortem brain are decreased in several neurological diseases. In BH_4 biosynthesis, GTP cyclohydrolase (CH) converts GTP to dihydroneopterin triphosphate (NH_2P_3); NH_2P_3 is converted to 6-pyruvoyltetrahydropterin (6-PPH_4) by 6-PPH_4 synthase; 6-PPH_4 is converted by one or two enzymes to BH_4, the last enzyme being sepiapterin reductase (SR). MPTP (10 mg/kg i.p., once each day for 5 days; sacrifice on day 17) was administered to male C57/Bl mice and 6-OHDA (8 ug in left nigra) to rats (sacrifice on day 30). MPTP decreased TH by 50% and DA and metabolites by greater than 70%; decreased BH_4 and CH by 50%, and did not alter 6-PPH_4 synthase or SR activity. These same parameters are being currently being measured in rat striata after 6-OHDA. The preliminary results with MPTP suggest that 6-PPH_4 synthase and SR are not predominantly located in nigrostriatal dopamine neurons. In contrast, there is significant localization of TH, CH, and BH_4 in striatal dopamine terminals. It is unclear whether BH_4 biosynthesis occurs in other non-aminergic cells in striatum, however, the large decrease in CH and BH_4 after MPTP suggests that BH_4 synthesis occurs largely in striatal dopamine terminals. 6-OHDA is being utilized to confirm these findings in the rat. It is possible that 6-PPH_4 synthase and SR are necessary for metabolic pathways not related to BH_4 biosynthesis, although other functions have yet to be described.

368.3 STUDIES ON THE MITOCHONDRIAL UPTAKE OF THE 1-METHYL-4-PHENYL-PYRIDINIUM SPECIES. D.M. Placenti*, J.B. Hwang*, R.E. Heikkila and W.J. Nicklas (SPON: R.C. Duvoisin). Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

It is generally accepted that 1-methyl-4-phenylpyridine (MPP^+), the metabolite of the neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is the actual toxic agent which causes degeneration of the dopaminergic nigrostriatal pathway. The ultimate mechanism by which MPP^+ causes cell death is not clearly established. It has previously been determined that MPP^+ inhibits NADH-linked oxidation at Site 1 of the mitochondrial electron transport chain. This inhibition requires a concentrative uptake of MPP^+ into mitochondria which is driven by the membrane potential. To study this uptake process further, we have examined ^3H-MPP^+ uptake and MPP^+-inhibited mitochondrial metabolism using analogs of MPP^+, non-related organic cations such as acetylcarnitine (AcCn) and methionyllysine (MetLys), and pyridine-containing compounds such as nicotine (NC), chloroquine (CQ) and related compounds. Dopamine (DA) and specific DA uptake blockers had no effect on the mitochondrial uptake of ^3H-MPP^+. As expected, analogs of MPP^+ did inhibit this process; even analogs such as 1-methyl-4-t-butylpyridinium, which itself is a poor inhibitor of NADH-linked oxidation, inhibited MPP^+ uptake. Cations such as AcCn and MetLys (at concentrations up to 1 mM) did not decrease the metabolic toxicity of MPP^+ or its uptake. NC did inhibit the uptake of ^3H-MPP^+; however, at concentrations used (1-2 mM) it partially uncoupled the mitochondria, thereby collapsing the membrane potential. Since uptake of MPP^+ is dependent upon maintenance of the membrane potential, this may explain the effects of NC. CQ, on the other hand, decreased both the uptake of ^3H-MPP^+ and the MPP^+-induced inhibition of NADH-linked oxidation without uncoupling oxidative phosphorylation. The effective range for CQ action was between 0.5-5 mM. Studies using alternate sequential additions of CQ or MPP^+ indicated that the decrease in the MPP^+-induced inhibition of Site I activity was due to the inhibition by CQ of MPP^+ uptake into the mitochondria. Analogs of CQ (quinacrine and quinidine) did not behave similarly. It is not yet known whether this CQ effect has any role in the reported protection by CQ of MPTP toxicity in primates [D'Amato et al., Life Sci. 40:705, 1987]. However, the above data on substances which affect ^3H-MPP^+ uptake indicates that compounds such as CQ or uncoupling agents, in general, might be useful in testing the hypothesis that inhibition of NADH-linked mitochondrial respiration is an important mechanism in mediating MPTP neurotoxicity.

368.4 MONOAMINE OXIDASE-A (MAO-A) AND THE NEUROTOXICITY OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP). A. Giovanni*, P.K. Sonsalla, B.A. Sieber*, L. Manzino*, D. Placenti*, and R.E. Heikkila (SPON: J.R. Ravens). Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

MPTP is neurotoxic to nigrostriatal dopaminergic neurons in experimental animals including primates, dogs and mice. It has been shown, both in vitro and in vivo, that MPTP is metabolized by monoamine oxidase-B to a dihydropyridinium intermediate ($MPDP^+$) which in turn spontaneously oxidizes to form the 1-methyl-4-phenylpyridinium species (MPP^+). It has also been shown that pretreatment of mice with a dose of deprenyl which selectively and extensively inhibits MAO-B, but which does not affect MAO-A (i.e., 2.5 mg/kg), completely protects mice from the neurotoxic actions of MPTP. In contrast, pretreatment of mice with a dose of clorgyline which selectively and extensively inhibits MAO-A, but which has no significant effect on MAO-B activity (i.e., 2.5 mg/kg), is not protective. This has led to the conclusion that MAO-B, but not MAO-A, plays an important role in the bioactivation of MPTP.

However, based on experiments in vitro with pure MAO-A and MAO-B and with mouse whole brain tissue preparations, it is clear that MPTP can be oxidized by both MAO-A and MAO-B. For example, the K_m value for MPTP oxidation by pure MAO-A is slightly but significantly lower than its K_m value for oxidation by pure MAO-B. In contrast, the V_{max} value for MPTP oxidation by pure MAO-B is much higher than its V_{max} value for oxidation by pure MAO-A. Similar results are obtained in mouse brain tissue preparations. Thus, based on K_m values, MPTP is a better substrate for MAO-A, and based on V_{max} values, it is a better substrate for MAO-B. It is reasonable to speculate that MAO-A might play a role in MPTP bioactivation in vivo when the MPTP concentration is low (at or below the K_m value for oxidation by MAO-A). In support of this concept, we have found that although deprenyl pretreatment of mice (2.5 mg/kg) greatly inhibits the formation of ^3H-MPP^+ from ^3H-MPTP, the pretreatment of mice with a combination of deprenyl and clorgyline (each at 2.5 mg/kg) appears to inhibit ^3H-MPP^+ formation to a greater extent. Moreover, clorgyline alone appears to inhibit ^3H-MPP^+ formation slightly. These observations suggest that some MPP^+ formation is being mediated in vivo by MAO-A. It follows that there might be some circumstances when the bioactivation of MPTP by MAO-A would conceivably play a significant role in the neurotoxic process.

368.5 RELATIONSHIPS BETWEEN MPTP-INDUCED NEUROTOXICITY AND THE LEVELS OF MPTP METABOLITES IN THE BRAINS OF SEVERAL STRAINS OF MICE. P.K. Sonsalla, D.M. Placenti*, L. Manzino* and R.E. Heikkila, Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

It has been shown that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is neurotoxic to nigrostriatal dopaminergic neurons in several species of animals including mice. We have recently reported that there are marked strain differences in the susceptibility of mice to MPTP. For example, C57 black mice were very sensitive to MPTP, whereas CF-W mice were moderately affected and CD-1 mice were relatively unaffected by MPTP treatment (4 injections, 20 mg/kg/injection, at 2 h intervals; Sonsalla and Heikkila, 1986). The present experiments were conducted to determine possible reasons for this differential sensitivity. MPTP neurotoxicity depends on 1) the monoamine oxidase-B (MAO-B)-catalyzed oxidation of MPTP and the formation of the 1-methyl-4-phenylpyridinium species (MPP^+) and 2) the active uptake of MPP^+ into nigrostriatal dopaminergic neurons. Thus, we measured brain MAO-B activity, the in vitro oxidation of MPTP by MAO, and neostriatal MPP^+ transport in several strains of mice (C57 black, CF-W and CD-1). In addition, brain levels of MPTP and MPP^+ were determined in these strains of mice following the systemic administration of radioactive MPTP.

Neostriatal MAO-B activity (using C^{14}-benzylamine as substrate) was only slightly less in CF-W and CD-1 mice than in C57 black mice. In addition, the in vitro oxidation of MPTP by MAO-B in whole brain mitochondrial preparations from CF-W or CD-1 mice was only slightly less than that observed in preparations from C57 black mice. However, after MPTP treatment (20 mg/kg, s.c.) whole brain levels of MPP^+ were 28% and 41% less in CF-W and CD-1 mice, respectively, than were levels in the brains of C57 black mice. It seems unlikely that the small differences in brain MAO-B activity among the various strains alone could account for the observed differences in brain levels of MPP^+ or in the neurotoxicity of MPTP. This is particularly apparent with CF-W and CD-1 mice, which have similar levels of MAO-B but are differentially affected by MPTP treatment. There were no differences in ^3H-MPP^+ transport into neostriatal synaptosomes from C57 black, CF-W or CD-1 mice, indicating that differential MPP^+ transport into dopaminergic neurons is not a factor associated with sensitivity differences. In conclusion, the sensitivity of different strains of mice to MPTP appears to correlate with brain levels of MPP^+. Additional data regarding the biodistribution and pharmacokinetics of MPTP and MPP^+ in the brains of these mice will be presented.

368.6 THE ROLE OF MONOAMINE OXIDASE-A IN THE BIOACTIVATION OF SEVERAL MPTP ANALOGS. R.E. Heikkila, S.K. Youngster*, B.A. Sieber*, P.K. Sonsalla, K. McKeown* and T.P. Singer*, Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854 and VA Medical Center, San Francisco, CA 94109.

It has been shown that MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) administration results in a destruction of the dopaminergic nigrostriatal pathway in experimental animals including mice, dogs and monkeys. MPTP itself is a protoxin which is metabolized by monoamine oxidase-B (MAO-B) to a dihydropyridinium intermediate which spontaneously oxidizes to the pyridinium species 1-methyl-4-phenylpyridinium (MPP^+). MPP^+ formation can be significantly attenuated and MPTP-induced neurotoxicity can be prevented by pretreatment of mice with inhibitors of MAO-B (e.g. deprenyl) prior to MPTP administration. In contrast, pretreatment of mice with MAO-A inhibitors (e.g. clorgyline) affords no protection against MPTP-induced neurotoxicity and has only a slight inhibitory effect on MPP^+ formation. It follows that the oxidation of MPTP by MAO-B plays a major role in the neurotoxic process. In the present study we have determined the relative roles of MAO-A and MAO-B in the bioactivation of several MPTP analogs, including 2'methyl-MPTP and 2'ethyl-MPTP. We have also determined kinetic constants for the oxidation of the same MPTP analogs by pure MAO-A and MAO-B in vitro.

In contrast to the results obtained with MPTP, the dopaminergic toxicity of 2'methyl-MPTP was unaffected by pretreatment with deprenyl alone and was also unaffected by pretreatment with clorgyline alone (each 2.5 mg/kg). However, a combination of the two was fully protective against the neurotoxicity. In parallel experiments, deprenyl alone inhibited partially, clorgyline alone did not inhibit but the combination was very effective in inhibiting 2'methyl-MPP^+ formation from 2'methyl-MPTP. Pretreatment with deprenyl alone was ineffective whereas pretreatment with clorgyline alone afforded almost full protection against 2'ethyl-MPTP-induced neurotoxicity. Moreover, 2'ethyl-MPP^+ formation was markedly inhibited by clorgyline alone, but not by deprenyl alone. The neurotoxicity of other analogs of MPTP including 2'chloro-MPTP also was not prevented by deprenyl alone but was prevented by a combination of deprenyl and clorgyline. In vitro, all of these MPTP analogs are good substrates for MAO-A. These data demonstrate that not only MAO-B, but also MAO-A, can play a significant role in the bioactivation of MPTP-like neurotoxins and lend further support to the concept that pyridinium formation from the MPTP analogs is an important feature of the neurotoxic process.

369.1 CORTICAL EPINEPHRINE PROJECTIONS DEMONSTRATED BY RETROGRADE TRACING COMBINED WITH TYROSINE HYDROXYLASE AND PHENYLETHANOLAMINE N-METHYLTRANSFERASE IMMUNOCYTOCHEMISTRY. D.K. Beato*, W.J. Burke, T.H. Joh and J.H. Haring (SPON: P.A. Young). Depts. of Anatomy and Neurobiology and Neurology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104 and Dept. of Neurology, Cornell Univ. Med. Coll., New York, NY 10021.

Phenylethanolamine N-methyltransferase (PNMT) is the terminal enzyme in epinephrine (Epi) biosynthesis and is therefore a useful marker for Epi-containing neurons and their processes. Using a sensitive assay for PNMT, we have shown PNMT to be present in both rat and human cortex. PNMT immunocytochemistry has demonstrated an extremely sparse PNMT-containing plexus in the cerebral cortex, as well as a more prominent plexus of PNMT-positive fibers in the central amygdaloid nucleus of the rat brain. Since PNMT is known to be present in some neurons in the absence of the other enzymes required for Epi production, we have used a combination of retrograde tracing with immunocytochemistry for PNMT and tyrosine hydroxylase (TH) to determine whether the cortical PNMT-containing plexus could be using Epi as a transmitter.

Adult, male Sprague-Dawley rats anesthetized with sodium pentobarbital received either focal WGA-HRP injections of the amygdaloid complex or large WGA-HRP injections of the frontal cortex. Brainstem sections were reacted for the presence of HRP using TMB as the chromagen, and the resulting TMB reaction product was stabilized. Sections were flat embedded in Araldite, and HRP-labeled neurons in the Epi-containing cell groups were selected for study. Serial sections 1µm thick were cut and adjacent sections were stained for PNMT and TH immunoreactivity, respectively, using a postembedding immunocytochemistry protocol. Injections of the cerebral cortex resulted in the labeling of neurons in the C_1 Epi cell group bilaterally. Only a few (10-20) lightly labeled neurons were seen in each case, consistent with our previous observations of a very sparse PNMT plexus. Although neurons containing PNMT or TH immunoreactivity or HRP alone were seen, a subset of C_1 neurons was shown to contain all three markers, suggesting the presence of an Epi projection to the frontal cortex arising from the C_1 cell group. The amygdala injections resulted in neurons being labeled in C_1 and C_2. A number of these neurons also contained HRP, TH, and PNMT and were therefore classified as Epi neurons. Thus, the present study has provided direct anatomical evidence for the presence of an Epi-containing projection to cortical structures in the rat brain.
Supported by NIH grant AG00302.

369.2 **TYROSINE HYDROXYLASE-IMMUNOREACTIVE NEURONS IN THE PRIMATE SUBFORNICAL ORGAN: SPECIES SPECIFICITY.** J. Lory*, J.H. Kordower, G. Bing*, J.R. Sladek Jr. and D.M. Gash (Spon: R. Mangano) Dept. of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester N.Y. 14642, USA.

The advent of Falck-Hillarp and subsequently glyoxylic acid histoflourescence procedures allowed for the localization of catecholamine cell groups within the diencephalon and brain stem of the central nervous system. Studies in a variety of vertebrate species have discerned a general mammalian pattern of catecholaminergic nuclei. Using immunocytochemical localization for tyrosine hydroxylase, the rate limiting enzyme for catecholamine synthesis, we now report a species specific group of tyrosine hydroxylase-immunoreactive neurons contained within the subfornical organ.

Five new world Capuchin (Cebus apella) monkeys, three old world African Green (Cercopithicus aethiops) monkeys, and six rats were used in this study. The Capuchin monkeys and the rats were perfused with a PBS solution containing 4% paraformaldehyde and 0.1% glutaraldehyde. The African Green monkeys were perfused with a PBS solution containing 4% paraformaldehyde. Coronal frozen sections (30-40 µ) were incubated in the tyrosine hydroxylase primary antiserum (Eugene Tech.) at a dilution of 1:1000 at 4°C. Forty eight hours later the sections were processed with the unlabeled peroxidase method or via an immunoflourescence procedure utilizing Texas Red (Amersham; 1:800). Sections obtained from the Capuchin monkeys and the rats were processed simultaneously while the African Green tissue was processed separately.

In both species of monkeys, numerous tyrosine hydroxylase-immunoreactive perikarya were observed in the subfornical organ. These cells measured 20-30 um in diameter and possessed long beaded varicose fibers. In the Capuchin monkey, and to a lesser extent the African Green monkey, tyrosine hydroxylase-containing cell bodies tended to be localized in the lateral aspects of the subfornical organ with fewer cells in the central core. There appeared to be more cells in the Capuchin monkey as compared to the African Green primate. However, we are cautioned in this interpretation since perfusion protocols were different for these two monkeys and staining occurred at different times. In contrast, rats who were perfused and stained in an identical fashion as the Capuchin monkeys never displayed tyrosine hydroxylase-immunoreactive cell bodies in the subfornical organ. Immunoreactive fibers were commonly observed. However, these appear to be derived from an extrinsic source. When normal serum was substituted for either the primary or secondary antiserum, immunoreactivity was not observed.

This experiment documents a previously undescribed catecholamine cell group that appears to be species specific. Additonal monkeys are currently being processed for histoflourescence and for the secondary and tertiary enzymes of catecholamine biosynthesis to determine whether these cells contain dopamine, norepinephrine, or epinephrine.

369.3 TYROSINE HYDROXYLASE (TH)-IMMUNOREACTIVE NEURONS OCCUR WITHIN THE SPINAL CORD OF THE CHICKEN FROM HATCHING THROUGH ADULTHOOD. J.A. Wallace, T.C. Hoffman*, R.M. Mondragon*, R.R. Maez* and P.C. Allgood*. Department of Anatomy, University of New Mexico School of Medicine, Albuquerque, NM 87131.

Catecholaminergic (CA) neurons exist within the spinal cord of many lower vertebrate species, and have also recently been observed within the spinal cord of the adult rat. While CA neurons have not been detected by histofluorescence methods within the spinal cord of the adult chicken, we previously reported that TH-positive neurons are found throughout the spinal cord of late embryonic chicks (Anat. Rec. 214:140A, 1986). In the present study, we examined whether these TH-immunoreactive cells occur only transiently during embryonic development, or if they are present within the spinal cord of chickens throughout the period of maturation. The spinal cords of chickens were examined on the day of hatching, and at 1,3 and 6 months of age. At each age, the occurrence of the cells was determined at mid-cervical and upper lumbar cord levels. The cells were demonstrated by anti-TH immunocytochemistry on paraffin sections, employing the ABC peroxidase staining technique. Similar to the pattern found during embryogenesis, at hatching two populations of TH-positive cells were observed in cervical and lumbar cord segments. One group of cells was located beneath the central canal, and the other was situated within the dorsal horn of the spinal cord along the border between the gray matter and the lateral funiculus. Both populations of TH-immunostained cells were demonstrated at all post-hatching periods examined. With increasing age, no differences were seen in the general location of the cells nor in their staining intensity. On the other hand, the frequency of appearance of these cells decreased with the increasing age of the animals. However, this observation is most likely due to the dilution of the number of the TH-positive cells within the rapidly increasing mass of the cord as the chickens grow. Overall, it appears that the spinal cord TH-immunoreactive neurons persist in the chicken from embryonic periods to adulthood. We have also reported that developing TH-positive spinal cord cells cannot be visualized by CA histofluorescence methods unless the animals are pre-treated with monoamine oxidase inhibitors or loaded with L-DOPA (Anat. Rec. 218:144A, 1987). However, it remains to be determined whether these same cells, once they are mature, can be visualized immunocytochemically, utilizing antibodies against dopamine or norepinephrine.
Supported by NSF grant BNS-8511079 and NIH grant RR-08139.

369.4 **LUMBAR CORD DENSELY INNERVATES A SPECIFIC SUBREGION OF THE PERIAQUEDUCTAL GRAY WHICH PROJECTS TO PARAGIGANTOCELLULARIS AND PREPOSITUS HYPOGLOSSI, THE MAJOR AFFERENTS TO LOCUS COERULEUS (LC): POSSIBLE CONNECTIONS BETWEEN THE PERIPHERY AND LC.** G. Aston-Jones, M. Behbehani[1] and M. T. Shipley[2], Dept. Biol., New York University, NY 10003, and [1]Dept. Physiol. & [2]Dept. Anat. Cell Biol., U. Cincinnati School Med., OH 45627.

We have recently identified paragigantocellularis (PGi) in the rostral ventrolateral medulla as a major input to LC. PGi is predominantly excitatory on LC neurons (Ennis and Aston-Jones, this volume) and many PGi neurons projecting to LC are excited by noxious stimuli. Similar stimuli potently excite LC neurons, and these along with other results led us to propose that LC sensory responses may be mediated through PGi.

To test this hypothesis, the anesthetic lidocaine was microinjected into PGi and responses of single LC neurons to subcutaneous rear footpad stimulation (FS) were recorded. In all 5 LC neurons tested, the response to FS was abolished within 1 min after lidocaine injection (20-200 nl); recovery was obtained for 4 of these neurons, with responses returning within 10 min.

These results indicate that PGi may be a crucial component of LC responses to FS. However, we and others have found only sparse afferents to PGi from lumbar spinal cord, indicating that direct spinal projections to PGi may not mediate this response. We now report that: (i) injections of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) into lumbar spinal cord produced dense anterograde labeling in a restricted portion of the midbrain periaqueductal gray (PAG), corresponding to the medial and dorsal ventrolateral PAG of Beitz and Shepard (1985). (ii) WGA-HRP injections in this subregion of PAG revealed strong reciprocal connections with the PGi area (primarily ipsilaterally) that contains LC afferent neurons. (iii) These PAG injections also reciprocally labeled the prepositus hypoglossi (PrH) area, the other major afferent to LC. (iv) Injections of WGA-HRP into PGi that produced anterograde labeling in LC also demonstrated specific reciprocal connections with this same subregion of PAG, and with the dorsal PAG. (v) Stimulation of the medial/ventrolateral PAG synaptically activates neurons in PGi.

These anatomic results indicate that specific areas of PAG project to the two main afferents to LC, suggesting that PAG could play a key role in controlling LC activity, perhaps coordinating LC and other PAG functions (e.g., analgesia). Our findings also suggest a possible circuit for responses of LC neurons to footpad stimuli, proceeding from the lumbar spinal cord to PAG and from there to PGi and PrH which in turn project to LC. Additional studies are in progress to test this possibility.
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369.5 SOURCES OF THE NORADRENERGIC INNERVATION OF THE BED NUCLEUS OF THE STRIA TERMINALIS IN THE RAT. T. Duong, R.S. Fisher, C.R. Houser and A.B. Scheibel. UCLA Departments of Anatomy and Psychiatry, Mental Retardation Research Center and Brain Research Institute, Los Angeles, CA 90024.

The bed nucleus of the stria terminalis (BNST) in the albino rat is characterized by a dense noradrenergic (NA) innervation. The sources of this innervation have not been directly demonstrated. Using a combined retrograde tracer-immunocytochemical technique, we have found that the NA innervation of the BNST arises principally from the medullary groups (A1, A2) and locus coeruleus (A4, A6). Other noradrenergic groups (A5, A7) appear to make few if any contributions.

The right BNST of adult male Sprague-Dawley rats was injected with deactivated horseradish peroxidase conjugated to wheat germ agglutinin and colloidal gold (HRP-WGA-CG). After a 48-hour survival period, the animals were transcardially perfused with fixative, and the brains were cut in the sagittal or coronal plane. Sections were processed for the retrograde tracer by a silver enhancement method and further processed for DBH immunocytochemistry using a peroxidase-antiperoxidase method. This allowed us to view the HRP-WGA-CG retrograde tracer as black granules within DBH-positive neurons which were stained brown. The injection site was confined to the BNST which receives a dense plexus of DBH-positive varicose fibers in the ventrolateral portion and a light to moderate plexus throughout the rest of the nucleus.

Retrogradely-labeled DBH-positive neurons in the brainstem were found predominantly ipsilateral to the injection although a few were observed contralaterally.

Within the main portion of the locus coeruleus (A6), double-labeled neurons were distributed throughout the antero-posterior extent of the nucleus. These double-labeled cells were observed both in the dorsal and ventral divisions of the nucleus. They were mainly fusiform or bipolar in shape with their long axis running in the rostro-caudal direction of the nucleus. Some cells were also multipolar with no obvious direction of orientation. The dorso-caudal extension of the locus coeruleus (A4), which is located in the lateral corner of the anterior medullary velum, also contained double-labeled cells. These were mainly fusiform in shape. Ventral to this nucleus, double-labeled cells were observed in the subcoeruleus.

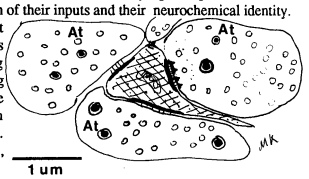
In the medulla, A1 is formed by a group of DBH-positive cells, lying dorsal to the lateral reticular nucleus. Double labeled cells were observed scattered throughout this NA nucleus. They were multipolar and their dendrites were oriented randomly. The A2 cell group consists of DBH-positive neurons mainly in and around the nucleus solitarius with a small number in the dorsal motor nucleus of the vagus. It extends from the level of the area postrema caudally past the obex. Medium-sized, double-labeled cells were observed in this nucleus. These neurons were multipolar with their dendrites oriented in a dorsomedial direction. Most were located within the nucleus solitarius portion of A2. A few were observed in the dorsal motor nucleus of the vagus. In the remaining two NA nuclei (A5, A7), double-labeled cells were rarely observed.

The BNST in the rat is thus characterized by a dense, convergent innervation originating from multiple NA brainstem nuclei. Supported by the David H. Murdock Foundation for Advanced Brain Studies.

369.6 ULTRASTRUCTURE OF NORADRENERGIC NEURONS IN THE CAUDAL VENTROLATERAL MEDULLA (A1 CELL GROUP) OF THE RAT. B. Nathan*, C. Gonzales*, M. Kalia (SPON. N. Moskowitz). Dept. Pharm., Thomas Jefferson Univ., Philadelphia, PA 19107

The A1 cell group in the caudal ventrolateral medulla comprises a compact population of noradrenergic (NA) neurons (Dahlstrom and Fuxe, '64) that show immunoreactivity with tyrosine hydroxylase (TH) and dopamine beta-hydroxylase (DBH). Light microscopic details of the rostrocaudal extent of this cell group and its relationship to cytoarchitecture of the medulla oblongata has been described (Kalia et al, '85). Since this cell group is located in a region of the detailed brainstem that is also occupied by numerous bulbospinal and visceral efferent neurons, analysis of the ultrastructural details of this region is necessary to understand the functional importance of these NA cells. We examined the ultrastructure of the A1 cell group in the rat, at a level 0.8 mm caudal to the obex, following fixation with acrolein (3.75%) in 2% paraformaldehyde in phosphate buffer (Pickel, '85). We analyzed tissue immunoreacted with an antibody directed against the more soluble enzyme-TH. No colchicine pretreatment nor the addition of Triton X-100 to increase the immunoreactivity and penetration of the antibody, respectively, was used.

Perikarya of the A1 cell group are medium sized with multiple spiny processes. The nucleus is large, centrally placed, with one to two shallow invaginations and a prominent nucleolus. The average nuclear-cytoplasmic ratio was 1:2. The most characteristic feature of the cytoplasm of A1 neurons was the presence of prominent, well-organized stacks of rough endoplasmic reticulum. A well-developed Golgi apparatus, free ribosomes, lysosomes, multivesicular bodies and large numbers of granular and agranular vesicles of different sizes were found. The soma was frequently associated with a glial cell that invested the A1 neuron over approximately 50% of its plasma membrane. The rest of the somatic surface was occupied by large axon terminals of which 2 to 3 made synaptic contact with the A1 cell in a single ultrathin section. **Dendrites** of A1 cells could be followed from the labeled neuron and were scattered in the neuropil in the immediate vicinity of the labeled cell. Most synaptic contacts with A1 dendrites were located on small dendrites or dendritic spines with a characteristic rosette arrangement (see drawing below) where 2 to 3, large unlabeled axon terminals, containing pleomorphic vesicles were seen simultaneously contacting the small A1 dendrite or dendritic spine. No dendro-dendritic synapses were seen. Dahlstrom and Fuxe ('64) had postulated the existence of NA axon terminals contacting NA dendrites in this region. We were able to demonstrate one to two examples of this arrangement. Labeled axons in the A1 cell group were invariably small (0.5-1 µm in diameter) and myelinated. A number of the ultrastructural features of the noradrenergic A6 cell group described by Pickel et al ('86) are similar to those seen in this study of A1 cells. Dual labeling techniques in the A2 cell group have revealed the existence of serotonergic (Pickel et al, '84a), peptidergic (Pickel et al, '79) and GABAergic (Pickel et al, '84b) nerve terminals contacting NA dendrites. The findings that NA dendrites in the A1 cell receive such a dense collection of synaptic inputs is new and needs to be evaluated in the context of the origin of their inputs and their neurochemical identity. The A1 region of the medulla oblongata does not receive afferents from the X and the IX nerves and therefore implicating centrally originating axons as sources of these, which by terminating in the A1 NA cell group provide the morphologic basis for the central role this region must play in central regulatory function. (Supported by USPHS Grants HL30991, HL31997, HL33632 to MK.)



369.7 INCERTOHYPOTHALAMIC A13 DOPAMINE SYSTEM. M.K. Sanghera, S. Grady*, W. Smith and D. Woodward. Depts, of Psychiat. and Cell Biol., Univ. of Texas Health Sci. Cntr., Dallas, TX. 75235.

The incertohypothalamic or nucleus A13 dopamine (DA) system has been implicated in the regulation of Luteinizing Hormone. Since gonadotrophin secretion is a sexually dimorphic function, the possibility that this nucleus may exhibit sexual dimorphism was investigated. A computer graphic system interfaced to a microscope was used to count, and measure the diameters of DA neurons labelled with antibodies raised to tyrosine hydroxylase (TH) and, display data in the 3-dimensional space of the nucleus. Four adult male, and female rats (in estrus), were deeply anesthetized with an interperitoneal injection of Nembutal. The brains were fixed via cardiac perfusion of saline and formalin, removed and then placed placed in formalin for 3-4 days. Frozen serial sections (50um) of the hypothalamus were taken throughout the caudal and rostral extent of the A13 and stained for TH (a gift from Dr J. Porter, UTHSCD). Data from every section containing A13 DA neurons were entered into the computer. The total number (± SEM) of TH-labelled cells in the nucleus in the male were 1296 ± 69 vs. 1252 ± 48 in the female (corrected for spurious counts, by the method of Abercrombie). The vast majority of cells in the A13 nucleus were of the large diameter, darkly stained, fusiform type. There was no differences in the diameters of these large cells (15.12 um ± 0.69 vs 15.8 um ± 0.32 in males and females respectively), nor in the smaller, rounded and lightly staining cells (8.82 um ± 0.30 vs. 7.00 um ± 0.23, male and female respectively). In addition to these two known populations of TH labelled cells in the A13 region, we found a hitherto third population of cells which were intermingled throughout the nucleus with the large fusiform type. These cells were somewhat more darkly stained than the small rounded cells, had little or no dendritic arborization and were of intermediate diameter in both sexes; (10.59 um vs. 0.44 in the male compared to 11.00 um ± 0.40 in the female). The data from this study indicate that (1) from numberical estimation of DA cell number there is no sexual dimorphism in the A13 DA system and (2) there is be an hitherto undescribed third population of TH labelled cells. Supported by Grant NS-24290-01.

369.8 THE DISTRIBUTION AND POSSIBLE CELLULAR ORIGINS OF VASOACTIVE INTESTINAL PEPTIDE (VIP) AND PEPTIDE HISTIDINE ISOLEUCINE AMIDE (PHI) IN THE RAT SUPERIOR CERVICAL GANGLION (SCG). C.A. Sasek, S. Landis and R.E. Zigmond. Depts. of Pharmacol., Harvard Med. School, Boston, MA 02115 and Case Western Reserve School of Med., Cleveland, OH 44106.

Studies on the acute effects of stimulation of the preganglionic cervical sympathetic trunk (CST) on tyrosine hydroxylase (TH) activity in the rat SCG have suggested the involvement of a noncholinergic preganglionic neurotransmitter in the stimulation of TH activity (TINS 8: 63, 1985). Secretin, VIP and PHI, all members of the secretin family of peptides, have been shown to increase TH activity in the SCG, suggesting that one or more of these peptides may be involved in the transsynaptic regulation of TH. A small number of neural processes containing VIP immunoreactivity (IR) has been reported to exist in the rat SCG. The purpose of the present study was to: 1) examine the distribution of these fibers in the SCG; 2) determine whether IR for other peptides of this family is also present; and 3) determine whether any such peptidergic fibers originate from preganglionic neurons of the intermediolateral cell column (IML). IR for VIP and PHI, but not for secretin, was found in the SCG. The distribution of VIP and PHI IR appeared identical in all respects. Large numbers of immunoreactive fibers and varicosities were present throughout the ganglion and its pre- and postganglionic nerve trunks. Dense clusters of immunoreactive fibers were also occasionally seen, as were labeled principal neurons. Examination of serial sections stained alternately for VIP or PHI IR revealed that at least some principal neurons contained both PHI and VIP IR. To determine if any of the immunoreactive fibers in the SCG originate from preganglionic cells in the IML, the CST was ligated. This resulted in a build up of VIP and PHI IR on the side of the ligature proximal to the spinal cord. Furthermore, when sections through thoracic spinal cord segments 1-3 of colchicine-treated rats were examined, both VIP and PHI immunoreactive cells and fibers were found in the IML. Tract tracing studies are in progress to determine if these peptide-containing cells do indeed innervate the SCG. These results demonstrate that two members of the secretin family of peptides are present in the SCG and that there is both an intrinsic and an extrinsic source for the peptide IR, with the latter possibly being the preganglionic sympathetic cells of the IML. These studies provide an anatomical basis for the hypothesis that VIP- and PHI-like peptides are released by preganglionic neurons and participate in the regulation of TH activity. (NS12651)

369.9 SECRETIN AND VASOACTIVE INTESTINAL PEPTIDE (VIP) STIMULATE TYROSINE HYDROXYLASE (TH) ACTIVITY IN SYMPATHETIC NERVE ENDINGS. M.A. Schwarzschild and R.E. Zigmond. Department of Pharmacology, Harvard Medical School, Boston, MA 02115.

Secretin and VIP stimulate TH activity in the rat superior cervical ganglion (SCG) (Ip et al., PNAS 79: 7566, 1982). TH activity in this ganglion is located primarily in the cell bodies and dendrites of sympathetic neurons. The purpose of the present study was to determine whether these peptides also increase TH activity in the terminals of these neurons, their primary sites for norepinephrine release. Rat irides and slices of rat submaxillary gland were incubated in the presence of the dopa decarboxylase inhibitor brocresine (30 μM). Control rates of dopa synthesis were 400 and 40 fmol/mg protein/min in the tissues, respectively. Dopa synthesis was reduced by more than 97% in tissues taken from animals that had had both SCG removed two days previously. Dopa synthesis was also blocked completely in submaxillary slices when α-methyl-p-tyrosine (0.2 mM), an inhibitor of TH activity, was included in the incubation medium. These results indicate that dopa accumulation in these end organs reflects the activity of TH in sympathetic nerve endings. In certain experiments, following incubation of tissues with secretin or VIP, TH activity was measured in tissue homogenates at pH 7.0, in the presence of a subsaturating concentration of the cofactor 6-methyl-tetrahydropterin (30 μM).

Both secretin and VIP stimulated dopa accumulation 4-fold in the intact iris. The EC_{50}'s of the two peptides were 30nM and 1 μM, respectively. Incubation of irides with either secretin or VIP (10 μM) also led to a 3-fold increase in TH activity measured in iris homogenates. In submaxillary slices, 10 μM VIP stimulated dopa acummulation (1.5-fold, $p<0.05$) only if the kallikrein-protease inhibitor, aprotinin (500 KIU/ml) was included. 1 μM VIP had no effect. Secretin produced a 4-fold maximal stimulation with an EC_{50} of 100 nM in these slices in the presence of aprotinin. Preliminary data suggest that secretin and VIP (10 μM) also stimulate dopa synthesis measured in situ and TH activity measured in homogenates in a third end organ of the SCG, namely the pineal gland. In submaxillary gland slices, the peptidergic stimulation of TH activity was not affected when Ca^{++} was excluded from the incubation medium, suggesting that secretin and VIP produce their effects on TH activity by acting directly on sympathetic neural processes, rather than by releasing substances from nearby neurons. Potential sources of peptides that might be involved in such a regulatory mechanism in vivo include (1) nearby parasympathetic or sensory nerve endings or (2) the general circulation. VIP-like immunoreactivity has been reported to be present in the rat submaxillary gland and iris, probably in postganglionic parasympathetic neurons. (Supported by NS12651)

369.10 ANTAGONISTS OF VOLTAGE-SENSITIVE CA^{++} CHANNELS BLOCK THE ACUTE INCREASE IN TYROSINE HYDROXYLASE ACTIVITY PRODUCED BY K^+ DEPOLARIZATION IN BOTH THE SUPERIOR CERVICAL GANGLION AND THE IRIS. A.R. Rittenhouse and R.E. Zigmond. Department of Pharmacology, Harvard Medical School, Boston, MA.

Tyrosine hydroxylase (TH) activity in the rat superior cervical ganglion (SCG) increases acutely following antidromic stimulation or depolarization with high K^+ (55 mM). In both cases, the increase is blocked by lowering Ca^{++} and raising Mg^{++} concentrations in the medium. Here we report that activation of TH in sympathetic neurons in the SCG by K^+ depolarization is dependent on Ca^{++} influx through voltage-sensitive Ca^{++} channels and that a similar mechanism is present in the terminals of these neurons located in autonomic end organs.

Ganglia were decentralized to remove preganglionic nerve terminals and were depolarized in vitro 4 days later with 55 mM K^+. Depolarization increased the rate of dopa synthesis in situ 3.1-fold. This increase was blocked by 69% and 61% by the addition of nimodipine (10 μM) or nitrendipine (10 μM) respectively, two blockers of voltage-sensitive Ca^{++} channels. Further studies indicated that a lower concentration of nimodipine (3 μM) was as effective as 10 μM.

To determine whether increased neural activity also increases TH activity in sympathetic nerve terminals, the preganglionic trunks of the SCG were stimulated unilaterally or bilaterally in vivo at 10 Hz for 15 min, using supramaximal depolarizing current. At the end of the stimulation period, the SCG and specific end organs containing terminals of neurons of the SCG were quickly removed, frozen, and stored at -80°C. TH activity, measured in tissue homogenates, increased an average of 4-fold in the SCG and 4 to 6-fold in the three end organs examined, the iris, pineal gland, and submaxillary gland. To determine whether the increase in TH activity in nerve terminals was dependent on Ca^{++} influx through voltage-sensitive Ca^{++} channels, irides were incubated for 15 min in high K^+. Hexamethonium (3 mM), atropine (6 μM), and phentolamine (10 μM) were present in the medium to block possible indirect effects on TH activity mediated by acetylcholine or norepinephrine release and subsequent stimulation of presynaptic receptors. High K^+ stimulated dopa synthesis in situ 3-fold, and this effect was maximal at 30 sec. The increase in enzyme activity was totally blocked in low Ca^{++} medium and was blocked by 75% in the presence of nimodipine (10 μM). The results suggest that the increase in TH activity produced by K^+ depolarization of both cell bodies and terminals of sympathetic neurons is mediated by an influx of Ca^{++} through voltage-sensitive Ca^{++} channels. (Supported by NS12651)

369.11 COLLIDINE (2-4-6 TRIMETHYL PYRIDINE) RELEASES TRITIATED NORADRENALINE (3H-NA) TAKEN UP BY RAT PINEAL AND VAS DEFERENS. J.L.Tomsig* and A.Pellegrino de Iraldi. Instituto de Biología Celular, Facultad de Medicina de Buenos Aires, Paraguay 2155, 1121 Buenos Aires, República Argentina.

Previous work in our laboratory demonstrated that collidine(2-4-6 trimethyl pyridine) used as a buffer in fixation procedures, abolishes the core osmiophilia and chromaffin reaction of granular synaptic vesicles in rat pineal and vas deferens nerves. This same effect was observed when tissues were briefly incubated with collidine and fixed thereafter. Those findings strongly suggested that the absence of core reactivity was due to a depletion of monoamines present in the synaptic vesicles by collidine.The results obtained with the use of collidine buffer, in conjunction with the well established histochemical procedures, could be also explained by assuming a higher rate of penetration for collidine than for glutaraldehyde or osmium tetroxide fixatives. In the present study we further investigated the effect produced by collidine in rat pineal and vas deferens previously incubated with 3H-NA. We found that collidine rapidly releases the exogenously added neurotransmitter from those tissues. The pineal gland bisected and the vas deferens trimmed in pieces of about 5 mg, were incubated separately in a Krebs modified solution containing 0.1 μM 3H-NA and gassed with 95% O_2:5% CO_2. The composition of the Krebs solution was (g/l) NaCl 6.9; KCl 0.35; $CaCl_2$ 0.28; $MgCl_2$ 0.11; NaH_2PO_4 0.12; $NaHCO_3$ 2.1; glucose 2; ascorbic acid 0.002; EDTA 0.0015; pH 7.4. After washing in the Krebs solution for 75 min, at 37°C, to allow the release of unspecific retained 3H-NA tissues were incubated in the following solutions:1)80 mM collidine buffer pH 7.4; 2) 80 mM cacodylate buffer pH 7.4; 3) modified Krebs solution; 4) 2.5% glutaraldehyde in 80 mM collidine buffer pH 7.4; 5)2.5% glutaraldehyde in 80 mM cacodylate buffer pH 7.4 and 6) 2.5% glutaraldehyde in 80 mM phosphate buffer pH 7.4. Samples of 0.5 ml were taken each 5 min for 30 min. Radioactivity of the samples was monitored in 15 ml of a solution 60/40 of toluene and methoxyethanol plus 3% 2,5-diphenyl oxazol (PPO).The tissues were homogenized in 10 volumes of 0.4N perchloric acid containind 1 mg/ml EDTA and 1.25 mg/ml Na_2SO_3. Collidine increased 3H-NA release 100 fold related to the initial value while the release elicited by cacodylate buffer or Krebs solution was almost negligible. Glutaraldehyde in collidine increaed the release 20 fold whereas by using cacodylate buffer or Krebs solution it is only increased two fold. Summarizing, collidine has a striking release-effect on stored 3H-NA which is greater when used alone than when mixed with glutaraldehyde. These studies are in agreement with histochemical studies previously reported and support the conclusion that collidine has a releasing effect on stored monoamines. This work was supported by a grant from the Cancejo Nacional de Investigaciones Científicas y Técnicas, R.Argentina.

369.12 **QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF α_1- AND β-ADRENERGIC RECEPTORS IN INTRAOCULAR RAT CEREBELLAR GRAFTS.** N.R. Zahniser, P. Curella*, D.M. Burnett*, J.A. Miller*, M. Eriksdotter-Nilsson* and A.-C. Granholm*. Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262 and Dept. Histology, Karolinska Institute, Stockholm, Sweden.

Electrophysiological responsiveness of cerebellar Purkinje cells to norepinephrine is diminished in aged rats. In order to determine whether intrinsic or extrinsic factors are responsible for this diminished responsiveness, we have begun studies of noradrenergic receptors in grafts of fetal rat cerebellum maintained in oculo. In the present experiments, α_1- and β-adrenergic receptors were studied in cerebellar grafts prepared from 15-day-old fetal rats (crown-rump length 12-14 mm) after 2 months in the anterior chamber of the eye of adult recipients. The cerebellar anlage continued their growth and development in oculo and had a final approximate diameter of 3 mm and weight of 2-5 mg. Routine histological analysis showed that these grafts possessed the typical trilaminar cortical microfolia and white matter areas previously described in such transplants. 2-[β(4-hydroxyphenyl)-ethylaminomethyl]-tetralone (BE 2254 or HEAT) was radioiodinated to [^{125}I]BE 2254 (IBE) and used to measure α_1-adrenergic receptors in 10μm sections with quantitative autoradiography (QAR). Specific binding of IBE was defined with 1μM prazosin, while β-adrenergic receptors were measured using specific binding of [^{125}I]-pindolol (IPIN) as defined with 1μM l-propranolol. Protein levels were measured in the same tissue sections using a densitometric assay and the QAR system. The α_1-adrenergic receptors were uniformly distributed throughout the grafts. In contrast, in the same grafts, the β-adrenergic receptors showed a more patchy or punctate distribution. At a concentration of IBE (200 pM) four-fold higher than its Kd value, the density of α_1-adrenergic receptors was 20 fmol/mg protein; a similar density of β-adrenergic receptors (30 fmol/mg protein) was observed at a concentration of IPIN (200 pM) equal to twice its Kd value. Similar experiments in intact cerebellum of young rats showed two-to-three-fold higher overall densities of both types of receptors. These results demonstrate that by using QAR, α_1- and β-adrenergic receptors can be localized and measured in intraocular grafts of rat cerebellum. Alterations in receptor properties may explain changes in electrophysiological responsiveness that occur with aging, and these can now be explored with this model. (Supported by USPHS AG 04418.)

370.1 ANTIAGGRESSIVE EFFECTS OF 5HT-1a AGONISTS. R.M. Carelli*, J.M. Liebman and G.C. Wagner. Psychology Dept., Rutgers Univ., New Brunswick, NJ and Pharmaceuticals Division, CIBA-GEIGY Corp., Summit, NJ

The serotonergic agonists 8-OH-DPAT, gepirone and buspirone have all been shown to displace serotonin from 5HT-1a receptors and, in addition, are all effective at increasing behavior in conflict paradigms and at reducing the aggressive behavior of mice. The following studies were conducted in an effort to determine the degree to which these compounds exert a differential effect on two measures of aggression, the resident-intruder and target biting paradigms.

In the resident-intruder paradigm, adult male Rockland-Swiss resident mice were housed individually in pan cages and exposed to standard, bulbectomized mice of the same sex, age and strain for 10-min test sessions. 8-OH-DPAT, gepirone and buspirone were administered IP in a corn-starch vehicle (pretreatment times = 20 min for 8-OH-DPAT and 30 min for gepirone and buspirone). A full dose-response curve for each compound was obtained using a repeated measures design with drug administration occurring no more often than twice/week. The vehicle was occasionally substituted for a drug dose. Intruder mice were drawn from a large population and the brief test session resulted in no injury to the subjects.

It was observed that 8-OH-DPAT, gepirone and buspirone all decreased the frequency of attack behavior (ED50 = 0.5, 3.2 and 5.0 mg/kg, respectively) and increased the latency to the first attack (ED50 = 0.6, 3.8 and 6.0 mg/kg, respectively). The drug vehicle had no effect on the aggressive behavior, which remained stable through the course of the study.

In the target biting paradigm, mice were placed in a small plastic cylinder and administered a mild 0.15 msec, 2.0 mA tail shock on a fixed-time 2-min schedule for 20-min daily sessions 5 days/week. These shock parameters were chosen as the minimal required to engender stable rates of behavior. Under baseline conditions, mice bit an inanimate target at three distinguishable rates: a high target biting rate immediately after the shock; an intermediate target biting rate during the intershock interval; and a low target biting rate during a 15-sec tone which signalled the impending shock. 8-OH-DPAT, gepirone and buspirone were injected IP as described no more often than twice/week.

It was observed that the administration of 8-OH-DPAT, gepirone and buspirone did not alter target biting behavior at doses as high as 1.6, 16.0 or 8.0 mg/kg, respectively.

These observations indicate that 5HT-1a agonists do exert a differential effect on aggressive behavior as assessed by these two paradigms and that the resident-intruder paradigm may be a sensitive procedure for assessing 5HT-1a agonist activity.

370.2 1-(2,5-DIMETHOXY-4-IODOPHENYL)-2-AMINOPROPANE HCL (DOI), A SELECTIVE 5-HT_2 AGONIST, EXERTS AN ANOREXIC ACTION IN RATS THAT IS PREVENTED BY LY53857, A SELECTIVE 5-HT_2 ANTAGONIST. L.E. Schechter and K.J. Simansky. Dept. Pharmacology, Medical College of Pennsylvania, Philadelphia, PA. 19129

Peripheral and central administration of serotonergic agonists can inhibit feeding in rats. Although the receptor subtypes responsible for serotonergic anorexia have not been studied systematically, some workers have suggested a role for central 5-HT_{1b} receptors(Dourish et al., Appetite, 7(suppl):127,1986). Recent data, however, indicate that the anorexic effect of peripherally administered 5HT is sensitive to 5-HT_2 receptor blockade (Fletcher and Burton, Psychopharmacol., 89:216,1986;Massi, Pharm.Biochem. Beh.,1987,in press; Simansky et al., this meeting). The present study therefore tested the effects of a selective 5-HT_2 agonist, DOI (Shannon,et al., Eur.J.Pharmacol., 102:23,1984) on milk intake in food-deprived rats.

Male Sprague Dawley rats(350-450 g) were housed individually with free access to fresh tapwater and maintained on a sweetened milk diet which was presented for 6 hrs. (1100-1700). The amount of milk consumed was measured for 30 min beginning 6 min following the ip administration of DOI or its vehicle. Controls ingested 21.4±1.6 ml during this test interval. DOI (0.1,1,2,4,8,10 uMol/kg;n=6-7/group) inhibited food intake in a dose-related fashion($p<.01$). For example, 2 uMol/kg reduced food intake by 46%($p<.01$) whereas 10 uMol/kg decreased intake by 81%($p<.01$). The ID_{50} estimated by regression was 3 uMol/kg.

Consistent with a role for 5-HT_2 mechanisms in DOI-induced anorexia, 60 min pretreatment with 6 uMol/kg of the selective 5-HT_2 antagonist, LY53857(Cohen et al.,JPET,235:319,1985), prevented the inhibition of food intake by an equimolar dose of DOI(VEH/DOI,-67%;LY53857/DOI,-11%,$p<.01$). A more extensive study showed that this antagonism by LY53857 (6uMol/kg, ip;n=7/group) was dose related($p<.01$). A dose as small as .26 uMol/kg of LY53857 markedly antagonized the anorexic action of 6 uMol/kg DOI(VEH/DOI,3.3±1.1ml; LY/DOI,13.3±3ml;$p<.01$). By comparison, 60 min pretreatment with 6 uMol/kg of xylamidine tosylate, a peripheral 5-HT_2 antagonist, did not prevent the anorexic action of an equimolar dose of DOI(VEH/VEH,21.3±1.4ml; XYL/VEH,20.3±2.0ml; VEH/DOI,6.3±1.4ml; XYL/DOI,5.0±1.5ml). This outcome contrasts with the potent antagonism by xylamidine of anorexia produced by peripheral 5HT and 4HT(Simansky,et al.,this meeting). Thus, the present study appears to implicate central 5HT-2 mechanisms in the anorexic actions of DOI. Further studies are investigating the behavioral and pharmacological specificity of this effect of DOI.(Supported by USPHS Grant MH41987-01 to KJS)

370.3 FENFLURAMINE AS A DISCRIMINATIVE STIMULUS: EFFECTS OF SEROTONIN (5-HT) AGONISTS AND ANTAGONISTS J. D. Smith* and R. Young* (SPON:J.H.Jonhson) Dept of Medicinal Chemistry, Sch of Pharmacy, MCV/VCU, Richmond, VA 23298.

The anorectic fenfluramine (FEN) exerts CNS effects predominantly through the release of endogenous 5-HT. Released 5-HT should interact at 5-HT receptors in a nonselective manner. The existence of at least two major populations of central 5-HT receptors, 5-HT1 and 5-HT2, is well established, and the possible existence of other populations (e.g. 5-HT3) and/or subtypes (e.g. 5-HT1A, 5-HT1B) of 5-HT binding sites have been proposed. Several site-selective and nonselective 5-HT agents have been identified; these agents include 8-OH DPAT (5-HT1A selective), TFMPP and mCPP (5-HT1B), MK 212 and 5-OMe DMT (5-HT nonselective), and quipazine (5-HT2).

In the present study, a drug discrimination procedure was used to examine the relationships between FEN and the above agents. Rats were trained to discriminate 1.5 mg/kg of FEN from saline in a two-lever operant choice task. The ability of the FEN-stimulus to substitute (i.e., generalize) to these agents was then examined. The FEN-stimulus (ED50 0.34 mg/kg) generalized to mCPP (ED50 0.56 mg/kg), MK 212 (ED50 0.69 mg/kg), 5-OMe DMT (ED50 7.19 mg/kg), and quipazine (ED50 1.31 mg/kg), and partially generalized to TFMPP (2.0 mg/kg=72% FEN-appropriate responding); a higher dose of TFMPP resulted in disruption of behavior (i.e., no responding). FEN-stimulus generalization did not occur to 8-OH DPAT (saline-like responding between 0.10 and 0.75 mg/kg).

In a series of antagonism tests, the FEN-trained rats were injected with various doses of ketanserin (KET, 0.05-10.0 mg/kg), a selective 5-HT2 antagonist, or 8-OH DPAT (0.01-0.50 mg/kg) prior to FEN (1.5 mg/kg) administration. Neither compound, over the dose range tested, completely attenuated the stimulus effects of FEN. Partial antagonism (i.e., ca. 50%) was observed however with 1.0 mg/kg of KET or 0.20 mg/kg of 8-OH DPAT. Interestingly, the administration of a combination of each dose of these drugs prior to FEN injection resulted in complete attenuation of the FEN-stimulus. The results suggest that (1) FEN probably produces a 5-HT nonselective stimulus effect and (2) in FEN-trained animals 8-OH DPAT appears to act as a 5-HT antagonist.

370.4 BEHAVIORAL ASSESSMENT OF 8-HYDROXY-2-(DI-N-PROPYLAMINO) TETRALIN(8-OH DPAT) IN TESTS FOR ANXIOLYTIC ACTIVITY. R. YOUNG* (SPON: G. METCALF). Dept of Medicinal Chemistry, Sch of Pharmacy, MCV/VCU, Richmond, VA 23298.

The behavioral effects of 8-OH DPAT, a site selective serotonin (5-HT1A) agent, were evaluated in two tests sensitive for anxiolytic activity. In the first test, rats were trained to lever-respond for sweetened milk on a multiple variable interval-fixed ratio (VI-FR) schedule of reinforcement. In the FR component a brief electric shock accompanied the presentation of reward (i.e., conflict procedure). The introduction of the punishment contingency markedly reduced rates of responding during the FR portions of the schedule. Treatment of these rats with diazepam (DZP) or 8-OH DPAT, administered intraperitoneally, significantly increased responding that was suppressed by shock over a five-fold (i.e., 1.0-5.0 mg/kg) and three-fold (i.e., 0.25-0.75 mg/kg) range of doses, respectively. In comparison, orally administered DZP or 8-OH DPAT significantly increased rates of punished responding across a 14-fold (i.e., 2.5-35.0 mg/kg) and 25-fold (i.e., 1.5-40.0 mg/kg) range of doses, respectively. By either route of administration, 8-OH DPAT produced maximum increases in punished responding that were smaller (400%) than those produced by DZP (600%).

In a second series of tests, rats were trained to discriminate either 2.0 mg/kg of DZP, 0.75 mg/kg of S(+)-amphetamine, or 3.0 mg/kg of ipsapirone (TVX Q 7821) from saline using a two-lever operant task. In tests of stimulus generalization, neither the DZP-stimulus nor the amphetamine-stimulus generalized to 8-OH DPAT or buspirone. In contrast, the ipsapirone-stimulus (ED50 = 0.87 mg/kg) generalized to 8-OH DPAT (ED50 = 0.09 mg/kg), buspirone (ED50 = 0.39 mg/kg), and gepirone (ED50 = 2.86 mg/kg) but not to diazepam. The results suggest (1) that 8-OH DPAT may possess anxiolytic properties which differ qualitatively from those produced by benzodiazepines and (2) the possible involvement of serotonergic systems in mediating the effects of 8-OH DPAT.

370.5 STIMULUS EFFECTS OF SECOND GENERATION ANXIOLYTICS: THE ROLE OF SEROTONIN. M.E.Pierson,* R.Young,* and R.A. Glennon* (SPON: G. King) Dept of Medicinal Chemistry, Sch of Pharmacy, MCV/VCU, Richmond, VA 23298

Unlike benzodiazepine (BDZ) anxiolytic agents, second generation anxiolytics such as buspirone (BUS), gepirone (GEP), and ipsapirone (IPSAP) display a low affinity for central BDZ receptors. In contrast, preliminary binding, drug discrimination, and other pharmacological data suggest that they may act via interaction at a particular population of serotonin receptors (i.e. 5-HT1 sites). The purpose of the present investigation was to further examine the role of 5-HT in the stimulus effects produced by BUS, GEP, IPSAP, a common metabolite (1-PP), and the BDZ diazepam. To this extent, male Sprague-Dawley rats were trained to discriminate ip administration of either the 5-HT1A agonist 8-OH DPAT (0.4 mg/kg), the 5-HT1B agonist TFMPP (0.5 mg/kg), or the non-selective serotonergic agent fenfluramine (1.5 mg/kg) from saline using standard two-lever operant techniques. The 8-OH DPAT stimulus generalized to BUS (ED50 1.5 mg/kg), GEP (ED50 2.1 mg/kg), and IPSAP (ED50 2.6 mg/kg) but all three agents were significantly less potent than 8-OH DPAT itself (ED50 0.17 mg/kg). The 8-OH DPAT stimulus did not generalize to 1-PP or diazepam (which produced saline-appropriate responding at doses of up to 1.0 and 2.5 mg/kg, respectively, and disruption of behavior at slightly higher doses). The TFMPP-stimulus (TFMPP ED50 0.2 mg/kg) did not generalize to any of the agents; all agents produced disruption of behavior at the highest doses evaluated. Each of the agents examined in fenfluramine-trained animals (fenfluramine ED50 0.34 mg/kg) resulted in partial generalization; (agent, maximal drug-appropriate responding and mg/kg dose): BUS (51%, 7.25), IPSAP (48%, 11), 1-PP (52%, 40). Higher doses produced disruption of behavior.

Additionally, TFMPP was administered (po) to rats trained to respond on a multiple VI-FR schedule of reinforcement. During the FR portion, a mild electric shock coincided with the presentation of reward (conflict procedure). In contrast to results with 8-OH DPAT and diazepam (these proceedings), TFMPP had no effect on punished responding.

The results of the present study support the idea that the mechanism of action of the BDZs may differ from that of the second generation anxiolytics and that the latter may involve a 5-HT, and in particular a 5-HT1A, mechanism. (Supported in part by VCU GIA #86/046)

370.6 FAWN-HOODED RAT STRAIN IS FUNCTIONALLY SUBSENSITIVE TO SEROTONIN AGONISTS RELATIVE TO SPRAGUE-DAWLEY AND WISTAR STRAINS. C.S. Aulakh*, P. Wang*, K.M. Wozniak* and D.L. Murphy. (Spon: S.N. Pradhan). Lab. of Clinical Science, National Institute of Mental Health. N.I.H. Clinical Center, 10/3D41, Bethesda, MD 20892

Fawn-Hooded (FH) rats possess a platelet storage pool deficiency analogous to that in the Chediak Higashi syndrome of humans (Meyers et al., Am. J. Pathol., 106:364,1982). Their blood platelets contain decreased numbers and contents of dense granules, decreased concentrations of serotonin (Tschopp and Weiss, Thomb. Haemorrh., 32:670, 1974) and possibly diminished uptake of serotonin (Stewart et al., Neurology, 33:176, 1983). The present study was undertaken to examine the possibility that FH rats may have altered CNS serotonergic function.

The food intake suppressant effects of three serotonin agonists, m-chlorophenylpiperazine (m-CPP, a selective 5-HT_{1B} agonist), 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT, a selective 5-HT_{1A} agonist) and fenfluramine (a 5-HT releasing agent) were compared in three different rat strains: Wistar, Sprague-Dawley (SD) and FH rats. The animals were housed individually and had free access to water. The animals were trained to take their daily food from 10 a.m. to 2 p.m. At the end of the first hour of food access the remaining food was weighed, and the difference from the original amount constituted one measure of food intake. Administration of all three serotonin agonists produced dose-dependent decreases in one hour food intake in all three strains. FH animals were significantly less sensitive to the food intake suppressant effects of all three serotonin agonists than either Wistar or SD rats. Body weight gain over the nine week course of the study was also significantly less in FH animals than either Wistar or SD animals. We will also present the data comparing the effects of various doses of m-CPP on body temperature, neuroendocrine changes (prolactin, corticosterone and growth hormone) and brain levels of m-CPP in these three rat strains.

In summary, this study supports some other data that FH rats, a strain with a peripheral platelet serotonin storage pool disorder, also possesses altered central nervous system serotonergic function. Further analysis will seek to identify other altered behavioral and neuroendocrine responses to serotonergic agents in the FH strain and clarify the biochemical nature of these defects. Finally, the comparable anorexic responses of Wistar and SD strains to serotonin agonists in the present study suggests that conclusions about serotonergic function made from one of these strains can be extrapolated to the other.

370.7 TEMPERATURE EFFECTS OF m-CHLOROPHENYLPIPERAZINE (m-CPP) AND 8-HYDROXY-2-(Di-n-PROPYLAMINO)TETRALIN (8-OHDPAT) IN THE RAT AND THEIR MODIFICATION BY ANTIDEPRESSANT TREATMENTS. K.M. Wozniak*, C.S. Aulakh*, J.L. Hill* and D.L. Murphy (SPON: N.A.Garrick). Lab of Clin. Science, NIMH, NIH, 10/3D41, Bethesda, MD 20892

The putative 5HT_{1A} agonist 8-OHDPAT and 5HT_{1B} agonist m-CPP produce hypo- and hyperthermia in the rat, respectively. We attempted to analyze the neuropharmacological components of these physiologically opposite effects on temperature with various receptor antagonists. These included clonidine, propanolol, naloxone, pindolol, haloperidol, ritanserin, methiotepin, metergoline and phenoxybenzamine.

Groups of 6 or more male Wistar rats (250 g) were pretreated with one of the above antagonists and challenged 15 min later with a dose of 8-OHDPAT (0.25 mg/kg s.c.) or m-CPP (2.5 mg/kg i.p.), which was chosen from a prior dose-response evaluation. Rectal temperature measurements were recorded at time periods up to 120 min after agonist injection. Pretreatment with methiotepin abolished the hypothermic response to 8-OHDPAT but was ineffective on the hyperthermia induced by m-CPP. Haloperidol, propranolol and pindolol partially attenuated the 8-OHDPAT induced hypothermia but also were inactive against m-CPP induced temperature changes. Metergoline was found to be the only antagonist of those tested that abolished m-CPP induced hyperthermia. These findings demonstrate that different response systems are involved in the opposing effects of 8-OHDPAT and m-CPP on rat body temperature.

Further groups of 5-6 animals were implanted with osmotic minipumps that delivered clorgyline (1 mg/kg/day), imipramine (5 mg/kg/day), chlorimipramine (5 mg/kg/day) or saline and challenged with the test dose of 8-OHDPAT or m-CPP after 21 days of treatment. Rectal temperature measurements were likewise recorded at time periods up to 120 min after agonist or saline injection. Chronic clorgyline administration attenuated both the hypo- and hyperthermic responses to 8-OHDPAT and m-CPP challenge, respectively, whereas the chronic administration of the tricyclics, chlorimipramine and imipramine, attenuated only the m-CPP induced changes in temperature.

These data demonstrate that changes in rat rectal temperature can be useful in the assessment of (1) the specificity of allegedly selective receptor agonists, and (2) the adaptational changes in serotonergic mechanisms during long-term antidepresant treatment.

370.8 EFFECTS OF OLFACTORY BULBECTOMY ON SEROTONIN RECEPTOR MECHANISMS INVOLVED IN NEUROENDOCRINE AND THERMAL REGULATION IN THE RAT. J.F. Nash*, C.A. Stockmeier, M.T. Lowy, G.A. Gudelsky and H.Y. Meltzer (SPON: E. Nemeth), Department of Psychiatry. Case Western Reserve University, Cleveland, Ohio 44106

Olfactory bulbectomy (BULB-X) has been suggested to be an animal model of depression. Removal of or lesioning the olfactory bulbs produces several behavioral changes including hyperactivity, irritability, elevated basal serum levels of corticosterone (CST) and a deficiency in passive avoidance learning in group-housed rats. These behavioral effects of BULB-X can be normalized by the repeated administration of antidepressants such as amitriptyline, imipramine and mianserin. Furthermore, acute injection of the specific serotonin (5-HT) uptake blockers, fluoxetine or zimelidine, reversed the deficit in passive avoidance learning. BULB-X may alter 5-HT mechanisms resulting in physiological changes similar to those observed in depression and therefore may be a valid animal model of human depression. These studies were designed to examine the effect of BULB-X on serotonergic-mediated neuroendocrine and temperature responses. In addition, serotonin-2 (^{3}H-ketanserin) and beta-adrenergic (^{3}H-dihydroaprenolol) receptor binding was determined in the frontal cortex and hippocampus in an attempt to correlate changes in ligand-binding with physiological changes.

Male, Sprague-Dawley rats were placed in a sterotaxic apparatus and olfactory BULB-X or Sham-BULB-X was performed. The rats were individually housed for at least 21 days. No differences were seen in basal (0900 hr) serum CST levels between BULB-X and control (SHAM) groups. The rat dexamethasone (DEX) suppression test (DST) was conducted in which 2 ug/kg of DEX was administered at 0900 and serum CST measured at 1300 hr. Although DEX suppressed CST, neither BULB-X nor SHAM animals "escaped" the suppressant effect of DEX. Administration MK-212, a 5-HT agonist, elevated serum CST to the same extent in both treatment groups. Similarly no difference in serum CST was observed between BULB-X and SHAM groups following administration of the 5-HT_{1A} agonist, 8-OH-DPAT. Furthermore, BULB-X did not alter temperature changes following administration of either MK-212 or 8-OH-DPAT. BULB-X did not affect ^{3}H-Ket or ^{3}H-DHA binding in the frontal cortex. However, BULB-X significantly decreased ^{3}H-DHA binding in the hippocampus by 20% as compared to SHAM animals. These data suggest that BULB-X does not significantly alter these measures of 5-HT neuronal function but may affect neurotransmission via the beta-adrenergic receptor. Further characterization is necessary to validate the use of this animal paradigm of human depression.

370.9 5-HYDROXYTRYPTAMINE MODULATES LIGHT RESPONSE IN CRAYFISH RETINAL PHOTORECEPTORS. A. Picones* and H. Arechiga (SPON: J. Villarreal). Depto. de Fisiología, Biofísica y Neurociencias, CINVESTAV, México, D.F.

Various roles have been proposed for 5-hydroxytryptamine (5HT) in the crustacean nervous system (Kravitz et al. 1985, in Model Neural Networks and Behavior, A.I. Selverston, Ed. Plenum Press, p. 339). 5HT containing neurons have been identified in the crustacean optic ganglia (Elofsson, R. 1983. Cell Tissue Res. 232: 221). Preliminary observations have indicated that 5HT may influence the response to light of retinal photoreceptors (Arechiga et al, unpublished). In the present work we report evidences suggesting that 5HT has a facilitatory role of light response on crayfish retinal photoreceptors.

The experiments were conducted in adult crayfish Procambarus clarki, of either sex and in intermolt. Light responses, intracellularly measured in dark adapted isolated retinas, were enhanced in a reversible manner by topical application of 5HT (10^{-4} M). Both the initial rapid phase and the late slow phase of the receptor potential were similarly facilitated by 5HT. This effect lasted more than 30 min. Membrane conductance during the light response increased under 5HT in proportion with the enhancement of the receptor potential. With test light pulses of intensity at 50% values in the dynamic range of the V-log I curve, both potential and conductance changes attained values of about 40% higher than controls. There were no effects of 5HT on either resting membrane potential or membrane conductance in darkness.

An immunocytochemical survey was made of the lamina ganglionaris with rabbit anti-5HT as primary antiserum using whole mount tissue preparations and histological sections (16-18 μm). Immunoreactive perikarya and fibres were identified in the lamina. Fibres were distributed in a columnar arrangement between two more densely packed layers in the distal and the proximal margins of the neuropile. This pattern was more conspicuous when dissection and fixation of the eyestalk was done at night time.

The electron microscopy analysis of the preparations using a peroxidase-antiperoxidase method revealed immunoreactive fibres running parallel and in close apposition to photoreceptor axons.

These results support the notion of a physiological facilitatory role of 5HT in the crayfish retina.

370.10 ACTIVATION OF TRYPTOPHAN HYDROXYLASE FROM CORTEX AND MIDBRAIN BY ACUTE SOUND STRESS. M. C. Boadle-Biber, T-H. Phan* and K.C. Corley. Dept. of Physiol., Medical College of Virginia, Virginia Commonwealth Univ., Richmond, VA 23298.

Acute stress increases the turnover of 5-hydroxytryptamine (5-HT) in rat CNS. This effect has been observed with immobilization, footshock, fear and exposure to ether (see Weil-Fugazza et al., Brain Res. 297:247 1984). One important contributing factor to the enhanced 5-HT turnover observed in response to stress may be the activation of tryptophan hydroxylase (TrpH), the enzyme that is rate limiting in 5-HT synthesis and hence determines the overall rate of 5-HT formation. The enzyme from cortex is activated in response to electrical stimulation of the dorsal raphe nucleus in which the 5-HT cell bodies of origin are located (Boadle-Biber et al., Neurochem. Intl. 8:83 1986). We recently reported that sound stress (2 sec, 110 dB sound pulses, VI-1 min for 120 min) increases cortical TrpH activity, measured in vitro, by 50 % compared with sham stressed control male Fischer 344 rats (Boadle-Biber et al., Fed. Proc. 46:3780 1987). This increase in enzyme activity may result from enhanced serotonergic neuronal firing to phasic auditory stimuli (Heym et al., Brain Res. 1 232:29 1982). We now report that the increase in enzyme activity to sound stress is also observed with enzyme extracts from midbrain, which contains the 5-HT cell bodies of origin of cortical projections. This increase, like that of cortical enzyme, is reversed by incubation with alkaline phosphatase and is nonadditive with the increase in enzyme activity obtained under phosphorylating conditions. Further studies have shown that enzyme activity remains elevated when the rats are killed 30 min after termination of the sound stress, but returns to control levels by 1 hr. Graded increases in in vitro enzyme activity are obtained over a range of sound intensities (90 - 120 dB). Enzyme activity also becomes elevated when the duration of the stress is shortened from 120 to 60 or 30 min (110 dB) and when the length of sound stimulus is reduced to 100 msec instead of 2 sec (110 dB, 1 hr). Thus phasic auditory stimuli enhance TrpH activity from rostrally projecting 5-HT neurons in a manner that is indistinguishable from the increase in activity obtained with electrical stimulation. These observations together provide the first instance of a noninvasive stimulus that induces a rapid and reversible alteration in TrpH activity.

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BIOGENIC AMINES AND RECEPTOR REGULATION

371.1 ADDITIVITY OF NEURAL ADAPTATION OF BETA-ADRENERGIC AND $5HT_2$ RECEPTORS TO IMIPRAMINE AND ELECTROCONVULSIVE SHOCK IN RATS: Regional Selectivity and Time Dependency. I.A. Paul*, G.E. Duncan*, R.A. Mueller, J.S. Hong and G.R. Breese. BSRC, UNC Sch. Med. and LBNT, NIEHS, Chapel Hill and Res. Tri. Pk., NC. 27514.

In previous work, we have demonstrated that electroconvulsive shock (ECS) selectively alters neuropeptide levels in brain regions whereas imipramine (IMI) does not, suggesting that the two antidepressant treatments are mechanistically separate (Paul et al., Soc. Neurosci. Abst. 12:416, 1986). In light of this data, we have examined the hypothesis that IMI and ECS may also affect beta-adrenergic (B-AR) and $5HT_2$ receptors ($5HT_2$-R) via independent mechanisms. Male rats were treated once daily for 1, 2, 4, or 14 d. with IMI (20 mg/kg, i.p.), ECS, or both. Controls received saline or electrode placement without current. The rats were decapitated 16 h. after the last treatment, and the brains dissected. Tissues were assayed for B-ARs or $5HT_2$-Rs using 1 nM ^{3}H-dihydroalprenolol (DHA) and 0.5 nM ^{3}H-ketanserin (KET) respectively. Non-specific binding was determined in the presence of excess isoproterenol for DHA or methysergide for KET.

IMI alone rapidly decreased cortical DHA binding, producing a 15% decrease by day 2 and a 23% decrease by day 4. In hippocampus, the effects of IMI were not evident until day 4 (-22%). Conversely, ECS alone produced significant effects on DHA binding in hippocampus by day 2 (-21%) but did not affect cortex until day 4 (-17%).

KET binding was not significantly affected by either IMI or ECS alone until day 4, at which time both cortex and hippocampus showed similar binding changes in response to IMI (-15% to -20%). In contrast, by day 4, ECS elevated KET binding by 11% in cortex but, was without effect in hippocampus.

When IMI and ECS were combined, cortical DHA binding changes were additive and apparent by day 2 (-30%). The additivity continued through day 15, finally producing a 50% reduction in binding. Significantly, B-AR changes of this magnitude are not produced in this rat strain by either treatment alone. In hippocampus, additivity was not evident until the 14th day of treatment. Cortical KET binding also displayed an additive response to IMI and ECS by day 4, being nearly the sum of the 11% increase due to ECS and the 15% decrease due to IMI (i.e. -8%).

These studies demonstrate that the effects of IMI and ECS on both B-ARs and $5HT_2$-Rs are additive. Further, the effects of ECS and IMI differ as to onset as well as to region of greatest sensitivity. These data, coupled with our previous work, support the hypothesis that ECS and IMI induce neural adaptation by separate mechanisms. Supported by USPHS grants HD-03110, MH-33127 and MH-39144.

371.2 ELECTROCONVULSIVE SHOCK INCREASES ALPHA-1 ADRENERGIC RECEPTOR BINDING IN RAT CORTEX AND AMYGDALA, BUT NOT IN OTHER BRAIN REGIONS. L.J. Grimm[1], D.C. Perry[1], J.A. Blendy[2], C.A. Stockmeier[2] and K.J. Kellar[2]. Depts. of Pharmacology, [1]George Washington Univ. Washington D.C. 20037, and [2]Georgetown Univ. Schools of Medicine and Dentistry, Washington, D.C. 20007.

Repeated administration of electroconvulsive shock (ECS) increases the density of alpha-1 adrenergic receptors labeled by [^{3}H]prazosin ([^{3}H]PZ) in rat cerebral cortex (Vetulani et al., Brain Res. 275:392, 1983; McLeskey et al., Soc. Neurosci. Abstr. 12:414, 1986), but not in hippocampus or hypothalamus (McLeskey et al., 1986). We have now employed quantitative in vitro autoradiography to measure [^{3}H]PZ binding with greater anatomical precision after ECS. Male Sprague-Dawley rats received ECS (150 mA, 60Hz, 300 ms duration) once daily for 10 days. Control animals were handled similarly, but no current was delivered. One day after the last treatment rats were decapitated and brains removed. Frozen 10 μ sections were incubated for 60 min at room temp containing 1 nM [^{3}H]PZ (13.8 Ci/mmol), then rinsed 2 X 5 min in ice-cold buffer, dried and apposed to Hyperfilm (Amersham). Alternate serial sections had 10 μM phentolamine added to the incubation to assess non-specific binding. Quantitation was done with a Loats computerized densitometer.

In agreement with previous results in membrane preparations, cortical [^{3}H]PZ binding was increased in ECS treated animals (22% higher in frontal/parietal cortex and 26% higher in parietal/striate cortex; both increases significant at $p<0.001$, t-test). The increase appeared to be most pronounced in the outermost laminae. Of all other regions surveyed, only the amygdala (but not the lateral amygdaloid nucleus) showed a significant change with ECS (22% increase; $p=0.002$). Hippocampus, hypothalamus, thalamus, geniculate nuclei, caudate putamen, central gray and cerebellum showed no significant differences. Earlier studies which used [^{3}H]WB-4101 to label alpha-1 receptors did not find changes after ECS (e.g. Bergstrom and Kellar, Nature 278:464, 1979). This could have been due to the fact that, under conditions employed, [^{3}H]WB-4101 labels serotonin-1a receptors as well as alpha-1 receptors (Norman et al., Mol. Pharmacol 28:487, 1985). Or, it might be due to the existence of subtypes of alpha-1 receptors (alpha-1a and alpha-1b) that can be discriminated by [^{3}H]WB-4101 but not [^{3}H]PZ (Morrow and Creese, Mol. Pharmacol. 29:321, 1986). If the latter is true, then ECS might affect these alpha-1 subtypes differentially. Current studies are comparing the effects of ECS on [^{3}H]PZ and [^{3}H]WB-4101 on brain binding sites in parallel.

371.3 EFFECTS OF ELECTROCONVULSIVE SHOCK ON [^{3}H]PRAZOSIN AND [^{3}H]WB-4101 BINDING TO ALPHA-1-ADRENERGIC RECEPTORS IN RAT CEREBRAL CORTEX. J.A. Blendy*, C.D. Rossiter*, and K.J. Kellar. Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, DC 20007.

Repeated administration of electroconvulsive shock (ECS) increases alpha-1-adrenergic receptors labeled by [^{3}H]prazosin in rat cerebral cortex (Vetulani et al., Brain Res. 275:392, 1983; McLeskey et al., Soc. Neurosci. Abst. 12:414, 1986). This contrasts with previous studies that found that ECS did not alter alpha-1 receptors labeled by [^{3}H]WB-4101 (Bergstrom and Kellar, Nature 278:464, 1979; Deakin et al., Psychopharmacology 73:345, 1981). Two potential explanations for this difference in results with the two ligands are that, under the conditions that it was used, specific binding of [^{3}H]WB-4101, labels serotonin-1A receptors as well as alpha-1 receptors (Norman et al., Mol. Pharmacol. 28:487, 1985); and that [^{3}H]prazosin appears to label two subtypes of alpha-1 receptors, alpha-1A and alpha-1B, while [^{3}H]WB-4101 labels primarily the alpha-1A subtype (Morrow and Creese, Mol. Pharmacol. 29:321, 1986). To determine whether ECS alters one or both alpha-1 receptor subtypes, we compared the effects of repeated ECS (once daily; 12 days) on the specific binding of [^{3}H]prazosin (nonspecific binding defined with 10 uM phentolamine) in the absence and presence of 1 nM WB-4101, which should occupy most of the alpha-1A sites. In addition, we measured [^{3}H]WB-4101 binding to alpha-1 receptors exclusive of serotonin-1A receptors by using the presence of 60 nM prazosin to define nonspecific binding.

Repeated ECS increased total specific [^{3}H]prazosin binding in the frontal-parietal cortex by 28% ($p<0.001$). In the presence of 1 nM WB-4101, [^{3}H]prazosin binding was increased by 30% ($p<0.001$). In the same tissues, [^{3}H]WB-4101 binding was increased to a lesser (14%) but statistically significant extent ($p<0.02$). These studies suggest that both alpha-1A and alpha-1B receptor subtypes might be increased by ECS, but that the alpha-1B subtype may be more affected.

371.4 EFFECT OF ALTERATIONS IN CIRCULATING GLUCOSE LEVELS ON α_2-NORADRENERGIC RECEPTOR BINDING SITES IN DISCRETE HYPOTHALAMIC AREAS. M. Jhanwar-Uniyal* and S.F. Leibowitz (SPON: W.R. Shapiro). The Rockefeller University, New York, N.Y. 10021.

Noradrenergic (NE) stimulation, specifically of the paraventricular nucleus (PVN), elicits eating and induces release of circulating glucose. Previous evidence indicates that this NE response is mediated via α_2-receptors and is greatest at the onset of the active (dark) cycle, when PVN α_2-receptor density reaches its peak. Moreover, deprivation of food (as little as 1 hr), specifically in the early dark period, decreases blood glucose levels and PVN α_2-receptor density, while deprivation in the late dark period actually enhances α_2-receptor concentration. The potent hypoglycemic agent tolbutamide, like food deprivation, also down-regulates medial hypothalamic α_2 receptors in close association with the decline in blood glucose. The present biochemical study investigates this effect of tolbutamide, and subsequent glucose injections, both at the start and end of the dark cycle, on α_2-receptor activity in discrete hypothalamic sites.

Male albino rats were administered vehicle (propylene glycol), tolbutamide (i.p. 50 mg/kg) or tolbutamide + D-glucose (25%) at 5 min or 15 min prior to sacrifice, at the start of the dark or light cycles. The brains were quickly removed and four hypothalamic sites, namely, the PVN, dorsomedial nucleus, medial preoptic nucleus and perifornical lateral hypothalamus, were micropunched. Standard radioligand technique was employed, using the α_2-agonist ^{3}H-p-aminoclonidine (^{3}H-PAC; 2.0 nM for high affinity binding site). Non-specific binding was determined in the presence of phentolamine (50 µM). Serum glucose levels were estimated using a YSI glucose analyzer.

The results demonstrate the following: 1) Tolbutamide treatment, within 5 and 15 min after injection at dark onset, caused respectively a 27% ($p < 0.05$) and 42% ($p < 0.05$) drop in serum glucose and a 48% ($p < 0.01$) and 63% ($p < 0.01$) decline in ^{3}H-PAC binding in the PVN but not other hypothalamic sites. 2) Tolbutamide treatment at the start of the light cycle produced a 49% ($p < 0.05$) decline in circulating glucose levels and a 40% down-regulation of α_2-receptor density only in the PVN. 3) Administration of D-glucose to tolbutamide-injected rats restored circulating glucose and significantly increased PVN binding (+28%) towards baseline, at dark onset but not at light onset. 4) In contrast to the PVN, the perifornical lateral hypothalamus exhibited an increase in ^{3}H-PAC binding 15 min after tolbutamide at dark onset. This effect was unresponsive to glucose administration. These findings suggest a close interaction between circulating glucose and PVN α_2-receptors, which depends upon the specific time within the diurnal cycle.

371.5 **REGIONALLY SELECTIVE REDUCTIONS IN THE DENSITY OF CENTRAL BETA ADRENOCEPTORS AFTER CHRONIC INTRACEREBROVENTRICULAR INFUSION OF ISOPROTERENOL IN RATS.** **G.A. Ordway*, C. Gambarana* and A. Frazer. Depts. of Pharmacology and Psychiatry, Univ. of Pennsylvania School of Med. and VA Medical Center, Philadelphia, PA 19104.**

Repeated treatment of rats with antidepressant drugs or electroconvulsive shock causes a marked reduction in the density of beta adrenoceptors in various regions of the brain, particularly in areas like the cerebral cortex where beta-1 adrenoceptors predominate. Chronic administration of centrally active beta agonists might similarly "down-regulate" central beta adrenoceptors and, therefore, might have potential antidepressant action. The beta adrenoceptor agonist, l-isoproterenol (ISO) activates brain beta-1 and beta-2 adrenoceptors in vitro, but does not penetrate the brain after systemic administration. Consequently, in this study the effect of chronic infusion of ISO into the lateral ventricles of rats on the density of central beta adrenoceptors was examined. Stainless steel cannulas were implanted in the right lateral ventricle of rats. Cannulas were connected via polyvinyl tubing to Alzet^R osmotic minipumps implanted subcutaneously. Pumps delivered drug or Ringer's solution at a rate of 1.05 ul/hr for 7 days. Rats were decapitated 10 to 12 hours after infusions were terminated and the brains were removed. Brain regions were selected for analyses based on their relative densities of beta-1 and beta-2 adrenoceptors: frontal cortex (FC, beta-1:beta-2 = 80:20), cerebellum (CB, beta-1:beta-2 = 10:90) and hypothalamus (HY, beta-1:beta-2 = 50:50). Beta adrenoceptors were measured in homogenates of these areas using the beta antagonist, ^{125}I-iodopindolol (IPIN). Infusions of ISO (5 ug/hr for 7 days) resulted in significant decreases in the Bmax of the binding of IPIN in CB (vehicle, 37±1 fmol/mg protein; ISO, 23±3 fmol/mg protein; $p<.001$) and HY (vehicle, 21±2 fmol/mg protein; ISO 9±1 fmol/mg protein; $p<.001$), but not in the FC (vehicle, 64±3 fmol/mg protein; ISO, 66±3 fmol/mg protein). In rats infused with ISO, the K_D of IPIN was unchanged in the FC (vehicle, 124±10 pM; ISO, 141±14 pM) and in the CB (vehicle, 48±1 pM; ISO, 47±2 pM), but was significantly increased in the HY (vehicle, 82±7 pM; ISO, 148±15 pM; $p<0.001$). Previous studies have shown that IPIN has a K_D for beta-1 adrenoceptors of about 150 pM and for beta-2 adrenoceptors of about 50 pM. Given this, one interpretation of these data is that repeated infusion of ISO is causing a preferential reduction in the density of beta-2 adrenoceptors in the brain. As ISO is a potent and full agonist at both beta-1 and beta-2 adrenoceptors, our results may indicate that central beta-2 adrenoceptors are more susceptible to down-regulation by beta agonists in vivo than are beta-1 adrenoceptors. **(Supported by research funds from the Veterans Administration and USPHS grants MH29094, MH14654 and MH09497).**

371.6 IMIPRAMINE TRANSPORT IN CULTURED MAMMALIAN CELLS INDUCES DOWN REGULATION OF BETA-ADRENERGIC RECEPTORS BY AN AUTONOMOUS MECHANISM. P.G. Lysko* and R.C. Henneberry* (SPON: P.R. Lowenstein). Molecular Neurobiology Section, Laboratory of Molecular Biology, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

Long-term treatment of laboratory animals with tricyclic antidepressants (TCAs) has long been known to lead to down-regulation of cell-surface receptors, including beta-adrenergic receptors. We have reproduced in primary cultured granule cell neurons from rat cerebellum and in cultured rat C6 glioma cells grown for 24 hr in the presence of 10 µM imipramine the TCA-induced down-regulation of beta-adrenergic receptors seen in brains of experimental animals. By measuring a decrease in [^{3}H]CGP-12177 binding to beta-receptors, we have found that amines such as chloroquine, NH_4Cl, (+)-propranolol, and trifluoperazine will down-regulate beta$_1$ receptors as well as does imipramine. This is seen in both C6 glioma cells and in the granule cell neurons. However, this apparent down-regulation is neither homologous nor heterologous, but is independent of the receptor. Rather, this autonomous down-regulation appears to occur as a consequence of imipramine transport and/or uptake into dense lysosomal and light endosomal fractions, where we have measured imipramine, propranolol, and dihydroalprenolol uptake after separation of the acidic organelles on Percoll density gradients. Therefore, the apparent down-regulation of beta-adrenergic receptors seen here for imipramine and other amines may be effected through a receptor-autonomous mechanism in common with the well known inhibitors of receptor recycling, chloroquine and NH_4Cl, which alkalize acidic organelles to interfere with receptor recycling and intracellular trafficking.

371.7 D-1 DOPAMINE RECEPTOR CHANGES AFTER CHRONIC TREATMENT WITH SCH 23390 AND/OR HALOPERIDOL. S.A. Parashos*, P. Barone*, I. Tucci*, and T.N. Chase. Experimental Therapeutics Branch, National Institutes of Neurological and Communicative Disorders and Stroke, Bethesda, MD 20892.

Recent experimental evidence supports the existence of interactions between D-1 and D-2 dopamine (DA) receptor subtypes. In order to study the possible impact of these effects on the chronic blockade induced D-1 DA receptor upregulation, four groups of five male Sprague-Dawley rats were treated for three weeks with the selective D-1 antagonist SCH 23390 (0.1 mg/kg sc), the predominantly D-2 antagonist haloperidol (1 mg/kg sc), the combination of the two drugs at the same doses, and the combination of their vehicles (control group). Four days after the final administration of the drugs the animals were sacrificed and D-1 DA receptor binding was studied in the striatum and nucleus accumbens by means of quantitative receptor autoradiography using ^{125}I-SCH 23982. Optical densities were evaluated by computer-assisted densitometry. SCH 23390 treatment significantly increased Bmax by 33% in both areas ($p < 0.01$). Haloperidol did not alter D-1 DA receptor numbers; however after combined administration of SCH 23390 and haloperidol, the D-1 DA receptor Bmax was 15% lower than the one obtained after chronic administration of SCH 23390 alone in the striatum ($p < 0.05$) but not in the accumbens. Receptor affinity remained unaffected by any of the treatments. Taken together with recent behavioral findings, these results imply that D-1/D-2 DA receptor interactions occuring in the striatum but not in the accumbens may be involved in chronic blockade induced D-1 DA receptor upregulation and corresponding behavioral effects, an observation with potential implications in the prevention and pharmacotherapy of drug-induced extrapyramidal disorders.

371.8 QUANTITATIVE AUTORADIOGRAPHIC DEMONSTRATION OF INCREASED D1 RECEPTOR BINDING IN RAT BRAIN AFTER REPEATED ELECTROCONVULSIVE SHOCK L.J. Fochtmann, M. Aiso*, R. Cruciani*, J.M. Saavedra, W.Z. Potter*. Section on Clinical Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20892.

Repeated electroconvulsive shock (ECS) has been shown to increase a variety of dopamine mediated behaviors in rats. However, corresponding changes in binding to dopamine receptors remain to be demonstrated. Previous work in our group and other has shown the utility of quantitative autoradiography using the ligand ^{125}I-SCH 23982, a highly selective D1 receptor antagonist, to precisely localize D1 receptor binding in the rat brain with greater sensitivity than that afforded by conventional membrane binding techniques. The present study examined the effect of chronic ECS on binding to dopamine D1 receptors using ^{125}I-SCH 23892 for in vitro quantitative autoradiography.

Male Sprague-Dawley rats weighing 200 - 250 g. received ECS (80 mA for .5 sec via ear clip electrodes) or sham ECS (handled and ear clips applied only) every other morning for a total of 8 treatments. One day after the final treatment animals were sacrificed between 9 AM and 11 AM. Brains were immediately frozen in isopentane and 16 micron sections were subsequently incubated with 250 pM ^{125}I-SCH 23892. Sections were opposed against ^{3}H-Ultrofilm and autoradiographs analyzed by computerize microdensitometry with comparison to ^{125}I standards.

Our previous work using ^{125}I-SCH 23892 for in vitro autoradiographic assessment of D1 receptor binding reveals a single class of high affinity binding sites in the caudate-putamen (B_{max} 174 ± 7 fmol/mg protein), nucleus accumbens (B_{max} 175 ± 8 fmol/mg protein) and substantia nigra pars reticulata (B_{max} 329 ± 47 fmol/mg protein). Chronic ECS produced a significant (45%) increase over these control values in D1 receptor binding in substantia nigra pars reticulata. A similar trend was present in caudate and nucleus accumbens with increase in D1 receptor binding of 35% and 30% respectively.

Although the exact function of the D1 receptor system is continuing to be elucidated, it has been postulated to have a modulatory effect on the D2 receptor system. If this is correct the changes we observe in the D1 receptor binding with chronic ECS may relate to clinical observations on the efficacy of electroconvulsive therapy (ECT) in treating psychotic sympotoms of depression as well as mania. It may also explain the improvements in Parkinsonian symptoms seen after chronic ECT is given to patients with the "On-Off" Syndrome as well as in patients receiving ECT for concommitant Parkinson's disease and affective disorder.

371.9 METHIONINE ADMINISTRATION INCREASES THE NUMBER OF SEROTONIN (5-HT) AND DOPAMINE (DA) BINDING SITES IN RAT'S BRAIN. C.E. Greenwood and M.T. Silvestre-Lontok*. Dept. Nutritional Sciences, Fac. Medicine, Univ. of Toronto, Toronto, Ontario, Canada. M5S 1A8.

We previously reported a 10-15% increase in Bmax, but no change in Kd, of striatal D_2-DA (spiperone) binding sites following methionine (MET) administration (500 mg/kg, i.p., twice daily for 5 days) to rats. Preliminary studies indicate that cortical 5-HT receptor number is also affected by MET administration (7.6±.4 vs 9.9±.8 pmol/g, mean±SEM for saline vs MET, $p<0.05$). The hypothesized mechanism of action is that MET, via increased methylation of membrane phospholipids, increases membrane fluidity thereby allowing cryptic receptors to become available for ligand binding. Direct confirmation of this hypothesis is unavailable however, and it is possible that MET is mediating its effect on receptor availability by an alternate mechanism. For example, MET administration also reduced by approximately 30% brain levels of tryptophan (TRP) and tyrosine (TYR), precursors for 5-HT and DA, respectively, due to increased competition for brain amino acid uptake. Thus MET may be mediating its effect on monoamine receptors indirectly by diminishing monoamine synthesis and inducing 'upregulation' of the receptors. Therefore to determine whether this effect of MET administration on monoamine receptor availability was due to decreased monoamine synthesis, the effect of administering MET or an equimolar dose of valine (VAL) to reduce brain TRP and TYR to an equivalent extent, on monoamine levels and receptor binding was measured. VAL and MET reduced brain TRP levels similarly (4.5 ±.1^a, 2.7±.2^b and 3.3±.2^b µg/g for saline, MET and VAL, $p<0.01$) However, this reduction in brain TRP levels did not influence steady-state levels of either 5-HT or its metabolite 5-HIAA. While similar neurochemical changes were observed with MET and VAL treatments, the changes in 5-HT binding were specific to MET treatment. That is, Bmax, but not Kd, of cortical 5-HT binding sites was increased by 13% in MET (7.7±.9) but not VAL (6.3±.6) treated animals, compared to controls (6.8±.5, $p<0.05$). A similar trend in 5-HT receptor number was observed in striatum. DA metabolism was influenced by both MET and VAL injections. Striatal DA levels were reduced by 26% (5.7±.7^a, 4.2±1.0^b, 4.1±1.0^b µg/g for saline, MET and VAL, $p<0.05$). This decrease in DA levels was reflected in lower levels of the DA metabolite DOPAC (1.9±.6^a, 1.3±.3^b and 1.6±.4^a, $p<0.05$), however this only reached significance in the MET treated animals. The other major DA metabolite HVA was unaffected by either amino acid. VAL injections did not produce similar changes in D_2-DA receptor number previously observed with MET. Thus the results of these experiments suggest that MET is mediating its effect on receptor availability independently of its effect on monoamine metabolism and hence indirectly support our hypothesis that the mechanism of action of MET is at the membrane level. (Ontario Mental Health Foundation).

371.10 SEROTONERGIC AND NORADRENERGIC INFLUENCES ON SEROTONIN TYPE 2 RECEPTOR REGULATION IN THE RAT: BEHAVIORAL AND BIOCHEMICAL STUDIES. A.S. Eison and G. Gianutsos. Section of Pharmacology and Toxicology, School of Pharmacy, University of Connecticut, Storrs, CT. 06268.

Chronic treatment of rats with a variety of clinically-proven antidepressants reduces the density of cortical serotonin type 2 (5-HT-2) receptors as well as the head shake response to 5-HT agonists. Similar effects are observed following chronic treatment with 5-HT-2 antagonists. While the down-regulation of 5-HT-2 receptors is associated with chronic antidepressant treatment, it is unclear whether this effect requires presynaptic 5-HT innervation or postsynaptic interaction with the 5-HT-2 receptor. Recent evidence suggests that enhancements of noradrenergic (NE) transmission may also play a role in antidepressant-induced changes in 5-HT-2 sensitivity.

The present studies have examined the effects of serotonergic and noradrenergic denervation in rats on the 5-HT-2 mediated head shake response. The behavioral effects of chronic (14 day) administration of a 5-HT-2 agonist (quipazine) and an antagonist (ketanserin) in unlesioned rats were also studied. Three days following the 5-HT-lesion, head shakes were unaltered while they were significantly above control 7 days post-lesion and below control 14 days post-lesion. No changes in head shakes were observed 3 days following NE-lesion despite a reduction of NE levels to 26% of controls. However, head shakes were significantly enhanced 10 days after lesion in the presence of an 87% depletion of NE. This suggests that disruption of presynaptic NE input by itself cannot account for the observed facilitation of 5-HT-2 mediated behavior. Chronic administration of quipazine and ketanserin both resulted in significant reductions in the head shake response comparable to those produced after 14 days of treatment with imipramine. Further, to complement all behavioral studies saturation binding parameters for beta-adrenergic and 5-HT-2 receptors will be presented. These studies demonstrate that noradrenergic as well as serotonergic influences may serve to modify 5-HT-2 receptor sensitivity.

371.11 DECREASED [^{3}H]SCH23390 LABELING OF STRIATAL D-1 SITES AFTER NIGROSTRIATAL INJURY. J.F. Marshall, R. Navarrete* and J.N. Joyce. Dept. of Psychobiology, University of California, Irvine, CA, 92717.

The up-regulation of striatal dopamine D_2 receptors following removal of the dopamine (DA) innervation has been a much studied phenomenon, because of its function significance in clinical and animal studies and because it has facilitated an understanding of CNS receptor regulation. Autoradiographic studies from this laboratory reveal that denervation-induced D_2 up regulation occurs predominantly in the lateral caudate-putamen (CP) of rats and monkeys. The current project extends this investigation to an analysis of the denervation-induced changes in D_1 sites, using both quantitative autoradiography and homogenate binding.

Male rats (200 g) were injected with 6-hydroxydopamine (6-OHDA, 8 ug in 4 ul) in the left rostral ventral tegmental area. The uninjected side served as control. Animals survived 4 d, 2 wk, 2 mo, 4 mo, or 11 mo postoperatively. Incubations were performed in 50 mM TRIS buffer plus ions containing 1 nM [^{3}H]SCH23390. Non-specific binding was defined using 5 uM (+)butaclamol. Adjacent sections were used for D_2 binding ([^{3}H]spiroperidol) or for labeling of high-affinity DA transport sites ([^{3}H]mazindol).

Eleven months postoperatively, rats showed dramatic (30-60%) losses of [^{3}H]SCH23390 binding in the CP ipsilateral to the 6-OHDA injection. The same rats showed the expected 15-50% elevations of specific [^{3}H]spiroperidol binding. Quantifying the autoradiographs revealed that the lesion-induced D_1 decrease was greatest dorsomedially; the D_2 elevation was greatest laterally. Autoradiographic studies of D_2 binding done on animals killed 4 mo or 2 wk postoperatively confirm this finding. At 2 wk, however, the ipsilateral CP decline in D_1 sites was more modest (10-20%, depending on CP region) that seen at the much later time points.

Scatchard analysis of the [^{3}H]SCH23390 (0.05-2.50 nM) or [^{3}H]spiroperidol (0.03-1.5 nM) binding was performed to CP sections of rats killed 8 weeks postoperatively. The B_{max} values of [^{3}H]SCH23390 binding ipsilateral to the lesion was less (1027 fmol/mg P) than in the control CP (1562 fmol/mg P) without a change in K_D (1.1 nM). [^{3}H]spiroperidol showed higher ipsilateral B_{max} (736 fmol/mg P) than in the control CP (653 fmol/mg P) without change in K_D (0.47 vs 0.49 nM).

No 6-OHDA-induced change in D_1 binding was apparent in autoradiographs through CP of rats killed 4 d postoperatively, even though DA loss is complete by that time.

Finally, preliminary studies of the [^{3}H]SCH23390 (1.0 nM) binding to ipsilateral and contralateral CP homogenates (P_2 fraction) reveal a similar effect: a 21% ipsilateral loss of CP binding 2 wk after 6-OHDA injection.

371.12 PHYSIOLOGICAL AND PHARMACOLOGICAL HORMONAL MODULATION OF THE RAT STRIATAL D1 DOPAMINE RECEPTOR. D. Lévesque* and T. Di Paolo, (SPON.: C. Harnois) Department of Molecular Endocrinology, Laval University Medical Center, Ste-Foy, Québec, G1V 4G2 and School of Pharmacy, Laval University, Québec, G1K 7P4, Canada,

It is well known that striatal D2 dopamine receptors are modulated by estrogens. After ovariectomy, their is no change in [^{3}H]spiperone striatal binding sites to D2 dopamine receptors. In female rats, hypophysectomy decreases by 20-30% [^{3}H]spiperone binding to striatal D2 dopamine receptors. In the present study we have observed that ovariectomy decreases (20 %, $p<0.01$ vs intact female rat) [^{3}H]SCH 23390 density of D1 dopamine receptor to striatal tissue with no change of affinity. No difference in [^{3}H]SCH 23390 binding affinity and density to striatal D1 dopamine receptors was seen between intact male and female rats. The density of striatal D1 dopamine receptor labelled with [^{3}H]SCH 23390 of hypophysectomized male rats are significantly higher (15 %, $p < 0.01$) compared to those in the intact female, while the affinity remains unchanged. A chronic treatment with 17β- estradiol (E_2, 10 ug b.i.d. for two weeks) of ovariectomized rats increases the density of striatal [^{3}H]SCH 23390 binding sites (Table I). NaCl significantly increases the affinity of [^{3}H]SCH 23390 to striatal tissue in both the control and treated groups.

TABLE I [^{3}H]SCH 23390 binding to rat striata

Groups	Assay buffer	Kd (nM)	Bmax (fmole/mg of protein)
Control	0 NaCl	0.363 ± 0.029	409 ± 17
	+ NaCl	0.244 ± 0.017 **	556 ± 48 **
E_2	0 NaCl	0.321 ± 0.033	590 ± 20 **
	+ NaCl	0.207 ± 0.012 ##	677 ± 38 &

** $p < 0.01$ vs cont. 0 NaCl; & $p < 0.05$ vs cont. + NaCl;
$p < 0.01$ vs E_2 0 NaCl.

Chronic treatment with E_2 increases the density of striatal D1 dopamine receptors by 22% an increase similar to the one observed previously for the D2 dopamine receptor (20-30%). In contrast, ovariectomy affects D1 dopamine receptors while D2 dopamine receptors remain unchanged. In spite of their co-localization in striatal tissues, the interaction of these two types of dopamine receptors is still not well understood. Our results show, as for D2 dopamine receptors, that D1 dopamine receptors are modulated by hormones under physiological and pharmalogical conditions. However, the regulation of these two dopamine receptors seems to be different. Supported by the MRC.

371.13 THE STRIATAL AGONIST BINDING SITE OF THE D-2 DOPAMINE RECEPTOR FLUCTUATES DURING THE RAT OESTROUS CYCLE. P. Falardeau, M. Morrissette* and T. Di Paolo. Department of Molecular Endocrinology, Laval University Medical Center, Quebec G1V 4G2, and School of Pharmacy, Laval University, Quebec G1K 7P4, Canada.

Behavioral and biochemical observations indicate that estradiol, progesterone and prolactin can influence the function of the nigro-striatal dopaminergic system. Striatal D-2 dopamine antagonist and agonist binding sites were measured during the rat oestrus cycle and compared to ovariectomized rats. Adult Sprague- Dawley female rats demonstrating at least two consecutive 4-day estrous cycles were included in the experiment. Animals were sacrificed either in the morning for ovariectomized rats and for rats in oestrus (E), diestrus I (DI), diestrus II (DII), proestrus A.M. (PAM) and in late afternoon for rats in proestrus P.M. (PPM). As previously reported, we observe a surge of plasma prolactin concentrations in the afternoon of PPM while estradiol levels increase gradually to reach a peak on the day of proestrus. Progesterone concentrations are more phasic with one peak the day of D1 and one on PPM. While no variation of the antagonist binding site of the D-2 dopamine receptor labelled with [^{3}H]spiperone was observed during the rat oestrus cycle, we observe here fluctuations of the agonist sites measured with the competition for [^{3}H]spiperone binding by apomorphine. In DII, 35% of the dopamine agonist binding sites are in a high affinity state and this proportion corresponds to that observed in ovariectomized rats. In PAM, PPM, E and DI, we observe a large conversion ($p < 0.01$) of the dopamine agonist high affinity sites into the low affinity (high affinity site: 15%). No significant change in the sum of high + low dopaminergic agonist binding densities and in the inhibition constants (Ki) for either the high or low affinity site of the D-2 dopamine receptor was observed during the rat oestrus cycle. Thus, physiological fluctuations of hormones as occur during the oestrus cycle can modulate extrahypothalamic biogenic amine activity, namely striatal dopamine systems which are not involved in the control of hormone secretion. This may be post-synaptically through an interaction with guanine nucleotide binding proteins. Supported by the MRC.

371.14 HORMONAL MODULATION OF GROWTH AND DOPAMINE RECEPTORS IN 7315a PITUITARY TUMORS. M.A. Bernier* and T. Di Paolo (SPON: S. Radouco-Thomas), Department of Molecular Endocrinology, Laval University Medical Center, Ste-Foy, Québec G1V 4G2 and School of Pharmacy, Laval University, Quebec G1K 7P4, Canada.

The hormonal modulation of growth and D-2 dopamine antagonist and agonist binding sites was studied in 7315a pituitary tumors. The agonist high- and low-affinity states of the dopamine receptor were investigated with apomorphine competition for [^{3}H]spiperone binding to dopamine receptors in 7315a tumors grown in intact female rats while the antagonist site of the receptor was investigated with saturation of [^{3}H]spiperone binding. The affinity and density of the antagonist state and of the high- and low-affinity agonist states are observed to be similar in 7315a tumors as for intact pituitary tissue and are similarly modulated by sodium (NaCl) and guanine nucleotide (Gpp(NH)p). 7315a tumor growth is slower in ovariectomized compared to intact female rats while 17β-estradiol (20 μg, b.i.d.) treatment for 2 weeks and discontinued for 1 week or treatment for 3 weeks inhibits growth of these tumors. Prolactin concentration and density of dopamine receptors are higher in tumors grown in ovariectomized compared to intact female rats while both are decreased after 23 days of 17β-estradiol treatment. Estradiol treatment decreases the affinity of the high- and the low-apomorphine binding sites while their proportions remain unchanged. In another series of experiments, we have compared growth of 7315a tumors and their dopamine receptors in male and female rats. Tumors inoculated in female rats grew faster than those inoculated in male rats. Plasma prolactin concentration is higher in tumor bearing female rats compared to male rats with these tumors. By contrast, tumor prolactin concentrations are lower in tumors from female rats compared to tumors carried by male rats. Dopamine antagonist and agonist binding sites have more affinity and a higher density in tumors grown in male compared to female rats. The proportion of the high- and low-agonist affinity states of the dopamine receptor remains unchanged whether the 7315a tumor was grown in male or female rats. These results confirm the hormono-dependence of these tumors. In addition, they show a relationship between tumor growth and dopamine receptors in 7315a tumors. Supported by the National Cancer Institute of Canada.

371.15 RELATIONSHIP BETWEEN BRAIN DOPAMINE LOSS AND D2 DOPAMINE RECEPTOR SUPERSENSITIVITY IN MPTP-INDUCED PARKINSONIAN MONKEYS. T. Di Paolo[1], P. Falardeau[1] and P.J. Bédard[2]. [1]School of Pharmacy, Laval Univ., Quebec, QC G1K 7P4 and Dept of Molecular Endocrinology, Laval Univ. Medical Center, Ste-Foy, QC G1V 4G2; [2] Dept. of Anatomy, Fac. Med., Laval Univ., Quebec, QC, G1K 7P4, Canada.

There is now much evidence that MPTP produces a severe parkinsonian syndrome. It is well known that this neurotoxin induces a loss of dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations in the caudate nucleus and putamen. However, data on brain DA receptors in MPTP-lesioned animals is still scarce. The present study seeks the relationship between DA, DOPAC or HVA concentrations and [^{3}H]spiperone binding sites in caudate nucleus, putamen and nucleus accumbens following DAergic lesion with MPTP in monkeys. Four ovariectomized monkeys (Macaca fascicularis) received intravenously MPTP in doses of 0.66 to 1.5 mg/kg whereas 3 were kept as controls. The animals were sacrificed one to five months after the injection of MPTP by an overdose of pentobarbital. The brains were immediately taken out, dissected and frozen. DA, DOPAC and HVA levels and [^{3}H]spiperone binding site densities (Bmax) and affinities (K_D) were determined. DA depletion varied from 37% to 100%. A mean elevation in [^{3}H]spiperone binding density of 114.81 ± 4.66% of control ($p < 0.01$) was observed when DA depletion was at least 90% of control values. No change in [^{3}H]spiperone binding dissociation constant was observed after MPTP treatment. We examined the correlation between DA, DOPAC and HVA concentrations and [^{3}H]spiperone binding. The best fit was a logarithmic correlation ($y = -7.185 \ln x + 111.148$; $R = -0.538$, $p < 0.001$) between DA concentrations (x) and the number of spiperone binding sites (y) in all structures. The correlation remained significant when structures were analysed separately (caudate nucleus: $R = -0.500$, $p < 0.025$; putamen: $R = -0.452$, $p < 0.05$; nucleus accumbens: $R = -0.856$, $p < 0.005$). The correlation between DOPAC or HVA concentrations (x) and [^{3}H]spiperone binding (y) were best represented by linear (DOPAC: $y = -0.734 x + 117.675$, $R = -0.607$, $p < 0.001$; HVA: $y = -0.531 x + 121.372$, $R = -0.540$, $p < 0.001$) rather than logarithmic equations (DOPAC: $y = -4.384 \ln x + 106.283$, $R = -0.333$, $p < 0.05$; HVA: $y = -0.601 \ln x + 133.940$, $R = -0.310$, $p < 0.05$). DA concentrations were found to be decreased more than HVA levels which may represent an increase neuronal activity and DA turnover of the remaining neurons after MPTP. The appearance of receptor supersensitivity after MPTP lesion where maximal decrease in DA is observed may reflect a second stage of compensation which becomes operative only when the number of remaining nerve fibers becomes too small to insure transmission. Supported by the Parkinson Foundation of Canada.

371.16 REGIONAL SENSITIVITY OF MESOLIMBIC AND STRIATAL D-2 RECEPTORS TO CHRONIC NEUROLEPTIC TREATMENT. A.M. Szczepanik*, C.A. Wilmot and D.B. Ellis*. (SPON: S.K. Puri). Dept. Biological Research, Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ 08876.

Many recent studies have shown that the striatum is a heterogeneous structure in terms of function, innervation and distribution of dopamine (D_2) receptors. In the present study, quantitative autoradiography was used to determine the effect of chronic neuroleptic treatment on the number and affinity of D_2 receptors in several regions of the striatum and the n. accumbens. Male Wistar rats (150-200 g) were treated daily with 1 mg/kg haloperidol, 20 mg/kg clozapine or vehicle for 21 days, n=6 per treatment group, and decapitated after a 24-hour washout period. Brain sections (20 microns, freeze-dried) were incubated with 0.2 - 1.4 nM [3]H-spiroperidol (77-85 Ci/mmole) in the presence or absence of 10 µM sulpiride, and subsequently apposed to tritium-sensitive film for 10-12 days. The autoradiograms were analyzed with a video camera-based image analysis system (RAS-R1000). Changes in receptor number or affinity in the n. accumbens and four regions of the striatum, i.e., rostral, medial, lateral and caudal portions, were determined by Scatchard analysis. Readings from the n. accumbens indicated a marked rostrocaudal gradient of D_2 specific binding, from 60% to <20% specific binding; therefore, readings from the rostral half with the higher density of D_2 receptors were used for Scatchard analysis. Results indicate differences in the sensitivity of striatal regions to D_2 receptor up-regulation following chronic dopamine antagonist treatment. Increases in receptor number occurred in the caudal (peripallidal) and lateral regions of the striatum and the n. accumbens, whereas no changes occurred in the rostral portion of the striatum.

371.17 DIFFERENTIAL EFFECT OF HIGH VS LOW DOSES OF CYCLO (LEU-GLY) ON DOPAMINE FUNCTION. N. Lee*, F. DeLeon-Jones*, C. Melchior, J. Fields, J. Lee* and R.F. Ritzmann. Olive View Medical Center, Sepulveda VAMC, UCLA, Los Angeles, Ca. 91342-1495., Univ of Il, Chicago 60612, Hines VAMC, Hines, Il 60141.

Pro-Leu-Gly-NH2(MIF), and its structural analogue cyclo (Leu-Gly) (cLG) alter both dopamine (DA) mediated behaviors and physiological responses. CLG also alters the specific binding of high affinity D-2 DA receptors in striatal tissue. One unusual property of cLG, MIF and related peptides is a bell shaped dose response curve. To date, however, no data exists relating to the mechanism of this phenomena. The purpose of this study was to examine the ability of both low and high doses of cLG to alter the DA system in the striatum by investigating the peptide's effect on a stereotypic response to apomorphine (APO) and on DA receptor binding after 3 different post-injection time intervals. Male, Sprague Dawley rats were divided into three groups: control, low and high doses. These groups received s.c. injection of water, 8mg/kg cLG, and 24mg/kg cLG respectively. Stereotypic behavior was rated at 1, 5 and 14 days after a single dose of cLG. Behaviors were elicited by administration of APO (0.5mg/kg i.p.) and observed between 10-12 a.m. Stereotypic responses were monitored every 5 minutes for 30 minutes after APO. A positive score was given for an amimal exhibiting continuous sniffing. Parallel groups of rats were used for DA binding. Animals were sacrificed and striata removed at 1, 5, and 14 days after cLG. Data from our stereotypy study suggest that after the low dose of cLG, animals showed increased stereotypic responses to APO at 1 and 5 days with this effect disappearing by 14 days. In the high dose group cLG produced a desensitization to APO induced behavior at 1 day but no changes in behavior at 5 or 14 days. Antagonist binding studies at 1 day showed no significant difference among Bmax values of the three groups (28, 34 & 38 fmol/mg). However cLG increased Bmax in the high dose group at 5 days compared to low dose and control groups. At 14 days the Bmax was increased for both low and high dose groups. The mean affinity values (29-57 pM) did not change as a result of cLG treatment.

These results suggest that: 1) at low doses cLG is a positive modulator of DA response whereas at the high dose this same peptide acts a negative modulator. Exact mechanism is unknown, however, one could speculate that cLG at the high dose acts as an antagonist at the same receptor or has an effect on another system other than DA which counterbalances the DA effect. 2) our study of D-2 DA receptors in the striatum shows that there is no correlation between antagonist binding and behavioral changes. Thus further studies of this receptor and behavior should be attempted with agonist binding.

371.18 CHRONIC AGONIST AND ANTAGONIST TREATMENT ALTERS DOPAMINE RECEPTOR LINKED ADENYLATE CYCLASE IN ANTERIOR AND NEUROINTERMEDIATE PITUITARY. B. Borgundvaag and S.R. George, Departments of Medicine and Pharmacology, Univ. of Toronto, Toronto, Ontario, M5S 1A8 Canada.

A number of cellular responses have been documented following chronic exposure of receptor systems to agonists and antagonists. Prolonged exposure to receptor stimulating agents has been reported to result in decreased numbers of receptors or down-regulation. Similarily, chronic exposure to antagonists has been reported to result in increased receptor numbers or up-regulation. Up- and down-regulation of receptor systems is not limited to changes in receptor numbers and include cellular responses with functional changes in post-receptor effector systems. In the present study we report the effect of chronic D2 dopamine receptor stimulation and blockade on the receptor-linked adenylate cyclase system in pituitary. Adult female Sprague-Dawley rats were injected with either bromocriptine (5 mg/kg/day, s.c.), haloperidol (2.5 mg/kg/day, s.c.) or vehicle, for 2, 9 or 14 days. Following treatment, animals were sacrificed by cervical dislocation, neurointermediate lobe (NIL) and anterior pituitary (AP) dissected and frozen at -70°C until use. Adenylate cyclase activity was determined by measuring the rate of conversion of [^{3}H]-ATP to [^{3}H]-cAMP. In both NIL and AP, treatment with bromocriptine resulted in a decreased ability of forskolin and guanine nucleotides to stimulate cAMP production. The Vmax for stimulation of adenylate cyclase activity at 10 uM forskolin was decreased following agonist treatment, and positively correlated with treatment duration. This effect was more pronounced at higher concentrations of forskolin, so that adenylate cyclase stimulation by maximal concentrations of forskolin was reduced by 30%. Guanine nucleotide activation of adenylate cyclase was also attenuated by bromocriptine treatment, with a 20% reduction of maximal stimulation. In anterior pituitary and neurointermediate lobe of vehicle-treated animals, n-propylnorapomorphine (NPA 50 nM) produced an average 18% inhibition of forskolin stimulated enzyme activity. Following bromocriptine administration, this was reduced to 5%, a reduction of 60%. Bromocriptine treatment did not affect the affinity of the enzyme for ATP with a Km of approximately 0.25 mM. Treatment with haloperidol resulted in slightly increased Vmax values for forskolin stimulated enzyme activity with no change in Km. The effect of NPA (50 nM) to inhibit forskolin-stimulated adenylate cyclase was enhanced in both AP and NIL following haloperidol treatment. These results suggest that chronic dopamine receptor activation or blockade result in alteration of the post-receptor mechanisms linked to both stimulation and attenuation of adenylate cyclase activity in AP and NIL.

372.1 EFFECT OF CHRONIC CAFFEINE ADMINISTRATION ON ADENOSINE A1, ADENOSINE A2 AND BENZODIAZEPINE RECEPTORS IN SPECIFIC AREAS OF THE RAT BRAIN. M. Dugich*, M. Hawkins*, N. Porter, and M. Radulovacki (SPON: R. M. Virus). Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago, IL 60612.

Caffeine competitively inhibits agonist binding to both adenosine (Ado) A1 and A2 receptors in the rat CNS. In addition, caffeine may also inhibit the binding of benzodiazepines (BDZ) and, to some extent, the behavioral effects associated with these compounds. Chronic treatment with Ado antagonists, such as caffeine, can alter the sensitivity of Ado receptors in the CNS, and various regions of the brain are differentially affected. Since caffeine may also affect Ado A2 and BDZ receptors, we examined the effects of chronic treatment with caffeine on these receptor populations in different regions of the rat brain.

Male Sprague-Dawley rats were treated with caffeine (75 mg/kg/day, i.p.) or saline (.9%) for 12 days. Twenty-four hr after the last injection rats were sacrificed by decapitation and the brains were removed. Scatchard analysis of [^{3}H]-R phenylisopropyl Ado ([^{3}H-R-PIA) and [^{3}H] flunitrazepam were performed in cortical, cerebellar, and hippocampal membranes. Estimates of Ado A1 and A2 receptor number in striatal membranes were obtained by examining the difference between saturating concentrations of 5'-N ethylcarboxamide Ado ([^{3}H]-NECA) and [^{3}H]-R-PIA binding (Yeung and Green, Naunyn-Schmiedebergs Arch. Pharmacol. 325:218, 1984). Ado A1 receptor binding was significantly increased in cortical membranes from rats treated with caffeine (14%, $p<.02$, Student's t-test) with no change in Kd (1.2 nM). No significant changes in ligand binding to Ado A2 or BDZ receptors were observed.

These results suggest that the chronic administration of high doses of caffeine to rats selectively affects only Ado A1 receptors without producing changes in Ado A2 or BDZ receptors. It appears, therefore, that Ado A1 receptors are susceptible to the antagonistic action of caffeine suggesting that blockade of these receptors serves as a biochemical substrate for the stimulant effects of caffeine. (Supported by FED-AFOSR grant 85-0349)

372.2 DESENSITIZATION OF FUNCTIONAL ADENOSINE RECEPTORS FOLLOWING CHRONIC ADMINISTRATION OF DIAZEPAM. M. Hawkins*, W. Pan*, P. Stefanovich* and M. Radulovacki. Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago, IL 60612.

Benzodiazepines (BZD) have been reported to inhibit the reuptake of adenosine (ADO) in the brain (Phillis et al., Brit. J. Pharmacol. 70:341, 1980). We have previously shown that chronic administration of diazepam decreases agonist radioligand binding to ADO A_1 and A_2 receptors in hippocampal and striatal membranes, respectively (Hawkins et al., Soc. Neurosci. Abstr., Vol. 12, Part 2, p 800, 1986). These effects are presumably due to an increase in synaptic ADO levels caused by diazepam. The aim of the present study was to determine if functional ADO receptors are affected by treatment with diazepam. For this purpose, we examined ADO-mediated adenylate cyclase activity and agonist radioligand binding following diazepam treatment. In addition, as ADO may modulate the sleep-wake cycle (Radulovacki et al., J. Pharm. Exp. Ther. 228:268, 1984), we also examined the effects of diazepam on sleep in rats.

Male Sprague-Dawley rats (300-350 gm) were implanted with subcutaneous ALZET mini-osmotic pumps containing diazepam (10 mg/kg for 7 days) and cortical electrodes for recording of the electroencephalogram (EEG). Polygraphic recordings were carried out during diazepam treatment and for the first 24h of withdrawal, following which the animals were sacrificed. ADO A_1 receptor binding was determined from Scatchard analysis of [^{3}H] R-PIA binding, while A_2 receptor binding was estimated by the method of Yeung and Green (Naunyn-Schmiedeberg's Arch. Pharmacol. 325:218, 1984) and by Scatchard analysis of [^{3}H] NECA and [^{3}H] R-PIA binding. BZD receptor binding was performed with [^{3}H]-flunitrazepam (Medina et al., Eur. J. Pharmacol. 90:125, 1983). Adenylate cyclase activity was determined by the conversion of [α-^{32}P] d-ATP to [^{32}P] d-cAMP as described by Cooper and Londos (J. Cyc. Nuc. Res. 5:289, 1979). Diazepam produced significant alterations in the EEG on the four stages of the sleep-wake cycle only during the first 12h of drug withdrawal. Agonist binding to ADO A_2 receptors was reduced by 25% as measured by Scatchard analysis and by 18% as measured by the [^{3}H] NECA minus [^{3}H] R-PIA assay (Yeung and Green, ibid). In addition, ADO stimulation of adenylate cyclase activity was significantly attenuated in striatal membranes from diazepam-treated rats. No changes in ADO A_1 or BZD receptor binding were observed in cortical or hippocampal membranes.

The present studies suggest that functional ADO receptors are desensitized following chronic treatment with diazepam. This conclusion is based on the observation that decreased agonist binding to A_2 receptors is associated with an attenuation in the ADO-stimulation of adenylate cyclase activity, an effect mediated for the A_2 receptor. (Supported by FED-AFOSR grant 85-0349)

372.3 OPPOSING ROLE FOR D-1 AND D-2 DOPAMINE RECEPTORS IN THE NEOSTRIATAL CONTENT OF CYCLIC GMP. W. C. Boyar, H. S. Kim, and C. A. Altar. Neuroscience Res., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901.

D-1 and D-2 receptors in striatal slices have been differentiated by their opposing effects on the production of cyclic AMP (Kebabian and Calne, Nature 77:93,1979) and the activity of adenylate cyclase (Onali et al., Eur. J. Pharmacol. 99:127,1984). D-1 and D-2 receptors have been differentiated in vivo by their actions on dopamine release (Boyar and Altar, J. Neurochem. 48:824,1987) and behaviorally by their effects on grooming (Molloy and Waddington, Psychopharm. 69:409,1984). We studied whether i.p. administration (mg/kg) of a D-1 (SKF 38393, 10-50) or D-2 (LY 171555, 0.25-1) agonist or a D-1 (SCH 23390, 0.02-2) or D-2 (sulpiride, 20-100; haloperidol, 0.3-0.5) antagonist could be distinguished by changes in the striatal content of cyclic GMP.

Male mice (Tac:SW) were injected with either the 0.9% saline vehicle (10 ml/kg) or a drug and killed by microwave irradiation at 30 min (or 2 hr for sulpiride and its vehicle). cGMP levels were measured in the striatum by radioimmunoassay (DuPont-NEN Corporation).

The D-1 antagonist SCH 23390 produced dose-dependent decreases in cGMP levels. Maximal suppressions of 28% were obtained with the 2 mg/kg dose. Conversely, the D-2 antagonists sulpiride and haloperidol increased striatal cGMP by up to 63% and 111%, respectively.

The D-1 agonist SKF 38393 produced dose-dependent increases in cGMP up to 90% at the 50 mg/kg dose. The D-2 agonist LY 171555 did not change cGMP levels.

In D1-D2 interaction studies, the 82% stimulation of cGMP by SKF 38393 (40 mg/kg) was blocked by SCH 23390 (0.3 mg/kg) and potentiated to 276% of control values by haloperidol (0.3 mg/kg).

To summarize, neostriatal cGMP levels are modulated in a manner similar to that for cAMP: D-1 receptor agonists stimulate production of cGMP, whereas D-2 receptors are inhibitory (antagonists increase cGMP) or inactive (agonists) for cGMP production. GTP (1 μM) stimulates adenylate cyclase (Nomura et al, Eur. J. Pharm. 106:437, 1984) and decreases the binding affinity of D-1 (Schulz et al, J. Neurochem. 45:1601, 1985) and D-2 (Creese et al, Mol. Pharm. 16:69, 1979) agonists. Thus, the changes in cGMP content reported here might contribute to functional interactions between D-1 and D-2 receptors via changes in GTP or cGMP-mediated responses.

372.4 α_2-ADRENERGIC RECEPTORS MEDIATE THE INHIBITION OF ISOPROTERENOL-STIMULATED CYCLIC AMP PRODUCTION IN STRIATAL AND CORTICAL NEURONS IN PRIMARY CULTURE. D.E. Kemp*, R.H. Lenox, J. Ellis and S. Weiss. Neuroscience Research Unit, Department of Psychiatry, University of Vermont College of Medicine, Burlington, VT 05405.

In slices of cerebral cortex, α_2-adrenergic agonists inhibit forskolin-stimulated cyclic AMP production. However, the same agonists lead to augmentation of the β-adrenergic stimulation of cyclic AMP production, a response that is not observed in other tissues. In this study, the actions of adrenergic agents on the intracellular production of cyclic AMP was examined in another CNS preparation, cortical and striatal neurons in primary culture, generated from the fetal mouse brain. We have previously demonstrated that these neuronal cultures display most of the properties of mature CNS neurons. Exposure of striatal neurons to increasing doses of the β-adrenergic agonist isoproterenol (ISO) resulted in a five-fold increase in intraneuronal cyclic AMP; half-maximal activation (EC_{50}) was achieved with 10nM ISO. Norepinephrine (NE; EC_{50}, 160nM) produced only a 3-fold increase in cyclic AMP levels. When cyclic AMP production was maximally activated with 10μM ISO, co-incubation with increasing concentrations of NE resulted in a dose-dependent attenuation of ISO-stimulated cyclic AMP levels. At 100μM NE, ISO-stimulated cyclic AMP production was inhibited by 40%; half-maximal inhibition was obtained with 56 nM NE. In the presence of the α_2-adrenergic antagonist yohimbine (10μM), cyclic AMP productions due to NE (100μM) or ISO (10μM) plus NE (100μM) were identical to ISO alone. The islet activating protein from Bordetella pertussis selectively ADP-ribosylates and inactivates N_i, the inhibitory guanine nucleotide regulatory protein of adenylate cyclase. When striatal or cortical neurons were exposed to pertussis toxin (100ng/ml) overnight, there was no detectable difference between ISO- and NE-stimulated cyclic AMP production. In fact, both agonists were able to stimulate cyclic AMP productions up to 8-fold; basal levels were unaffected. These data suggest that α_2-adrenergic receptors mediate the attenuation of β-adrenergic-stimulated cyclic AMP production in striatal and cortical neurons in primary culture, and do so via the inhibitory guanine nucleotide regulatory protein of adenylate cyclase.

Supported by grant PHS RO1 AG05214, NSF-VT EPSCoR Project 5 grant, and a grant from the Cummings Memorial Fund of the American Federation for Aging Research. S.W. and J.E. are recipients of a Medical Research Council of Canada Fellowship and a New Investigator Research Award (PHS R23 20740), respectively.

372.5 **INTERACTIONS BETWEEN DOPAMINE D1 AND OPIOID RECEPTORS IN THE RAT STRIATUM.** M. Parenti, L. Rusconi*, V. Cappabianca* and A. Groppetti*. Department of Pharmacology, University of Milan, Sch. of Medicine, Milan, Italy.

Presynaptic interactions between opioid and dopamine (DA) neurons are well documented. A close functional link between the two systems has been suggested to exist also postsinaptically in the rat striatum where opioid and DA-D1 receptors seem to modulate in a negative and a positive manner respectively the activity of a common enzyme, i.e. adenylate cyclase (AC). There are evidences suggesting that opiates and DA may regulate the same AC mojety by a receptor-receptor interaction. In this respect we have found that while the degeneration of striatal cell bodies by kainic acid totally abolishes both opioid-and DA-D1 receptor modulation of AC, the intrastriatal injection of a selective cholinotoxin, ethylcholine mustard aziridinium ion (AF64A) does not significantly alter both receptor activities excluding their presence on acetylcholine neurons. Moreover experimentally-induced changes in one receptor activity often results in compensatory alteration of the other. Intrastriatal pertussis toxin injection leads to a functional impairment of opioid receptor inhibition of AC through ADP-ribosylation of the inhibitory guanine nucleotide regulatory protein Gi and causes an increased stimulation of striatal AC by DA. A similar effect is induced by prolonged in vivo treatment of rats with morphine. Moreover the post-natal development of DA-and opioid-induced regulation of AC provides further support to the functional interdependence between the two receptor systems: AC is very sensitive to DA activation at birth and progressively decreases to about one third at adult age while opioid inhibition shows an opposite behaviour being almost absent at birth and maximal at adult age.

The above results therefore suggest that a reciprocal functional interaction between striatal DA-D1 and opioid receptors can serve as a mechanism to properly control AC activity. Disfunction of this system can be important in the etiopathogenesis of extrapyramidal diseases.

372.6 CYCLIC AMP ACCUMULATION SUPERSENSITIVITY TO THE ALPHA-1 AGONIST PHENYLEPHRINE (PHE) IN RAT HIPPOCAMPUS FOLLOWING LESION OF THE SEROTONERGIC MEDIAN RAPHE NUCLEUS (MRN). H. Ladinsky°, R. Vinci*, M. Parenti+, P. Cicioni* and S. Consolo*. Istituto di Ricerche Farmacologiche "Mario Negri", +Dipartimento di Farmacologia, Università di Milano and °Istituto De Angeli, Milan, Italy.

Serotonergic raphe deafferentation elicits an up regulation of a nM (^{3}H)WB-4101 binding site in rat hippocampus for which NE displays high affinity and prazosin low affinity. Guanine nucleotides affect the nM binding of hippocampus alpha-1 adrenergic receptors suggesting that these sites are agonist in nature. In the present study we determined whether the Gpp(NH)p-reduced "agonist binding" of (^{3}H)WB-4101 implicates a receptor-adenylate cyclase interaction in the rat hippocampus. Thus, the effect of specific alpha-1 agonist PHE on cAMP production in hippocampal slices of sham-operated and MRN-lesioned rats was examined. The concentration-response curve of PHE-induced cAMP accumulation in hippocampal slices on the MRN lesioned group was shifted strongly to the left with the peak increase being reached at the concentration 200 times lower (0.5 uM) than that required to raise cAMP formation to the same extent in slices of the sham-operated group. cAMP accumulation supersensitivity began 8 days (35%) and reached a plateau (50%) at 21 days postlesion. This effect was direct as it was found also in crude homogenate preparations. In addition, the increase, and the decrease, in cAMP generated by isoproterenol and acetylcholine was not affected by MRN lesion. The alpha-1 antagonist WB-4101 was the most potent inhibitor of the PHE-induced increase in cAMP formation (IC_{50} = 1.2 x 10^{-7} M), 100-fold lower than that of prazosin, (IC_{50} = 1.4 x 1^{-5} M); yohimbine and propranolol, alpha-2 and ß-antagonists, respectively, inhibited only at high doses. 5-HT (10 uM) induced a marked rightward shift of the concentration-response of PHE-induced accumulation without exerting any effect of its own on the cAMP basal level of MRN lesioned rats. It is concluded that alpha-1 receptor activation affects hippocampal adenylate cyclase activity either directly or indirectly and this interaction is negatively modulated by the 5-HT system.

372.7 NORADRENERGIC POTENTIATION OF CEREBELLAR PURKINJE CELL RESPONSES TO GABA: EVIDENCE FOR MEDIATION BY AN INTRACELLULAR SECOND MESSENGER SYSTEM. B.D. Waterhouse, F.M. Sessler, J.-T. Cheng and H.H. Yeh. Dept. of Physiol. and Biophys., Hahnemann Univ. Phila. PA 19102 and Dept. of Neurobiol. and Anat., Univ. of Rochester Med. Ctr., Rochester, NY 14642.

Previous in vivo studies from our laboratory have consistently shown that iontophoretically applied norepinephrine (NE) can potentiate GABA-induced depressant responses of cerebrocortical, cerebellar and hypothalamic neurons. Additional experiments employing amino acid analogs and a variety of adrenergic agonists and antagonists have further suggested that this noradrenergic facilitating action is specific for GABA and results from the activation of a beta type adrenoceptor. The goal of the present studies was to determine if the cyclic AMP second messenger system might also be a component of the mechanism responsible for this NE modulatory action on GABA responses. In one set of in vivo studies, extracellularly recorded responses of individual cerebellar Purkinje (P) cells to iontophoretic pulses (5-55nA, 10 sec duration) of GABA or beta-alanine were examined before, during and after 8-bromo- 3', 5' cyclic AMP (Brom-cAMP) microiontophoresis. A second group of in vitro experiments examined P-cell responses to GABA (5-50 nA.) before, during and after NE iontophoresis or bath application of forskolin (10-30µM). Peri-event histograms which summed unit activity during amino acid applications were quantitatively evaluated to assess the effect of NE, cyclic AMP and forskolin on GABA-induced inhibition. In the intact rat brain, iontophoretic administration of Brom-cAMP caused a marked NE-like augmentation of P-cell responses to GABA in 13 of 17 (76%) cells tested. As with NE, Brom-cAMP was ineffective in enhancing P-cell inhibitory responses to beta-alanine, an agent which like GABA causes hyperpolarization, by increasing Cl^- conductance. In 11 of 17 (65%) neurons tested in vitro, iontophoretically applied NE markedly enhanced P-cell responses to GABA in a manner similar to that observed previously in vivo. Bath application of forskolin was also capable of potentiating GABA-induced inhibition in each of 3 cases tested. In summary, these results indicate that a membrane permeable analog of cyclic AMP and an agent which directly activates adenyl cyclase can mimic the previously observed GABA-potentiating actions of NE. Thus, these findings provide further support for the contention that noradrenergic enhancement of GABA inhibition results from a cascade of transmembrane events which includes beta receptor activation, adenyl cyclase stimulation and increased intracellular production of cAMP. (Supported by AFOSR-85-0155 and NS 18081 to B.D.W.)

372.8 CYCLIC GMP ANALOGUES INCREASE THE EXCITABILITY OF CEREBELLAR PURKINJE NEURONS IN CULTURE. T. Jacquin*, C.P. Crimi* and D.L. Gruol, Div. Preclin. Neurosci., Scripps Clinic and Res. Foundation, La Jolla, CA 92037.

Cerebellar Purkinje neurons (PNs) express high concentrations of cGMP dependent protein kinase which is thought to play an important role in neuronal physiology (Schlichter et al, PNAS, 1980). As a first step toward defining the functional role of this cyclic nucleotide in the PN, we have tested the effect of membrane permeable analogues (50-500 µM) on the spontaneous activity and membrane properties of PNs in a model system: modified organotypic cultures derived from rat cerebellar cortex. The culture system and the morphological and physiological properties of the cultured neurons have been described (Gruol, Brain Res. 263, 1983). In the first series of experiments, extracellular recording techniques were used so that the intracellular environment was not disturbed. Firing rate and pattern were quantitated by computer. In the majority of PNs studied with this technique (N=22), cGMP analogues applied to the recording medium (several analogues tested) increased the spontaneous firing rate and regularized the patterns of activity. Mean firing rates were 10.8±1.1 (±SEM) under control conditions and 12.3±1.2 in the presence of cGMP, which were significantly different at the $P<0.0005$ level (Paired-sample t-test). In five cells, the bursting component of the control activity patterns was enhanced. Mean values for % burst were 17±6 for control and 40±3 in the presence of cGMP. Similar results were observed in intracellular recordings using whole cell methods. The main effect of cGMP on the spontaneous activity recorded intracellularly was an increase in the frequency of burst discharges and synaptic events. The most pronounced effect of cGMP on membrane properties was an enhancement of excitability, as demonstrated by an increase in the number of spikes evoked by depolarizing current pulses or synaptic events. In TTX treated preparations (n=4) the anode break Ca++ spike was prolonged by cGMP. The effect of cGMP analogues on resting membrane potential and the voltage response evoked by hyperpolarizing current pulses was variable. Resting membrane potential was hyperpolarized (3-15mV) in 5 cells, depolarized (5mV) in 1 cell and unchanged in 8 cells. Input resistance measured with hyperpolarizing current pulses was decreased in 5 cells, increased in 2 cells and unchanged in 7 cells. These data demonstrate that the excitability of PNs can be altered by cGMP analogues and support a physiological role for cGMP and its kinase in the PN. The fact that several actions of the cGMP analogues were observed suggests that cGMP may have more than one site of action in the PNs. (Supported by NINCDS Grant NS21777 to DLG and a grant from the Fondation de l'Industrie Pharmaceutique pour la Recherche to T.J.)

372.9 FORSKOLIN INCREASES THE EFFICIENCY OF ELECTRICAL STIMULATION OF VASOPRESSIN RELEASE FROM RAT NEUROHYPOPHYSIS IN VITRO

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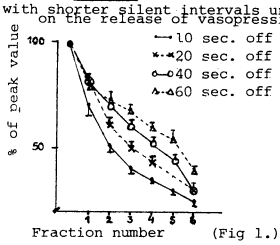
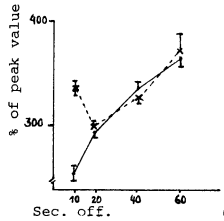
Electrical stimulation of rat neurointermediate lobe (NIL) in vitro characteristically results in an abrupt increase in the rate of vasopressin release above the pre-stimulation level, followed by a decline despite continuing stimulation. We have studied the rate of decline of vasopressin release during 40 min electrical stimulation as a function of the length of silent intervals between pulse trains. Pulses (0.2 msec/pulse, 16 Hz) were delivered as 20 sec trains with silent intervals varied from 10-60 sec between individual NILs. The perifusing medium was collected in fractions every 5 min and its vasopressin content measured by RIA. Release in the fractions following the peak was expressed relative to the peak value. Fig.1. shows that the decline in the rate of release was steepest with 10 sec silent intervals. Forskolin 10 uM added throughout the stimulation period increased the content of cAMP from 0.2 to 8.1±0.5 (SEM, N=4) pmoles/NIL. In the presence of forskolin the decline in the rate of vasopressin release was slower with silent intervals of 10 sec, but not of 20, 40 or 60 sec. Fig.2 shows the cumulative release (% of the peak) for different lengths of silent intervals, in the presence (×···×) or absence (●——●) of forskolin 10 uM. Thus, the efficiency of electrical stimulation (vasopressin released/single pulse) with 10 sec intervals was increased by 32 % in the presence of forskolin. In rats in vivo, during dehydration an increasing number of vasopressin neurons in supraoptic nucleus fire in bursts with inter-burst periods from 4 to 200 sec and the frequency of shorter periods increasing with the severity of osmotic stress. Increase in the efficiency of electrical stimulation with short silent intervals in vitro due to forskolin might reflect the presence of a cAMP-dependent mechanism in vivo counteracting the diminished efficiency of firing with shorter silent intervals under the conditions of high demand on the release of vasopressin.



(Fig 1.)



(Fig 2.)

372.10 **INTRAHIPPOCAMPAL DISTRIBUTION OF FORSKOLIN AND CALMODULIN SENSITIVE ADENYLATE CYCLASE ACTIVITY IN MALE RATS.**

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We investigated the intrahippocampal distribution of basal, forskolin (F) and calmodulin-sensitive (CaM) adenylate cyclase (AC) activity in membrane preparations to determine the relationship of these biochemical measures to the localization of F-binding site(s) (Worley, P.S. et al. Proc.Natl.Acad.Sci. 83:4053, 1986). AC activity was measured, in membranes prepared in either sucrose (SB, 300 mM) or imidazole (IB, 10 mM) buffers containing 2 mM EGTA and 5 mM EDTA, pH 7.4, by the conversion of ^{32}P-ATP to ^{32}P-cAMP (Salomon, Y. et al. Anal.Biochem.58:511, 1974).

Both basal and F-stimulated (10 uM) AC activity were higher in dentate gyrus (DG) than in either ammon's horn (AH) or whole hippocampal (H) membranes prepared in SB (basal = 157±30, 68±15 and 89±23; 10 uM F = 712±80, 429±99 and 295±58 pmoles cAMP/mg/min^{-1} respectively, n ⩾ 4). The difference between the response to F in DG and AH was due to an increased V_{max}(898±135 vs. 659±132 pmoles cAMP/mg/min^{-1}, n = 4, $p < 0.05$, paired t-test), and not a difference in the EC_{50} (2.26±0.35 vs. 4.92±2.66 uM, n = 4). The differential response to F (10 uM) was maintained along the dorsal-ventral axis of the hippocampus. No dorsal-ventral differences were noted in the F (10 uM) response in either DG or AH. Omission of GTP (10 uM) from the incubation medium had no effect on either basal or F concentration-response curves in DG or AH, although GTP increased hippocampal F-binding sites.

Addition of both Ca^{2+} (250 uM) and CaM (50 U/125 ul) was required to stimulate AC activity in H membranes prepared in IB (basal = 77±15 Ca^{2+}/CaM = 176±16 pmoles cAMP/mg/min^{-1}, n = 4). Ca^{2+}/CaM AC activity was significantly higher in DG than AH (248±50 vs. 106±21 pmoles cAMP/mg/min^{-1} respectively, n = 3, $p < 0.05$ paired t-test). A similar difference in basal activity was also noted (92±9 vs. 57±7 pmoles cAMP/mg/min^{-1} respectively, n = 3). In preliminary experiments (n = 2), addition of F (10 uM) had no effect on the AC response to Ca^{2+}/CaM in DG and less than additive effects in AH, suggesting that part of the AC response to Ca^{2+}/CaM and F may occur through a common limiting mechanism.

In general, our biochemical measurements of F-sensitive AC activity parallel the intrahippocampal distribution of F-binding sites, but the relationship(s) between GTP, Ca^{2+} and CaM effects on F-binding and AC response remain to be clarified. Stimulation of AC in DG granule cells has been implicated in long term potentiation, thus in vitro measurements of AC activity may add to our understanding of cAMP involvement in memory processes.

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372.11 NEURAL REGULATION OF ADENYLATE CYCLASE IN RAT BROWN ADIPOSE TISSUE. J.G. Granneman, Center for Cell Biology, Sinai Research Institute, Detroit, Mi 48235.

Many tissues decrease their responsiveness after exposure to increased levels of neural stimulation. In contrast, we have reported that cold exposure, which increased sympathetic nerve stimulation of brown adipose tissue (BAT) in rats, increased the responsiveness of adenylate cyclase to norepinephrine and fluoride. Furthermore, the sensitization of adenylate cyclase is prevented by surgical denervation, indicating this effect is in fact produced by neural stimulation.

We are examining the molecular basis of the increase in adenylate cyclase responsiveness and have found that exposing rats to 4°C for 3 days increased by 2-3 times the maximum velocity of adenylate cyclase activity to stimulation by guanylyl-5'-imidodiphosphate without altering the apparent affinity of the enzyme for the nucleotide. In addition, cold exposure increased maximal extent of cholera toxin-catalyzed [32]P-ADP ribosylation of the alpha subunit of the stimulatory regulatory protein of adenylate cyclase (G_s) in BAT membranes by 2-3 times but did not alter pertussis toxin labelling of the inhibitory regulatory protein. These results suggest that membranes of cold-exposed rats contain more functional G_s protein. However, direct immunological detection of G_s by Western blotting demonstrated that the total amount of G_s present in control and sensitized membranes is similar. Taken together, these results indicate that although control and sensitized membranes contain similar amounts of G_s, only a fraction of the total G_s present in control membranes is functional. Neural stimulation of BAT appears to increase by 2-3 times the fraction of existing G_s molecules that can stimulate cyclic AMP formation and serve as a substrate for cholera toxin. The exact mechanism by which neural stimulation increases the function of existing G_s molecules is not currently known, but may involve the interaction of G_s with membrane components that are unique to sensitized membranes. (Supported by NIH grant AM 37006).

372.12 NEUROMODULATION OF TYPE II CYCLIC AMP-DEPENDENT PROTEIN KINASE.

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The type II regulatory subunit of cyclic AMP-dependent protein kinase (R-II) is autophosphorylated through an intramolecular reaction with the catalytic subunit, but the physiological significance of this reaction is not fully understood. We have reported that the in vitro ^{32}P-autophosphorylation of R-II is increased in cytosolic fractions from denervated (DEN) solei compared to contralateral, control (CON) muscles of the rat. Also, the early onset (3 hr) and progression of this phosphorylative modulation was directly related to the denervation period and the length of the distal nerve stump. Therefore, this alteration is not solely due to the cessation of the impulse-directed release of acetylcholine with transection of the sciatic nerve.

Now, we show that the ratio of (+P)R-II (phosphorylated R-II) to (-P)R-II (dephosphorylated R-II) in vivo is markedly higher in DEN compared to CON solei. A rapid and direct experimental approach with precautions to prevent alterations during sample preparations is used to assess the relative content of (+P)R-II and (-P)R-II in vivo.. Solei are clamp-frozen in situ and put in liquid nitrogen upon removal, DEN (by cutting the nerve) and CON solei are kept paired during sample preparation, the muscles are rapidly homogenized with 10 mM potassium phosphate buffer pH 6.8, 1 mM EDTA, 1 mM 2-mercaptoethanol and 20 mM benzamidine to inhibit protein kinase, protein phosphatase and protease activities, and muscle cytosol (105,000g supernatant) is immediately treated in 2% sodium dodecyl sulfate (SDS) and 5% mercaptoethanol in a boiling water bath for 2 minutes. These samples are kept frozen until resolution of cytosol proteins by SDS-gel (7.5%) electrophoresis (SDS-PAGE) in the Laemmli system.

Afer SDS-PAGE and electrotransfer of the separated proteins to nitrocellulose sheets, (+P)R-II and (-P)R-II are visualized by autoradiography after immunodetection with rabbit anti-rat skeletal muscle R-II serum as the primary probe and either radioiodinated goat-anti-rabbit IgG or protein A as the secondary probes. The relative content of R-II isoforms is evaluated by soft laser densitometry of the autoradiographs.

The electrophoretic mobility of rat solei (+P)R-II is less than that of (-P)R-II to yield a difference of about 2,000 in their apparent molecular weights. When muscle cytosols are incubated with 1 mM ATP and 15 mM Mg or with 10 mM cyclic AMP before SDS-PAGE, then the mobility shift upon phosphorylation and dephosphorylation of R-II can be seen. Tissue and species differences in the occurrence of this phenomenon are well-recognized. The phosphorylation shift of rat solei R-II provides a direct experimental approach for evaluation of denervation-induced changes in the relative content of (+P)R-II and (-P)R-II.

Supported by the VA Medical Research Service.

372.13 cGMP PRODUCING NEURONAL NETWORKS IN THE HIPPOCAMPUS: A COMPARISON OF THE EFFECTS OF NITROPRUSSIDE AND POTASSIUM ON THE PRODUCTION OF cGMP IN RAT HIPPOCAMPAL SLICES INCUBATED IN VITRO AS REVEALED BY IMMUNOCYTOCHEMISTRY. J. de Vente±, J. Schipper±, L.Hudson,± and H.W.M. Steinbusch (SPON: W.J. Smeets). Dept. of Pharmacology, Vrije Universiteit, Medical Faculty, 1081 BT AMSTERDAM, The Netherlands.

Biochemical determination of cGMP revealed the presence of this cyclic nucleotide throughout the mammalian brain. The exact regional localization together with the differences between cellular concentrations in various cell types and the subcellular distribution of cGMP is, with a few exceptions, not known. Recently we described the use of a new cGMP antibody especially designed for application in immunocytochemistry (De Vente et al., Neuroscience, in press, 1987). This antibody was used in an in vitro study on the distribution of cGMP in rat hippocampal slices.

In this study male rats (180-220 g) were decapitated and the brains quickly removed. Hippocampal slices of either 100 or 300 um were prepared at 0°C in a normal isotonic salt solution. Slices were incubated for 30-60 min at 37°C and pH 7.4 in an atmosphere of 5%CO_2/95%O_2, and subsequently incubated in the presence of several pharmacological agents. The incubation was terminated either by adding fixative for immunocytochemical processing or by the addition of TCA for biochemical assay. cGMP was visualized in the 100 um slices using the newly developed antibody by a peroxidase-antiperoxidase procedure with a DAB/Ni intensification. The 300 slices were frozen and 10 um cryostat sections were collected on slides and processed for cGMP-immunoreactivity using a FITC procedure. Both immunocytochemical methods gave identical results. The staining was specific for cGMP as judged by conventional immunocytochemical controls.
At basal levels a weak cGMP staining was associated with blood vessels and cell bodies in the pyramidal layer of the hippocampus. In the presence of IBMX, (0.1-1.0 mM) cGMP immunostaining increased in blood vessels and cell bodies, whereas an occasional nerve fiber was observed. Sodium nitroprusside (10 uM) alone did not appreciable increase cGMP, but in combination with IBMX, theophylline or zaprinast (M&B 22.948) an intense staining of the blood vessel walls and a dense neuronal network throughout the hippocampus was observed. Treatment with K^+ (20 mM, isotonic replacement of Na^+) showed a circumscribed cGMP-immunoreactive neuronal network, principally located in or around the stratum lacunosum moleculare; this K^+ induced cGMP elevation was absolutely dependent on extracellular Ca^{2+}, whereas the cGMP response induced by the phosphodiesterase inhibitors or sodium nitroprusside was not. At an overall level the biochemical determination of cGMP in the hippocampal slices confirmed the immunocytochemical results. In conclusion, we found a widely distributed cGMP producing neuronal network which is regulated by at least two independent mechanisms.

372.14 TEMPORAL SPECIFICITY IN THE RESPONSE OF BRAIN ADENYLATE CYCLASE TO TRANSIENT STIMULI. Y. Yovell* and Y. Dudai. (SPON: V. Teichberg). Department of Neurobiology, Weizmann Institute, Rehovot 76100, Israel.

Studies in Aplysia and Drosophila have implicated adenylate cyclase in learning and short-term memory. One suggestion is that adenylate cyclase may serve as a molecular convergence locus for signals mediating conditioned stimuli (CS) and unconditioned stimuli (US) during associative learning (Eliot et al., Soc. Neur. Abs. 12: 400, 1986). This suggestion raises the hypothesis that adenylate cyclase may display temporally specific responses to converging stimuli, e.g., transmitter, G-activators or ions; and that such responses may underlie some elementary behavioral constraints of classical conditioning, e.g., the necessity for temporal pairing of CS and US. Until recently, direct probing of adenylate cyclase responses to transient and interacting stimuli was not feasible. We have recently developed a novel in vitro perfusion methodology for this purpose (Y. Yovell, Y. Dudai, E. Kandel and T. Abrams, in preparation). In this system, a synaptosomal membrane preparation is embedded on a filter in a small reaction chamber. The chamber is connected via a rotary valve to reservoirs containing assay solutions, from which it is continuously perfused. The perfusate is sequentially aliquoted and assayed for production of radiolabeled cAMP. A programmable pulse generator controls valve positions. Switching between reservoirs containing cyclase activators enables presentation of stimuli in a paired/unpaired manner. We have now employed this methodology to probe some temporal integrative properties of adenylate cyclase in a synaptosomal preparation from rat cerebral cortex. The response of adenylate cyclase to combinations of transients of Mg^{2+} and the GTP analog GTPγS, or to GTPγS and isoproterenol transients, revealed a temporally specific pattern: Paired presentations of GTPγS and Mg^{2+} transients resulted in greater cyclase activation then unpaired presentation. Conversely, paired presentation of isoproterenol and GTPγS transients yielded lower activation then their unpaired presentation. Latent "memory" of previous exposure to GTPγS was revealed by subsequent brief exposures to Mg^{2+} and transmitter analog; the latter, when following a GTPγS transient, caused additional persistent activation. Latent "memory" of previous exposure to transmitter analog was also revealed. In contrast, the cyclase complex did not exhibit "memory" of previous Mg^{2+} exposures. Although two of the stimuli used in these studies are non-physiological, they partially simulate properties of receptor and G-unit activation. We propose that asymmetric temporal integration of transient stimuli by the cyclase complex may play a role in modulation of synaptic plasticity, e.g. during elementary associative learning. (Supported in part by the US-Israel Binational Science Foundation, Jerusalem).

PRESYNAPTIC MECHANISMS IV

373.1 NON-QUANTAL RELEASE OF TRANSMITTER AT DEVELOPING NEUROMUSCULAR JUNCTIONS OF XENOPUS IN CULTURE. A.D. Grinnell, and S.H. Young. Jerry Lewis Neuromuscular Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Non-quantal, or 'leak' release of transmitter at the neuromuscular junction has been described in frog (Katz and Miledi, Proc.R.Soc.Lond. 196:59, 1977), mouse (Vyskocil and Illes, Pflugers Arch. 370:295, 1977), and Xenopus cells in culture (Sun and Poo, J.Neurosci. 5:64, 1985). In these studies, the presence of 'leak' release was assayed by slight hyperpolarizations of the muscle membrane potential after application of acetylcholine (ACh) receptor channel blockers to the junctions. Since the time course of the control-block-recovery cycles were long, detailed information on the temporal characteristics of the 'leak' have not been available. In our study, we have taken advantage of the high resting input impedence of Xenopus muscle cells (100 MΩ) in combination with the whole cell patch clamp technique to measure the 'leak' currents flowing across cell membranes of nerve contacted muscle cells in 1-day and 3-day old nerve-muscle cultures. The background current noise can be small enough (≤ 1 open ACh channel at -80 mV holding potential) so that 'leak' release can be measured directly and continuously, i.e., without addition of blockers. With these conditions, 'leak' release is seen to occur in episodes of current fluctuations separated by periods of quiet baselines (interrupted only by miniature endplate currents, MEPCs). As expected, application of α-bungarotoxin (20 μg/ml) to the bath blocks these episodic current fluctuations, leaving only the stable baselines. 'Leak' release is not often observed at very young (1-day culture) nerve-muscle contacts, occuring in only 1 out of 8 contacts. In continuing experiments with older (3-day) cultures, this fraction increases to 37% of contacts which show 'leak'. The leak does not occur continuously, but in episodes at an average rate of 0.75/min. Average episode duration is 1.2 sec. The frequency of MEPCs does not change during an episode of 'leak'. At one synapse, MEPC frequency was 1.50, 1.50, and 1.52 MEPCs/sec before, during, and after a large duration 'leak' episode.
Supported by NSF grant BNS 85-1079, and by grants from the Muscular Dystrophy Association.

373.2 QUANTAL RELEASE OF NEUROTRANSMITTER IS NOT ASSOCIATED WITH THE OPENING OF LARGE SINGLE CHANNELS ON THE NEURONAL MEMBRANE. S.H.Young,I.Chow,and A.D.Grinnell. Jerry Lewis Neuromuscular Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

In recent years, some investigators have challenged the traditional view that quantal release of neurotransmitter occurs through fusion of transmitter-containing vesicles with the neuronal plasma membrane. A common element of this challenge is the involvement of cytoplasmic transmitter in the quantal release process, although specific mechanisms for this proposed form of release are varied. We have examined one such mechanism-- that cytoplasmic acetylcholine (ACh) could be released through large channels in the neuronal plasma membrane in order to produce miniature endplate potentials (MEPPs). We see no evidence for such channels. Chow and Poo (J.Neurosci. 5:1076, 1985) have shown using cultured nerve and muscle cells of Xenopus that when a muscle cell is placed into contact with the soma of a neuron, MEPP-like depolarizations are seen in the muscle cell within 5 min. of contact (57% of contacts). In our experiments, a muscle cell was impaled with a microelectrode and placed into contact with soma of an isolated neuron with short (≤30μm) neurites not in contact with the muscle cell membrane. After the appearance of MEPPs, the soma was held under voltage clamp with a whole-cell patch clamp. The high resting membrane resistance of the soma (>200MΩ) provides high resolution recording of neuronal membrane currents (resolution of 2 pA and 1 ms), which are recorded simultaneously with muscle membrane potential. Neuronal currents from 8 nerve-muscle contacts which produced over 1,000 MEPPs were examined before, during, and immediately after each MEPP. No neuronal channels were observed in association with the MEPPs. Consideration of the size of ACh (M.W. 146), makes it seem unlikely that we would not detect the opening of a channel large enough to pass this cation, making it unlikely that such channels are involved in the production of MEPPs. Supported by NSF grants BNS 85-1079, BNS 84-19893 and by grants from the Muscular Dystrophy Association.

373.3 VOLTAGE/TIME DEPENDENCE OF K^+-INDUCED EXOCYTOSIS OF TRANSMITTER. C. Haimann*, R. Fesce*, F. Grohovaz* and B. Ceccarelli* (SPON: European Neuroscience Association). - CNR Ctr Cytopharmacol. - Ctr. Periph. Neuropath. - Dept. Pharmacol. - Un. Milano - Italy - 20129.

At the frog neuromuscular junction, exocytosis of quanta of ACh generally occurs near specialized structures called active zones. After 15 min in 20 mM K^+, however, vesicle fusions are observed all over the presynaptic membrane. This might reflect late onset of endocytosis, or the activation of latent fusion sites either by the prolonged depolarization or by some specific effects of potassium (J. Cell Biol. 81:178-192, 1979).

We have studied the changes in the distribution of vesicle fusion images on freeze-fractured presynaptic membranes from junctions fixed in 20 mM K^+ either 0, 1 or 5 min after exposure to 20 mM K^+. These changes were correlated with the rate of quantal secretion, measured by fluctuation analysis (J. Gen Physiol. 88: 25-57,1986) of intracellular recordings before and during fixation.

Mepp rate increased and began to decline from 30 to 60 sec after the addition of the fixative solution, until mepps disappeared within about 1½ min. During the decline (which might correspond to the fixation time) totals of 15,000, 20,000 and 30,000 quanta were secreted at 0, 1 and 5 min. Replicas from these junctions showed densities of 4.7, 3.3 and 4.0 fusions/μm^2, respectively. Almost all the fusions (96%) were located near the active zones at 0 min, 54% at 1 min, and only 26% at 5 min (i.e. fusions were distributed almost uniformly on the presynaptic membrane).

In spite of the changes in distribution, the densities of fusion images were similar at 0, 1 and 5 min. Since the quanta secreted did not decline with time it is unlikely that an important contribution to the number of fusions at late times arose from the onset of an endocytotic process absent at early times. Furthermore, asynchronous quantal secretion induced by hypertonic solution (150 mM sucrose) was equally intense and sustained, but fusions were predominantly located near the active zones at 5 as well as 15 min.

In preparations exposed to 20 mM K^+ for 5 min in the absence of Ca^{2+} and for 30 more sec in the presence of Ca^{2+} before fixation, fusions were uniformly distributed on the presynaptic membrane. This indicates that K^+ activates an additional population of sites for exocytosis through the prolonged depolarization.

The occurrence of fusions over the whole membrane is accompanied by a decreased density near the active zones. Such a decrease does not occur during prolonged electrical stimulation, suggesting that K^+-induced secretion undergoes partial inactivation. Preliminary data on the time course of mepp rates observed at the same junction with different concentrations of K^+ support this interpretation.

The changes in fusion localization might reflect different voltage/time dependences of two population of fusion sites (calcium channels ?). *partially supported by MDA grant, B.C.*

373.4 EFFECT OF INTRACELLULAR SODIUM ON STIMULATION-INDUCED CHANGES IN NEUROTRANSMITTER RELEASE. D.R. Mosier* and J.E. Zengel. Depts. of Neuroscience and Neurosurgery, Univ. of Fla. College of Medicine and Veterans Administration Medical Center, Gainesville, FL 32610.

Repetitive stimulation of presynaptic nerve terminals often leads to an increase in the amount of neurotransmitter released by a nerve impulse. A number of investigators have proposed that this increase in release results from an accumulation of intracellular Ca^{++}. It has also been suggested that Na^+ may play a role in stimulation-induced increases in release, either by a direct effect on release or indirectly by affecting intracellular $[Ca^{++}]$.

Previous studies in our laboratory have shown that reductions in extracellular Na^+ concentration not only elevate transmitter release, but also selectively enhance an early component of the increase in transmitter release which occurs during repetitive stimulation. To further investigate the role of sodium in stimulation-induced increases in release, we have examined the effects of agents known to alter intracellular $[Na^+]$.

End-plate potentials (EPPs) were recorded from frog (Rana pipiens) sartorius muscles under conditions of reduced quantal release (0.4-0.7 mM Ca^{++}, 5 mM Mg^{++}). The muscle nerve was conditioned with trains of 10 impulses at 20 impulses/sec. Changes in EPP amplitude were expressed as V(t), the fractional increase over the control EPP amplitude, such that

$$V(t) = (EPP_t/EPP_c) - 1$$

where EPP_c is the amplitude of the first EPP of the train and EPP_t is the EPP amplitude at time t during the train.

Addition to the bathing solution of 0.5-4 µM monensin, an ionophore known to raise intracellular $[Na^+]$, led to substantial increases in V(t) in five of ten preparations tested. The effect of monensin appeared to be primarily on an early component of increased release, similar to the effect of reduction in extracellular $[Na^+]$ described previously. In the remaining five preparations, either no change or a small decrease in V(t) was observed. No consistent effects were observed on control levels of transmitter release.

Another way to increase intracellular $[Na^+]$ is to inhibit the plasmalemmal Na/K ATPase with the cardiac glycoside ouabain. Although addition of 3-20 µM ouabain led to large increases in control EPP amplitude in eight of nine preparations, we observed no consistent changes in V(t). In most experiments, the effects of ouabain were irreversible and led eventually to failure of release.

The results of the monensin experiments support the suggestion that intracellular $[Na^+]$ may be involved in one or more processes underlying changes in transmitter release with repetitive stimulation. Although the results of the ouabain experiments appear to be inconsistent with this hypothesis, it is possible that additional effects of ouabain, e.g., on the nerve terminal action potential, may be acting to obscure the effect of increased intracellular $[Na^+]$ on stimulation-induced increases in release.

373.5 ADENOSINE IS AN ENDOGENOUS MODULATOR OF STIMULATION-INDUCED DEPRESSION AT THE FROG NEUROMUSCULAR JUNCTION. Stephen D. Meriney and Alan D. Grinnell. Jerry Lewis Neuromuscular Research Center, UCLA, CA 90024.

Adenosine has been shown to inhibit transmitter release (Ginsborg and Hirst, J. Physiol. 224:629 (1972)). ATP (co-released with ACh) is hydrolyzed by enzymes present in the junctional region, and adenosine becomes available for modulation of subsequent release from the presynaptic terminal (Rebeiro and Sebastiao, J. Physiol. 384:571 (1987)).

We have investigated the effect of adenosine in the context of stimulation-induced depression of neuromuscular transmission. Classically, depression has been attributed entirely to depletion of ACh from the presynaptic terminal. Identified terminals (visualized with peanut lectin-FITC) were studied in partially curarized cutaneous pectoris muscles of Rana pipiens by intracellular techniques. Following physiological characterization, the lengths of these junctions were quantitated by camera-lucida drawings of NBT-stained terminals.

The depression of transmitter release during 20 Hz nerve stimulation is closely correlated with the amount of ACh released/unit length. Strong terminals can depress by as much as 80% while weak ones frequently maintain facilitation during a 30 second tetanus. The effect of tetanic stimulation on endplate potential size is predominately a balance between facilitative effects and depression due to depletion of ACh. However, tetanic stimulation in the presence of adenosine deaminase (which hydrolyzes endogenous adenosine) can reduce depression by as much as 50%, often resulting in significant facilitation. Sensitivity to adenosine deaminase is correlated with release/unit length such that weak junctions are influenced to a greater extent than strong ones. It is not surprising that modulation by endogenous adenosine represents a larger percentage of stimulation-induced depression at weaker junctions, since these would not be expected to have significant depletion of ACh. A large amount of depletion-mediated depression at strong junctions may mask the effects of endogenous adenosine. We conclude therefore, that adenosine is a potent endogenous modulator of synaptic depression during repetitive nerve stimulation, especially when transmitter depletion is not severe.

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373.6 ACETYLCHOLINE RELEASE DURING REPETITIVE ACTIVITY AT THE FROG NEUROMUSCULAR JUNCTION. R.S. Manalis and M.B. Wildermuth*. Indiana-Purdue Univ., Fort Wayne, IN 46805.

One approach to the study of transmitter release has been to use certain chemicals, such as pharmacological agents or heavy metal ions, as specific probes to disrupt the release process. However, most of the electrophysiological studies that have been performed along this line have used rather simple experimental designs in which ACh release has been evoked following single shocks to the motor nerve. Such investigations have neglected the more subtle aspects of transmitter release which become apparent when ACh is released repetitively during a train of stimuli. Therefore, we have begun to perform such studies. The combined use of conventional electrophysiological and digital recording techniques allows for a systematic study of the kinetics of ACh release. Sciatic nerve-sartorius muscle preparations of the frog (Rana pipiens) were bathed in normal Ringer's solution containing (in mM): NaCl (111); KCl (2.5); $CaCl_2$ (2.0); tris maleate (4) (pH = 7.1-7.2; temp. = 15° C). Endplate potentials (EPPs) were recorded from curarized preparations ([dTC] = 4.31 µM. Trains of stimuli were applied to the nerve. For a given train, the stimulus frequency was usually constant and was within the range of 20-63 Hz. Stimulus trains lasted 4 sec and were applied every 8 min. Changes in evoked ACh release were measured electrophysiologically as variations in EPP amplitude. Moderate stimulus frequencies (30-40 Hz) applied to the nerve produce the following changes in ACh release: a rapid facilitation, a rapid depression and, finally, a slow depression. There is also a marked temporal summation which underlies the facilitation and rapid depression phases. The data also clearly show an inflexion point between the two phases of depression. EPP amplitudes were normalized relative to the maximally facilitated one in the train. This inflexion point as extrapolated to the Y-axis (normalized EPP amplitude) is referred to as the mobilization coefficient, which was found to be .5 at 40 Hz. Preliminary experiments have shown an inverse relationship between stimulus frequency and the time constant of decay during the rapid depression phase. This time constant (in sec) was 1.38, .960, .919, .525, .491, 1.22, and .290, respectively, at the following stimulus frequencies (in Hz): 20, 25, 31, 40, 50, 20 (repeat), and 63. (Supported by NSF grant BNS-8609047.)

373.7 MECHANISMS OF PRESYNAPTIC FACILITATION AT THE CRAYFISH OPENER NEUROMUSCULAR JUNCTION.
S.Sivaramakrishnan[1], G.D. Bittner[1] & M.S. Brodwick[2]. [1]Department of Zoology, University of Texas at Austin, and [2]Department of Physiology & Biophysics, UTMB, Galveston,Texas.

Presynaptic facilitation is defined as an increased probability of transmitter release resulting from repetitive stimulation of an axon terminal. Facilitation at many synapses may be regulated by both sodium and calcium ions. At frog neuromuscular junctions, for example, reduction of extracellular sodium increases quantal content (Birks & Cohen, Proc. R. Soc., Lond., B 170: 381, 1968), while an increase in intracellular sodium facilitates transmitter release at crayfish neuromuscular junctions (Atwood, Swenarchuk & Gruenwald, 1975, Brain Res., 100: 198,1975). Sodium is thought to act indirectly on transmitter release by raising the internal calcium concentration.It has been proposed that this secondary regulation of transmitter release by sodium ions could be due to either a competition between sodium and calcium ions at the neuromuscular junction or the presence of a sodium-calcium exchange mechanism in the presynaptic terminal.

We are investigating the sodium-dependence of presynaptic facilitation at excitatory neuromuscular junctions made by a single axon on opener muscle fibers in the crayfish walking leg. Two electrodes are inserted in the excitor axon close to a presynaptic terminal. One electrode is used to pass current into the terminal and the other to record membrane potential. A third electrode is used to record post-synaptic potentials intracellularly from an adjacent muscle fiber.

Partial reduction or complete removal of sodium ions from the bathing medium results in a hyperpolarization of the presynaptic terminal of the excitor axon and, usually, a decrease in the resting frequency of spontaneous miniature potentials (MEPPS). However, if the membrane potential of the terminal is clamped at its resting level, reduction of the external sodium concentration causes a large increase in the resting MEPP frequency. The lower the external sodium concentration, the greater the MEPP frequency.

The crayfish opener muscle is also innervated by a GABA-ergic inhibitor axon which makes synapses on both the excitor axon and on opener muscle fibers. We tested the possibility that the inhibitor is tonically active and that this tonic inhibition affects the MEPP frequency in low-sodium solutions. Addition of picrotoxin to a bathing medium containing normal sodium results in small changes in membrane potential of the excitor axon but has no effect on the resting MEPP frequency. When picrotoxin is added to a bathing medium containing low sodium and the membrane potential of the excitor axon clamped at its resting level, an increase in MEPP frequency is still observed. Tonic inhibition is therefore probably not present in this preparation.

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373.8 TIME COURSE AND CHARACTERISTICS OF STRETCH-INDUCED INCREASE IN TRANSMITTER RELEASE FOR FROG MOTOR NERVE TERMINALS. S. Hulsizer* and A. D. Grinnell, (SPON: S. Bodine-Fowler) Dept. of Physiology and Jerry Lewis Neuromuscular Research Center, Univ. of Calif. Los Angeles, Los Angeles, Calif, 90024.

In response to stretch, the presynaptic terminal of the frog sartorius neuromuscular junction increases transmitter release. Previous studies employing static changes in length have shown an increase of up to 100% in EPP size, MEPP frequency and quantal content with a 20% increase in stretch (Hutter and Trautwein, J. Physiol., 133:610-625, 1956 and Turkanis, J. Physiol., 230:391-403, 1973). However the degree of increase is quite variable for different junctions and little is known about the rate of onset of the phenomenon. With the aim of understanding better the mechanism of the effect and its physiological relevance, we have investigated the time course of the effect.

The preparation utilized both sartorius muscles from Rana pipiens, coupled in series by a string connecting the tibial tendons, with the pelvic ends pinned into a Sylgard dish such that both muscles were initially at rest length. The muscles were bathed in normal frog Ringer's solution with enough curare to block nerve-induced contraction (typically 3 uM). The left muscle was stimulated electrically inducing a contraction that stretched the right muscle. During the stretch, the nerve to the right muscle was stimulated via a suction electrode, producing EPPs that were recorded with a "floating" intracellular electrode. This electrode was placed near a terminal and recorded EPPs at rest length and at varying degrees of stretch, up to 115% of rest length. The interval of time from the onset of stretch to the EPP was varied in order to determine the rate of development of the response. At 18°C for any measured degree of stretch, the response reached steady state levels within 20 msec although no effect was seen at 10 msec. This effectively establishes that the stretch effect is fast enough to be of physiological significance in supplementing the spinal stretch reflex and suggests that second messenger systems are probably too slow to cause this effect. Further studies are underway to correlate the degree of stretch effect with morphological and physiological properties of the junction.

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373.9 LONG-TERM FACILITATION OF SYNAPSES BETWEEN NEURONS OF APLYSIA GROWN IN VITRO INVOLVES INCREASED TRANSMITTER RELEASE: A QUANTAL ANALYSIS. N. Dale*, S. Schacher and E.R. Kandel. (SPON: J.S. Eisenman) HHMI, Ctr. for Neurobiol. & Behav., Columbia Univ., and NYS Psychiat. Instit., NY, NY 10032.

Both short- and long-term sensitization of the gill withdrawal reflex in Aplysia involve corresponding short- and long-term facilitation of the synaptic connections between siphon sensory and gill motor neurons. Whereas the short-lasting synaptic facilitation involves an increase in the amount of the transmitter released by the presynaptic sensory neuron (Castellucci and Kandel, 1976), the mechanisms by which this same sensorimotor synapse can be facilitated in a long-lasting way remain unknown.

To determine whether long-term facilitation involves an increase in transmitter release, a quantal analysis was performed on sensorimotor synpases reconstituted in vitro, which can exhibit long-term facilitation following repeated applications of 5-HT (Montarolo et al., 1986). L7 motor cells were co-cultured with either one or two sensory neurons for 4-5 days. The amplitude of the synaptic connections between the sensory and motor neurons and the amplitudes of any spontaneously-occurring miniature synaptic potentials (mEPSPs) were measured before and 24 hours after 5 applications of 1 uM 5-HT. The amplitude of the mEPSPs ranged from 92 to 156 uV; they were characterized by a short rise time and a roughly exponential falling phase, giving them a shape very similar to that of the evoked EPSP. Five repeated applications of 5-HT caused a twofold facilitation of the synaptic connection; however, in no case did the mean amplitude of the spontaneous mEPSPs change significantly. Overall evoked EPSPs were facilitated by 113% (s.e.m. 36.8, n = 8), while the mean amplitude of the spontaneous mEPSPs showed a slight, but not significant, increase of 2.3% (s.e.m. 2.63, n = 8). Long-term synaptic facilitation is not therefore accompanied by an increase in the size of the unit quantum of transmitter released by the sensory neuron.

Amplitude histograms of EPSPs, evoked under conditions of low release (120 mM Mg, 7 mM, Ca), indicated that the size of the unitary evoked potential did not change with facilitation. Instead, there was a decrease in the number of failures (mean = -79%, s.e.m. 9.6, n = 6) and an increase in the number of multiple release events. This suggests that the size of the quantum not only stays constant, but that the quantal content of the EPSP increases during long-term facilitation. We therefore conclude that long-term synaptic facilitation, like short-term modulation of the same synapse, involves an increase in the amount of transmitter released by the sensory neuron. While our experiments allow us to rule out certain types of postsynaptic change (e.g., changes in receptor affinity or density), it remains likely that long-term facilitation does involve some postsynaptic alterations such as growth and formation of new contact points.

373.10 USE-DEPENDENT REGULATION IN A SYNAPTOSOMAL PREPARATION FROM THE SYMPATHETIC GANGLION. M. W. McCaman*, C. A. Briggs and D. A. McAfee. Div. of Neuroscience, Beckman Research Institute/City of Hope, Duarte, CA 91010 and Abbott Laboratories, Abbott Park, IL. 60064.

We are developing a preparation of synaptosomes from the rat superior cervical ganglion, a tissue that has been valuable in many neurophysiological studies. As little as one rat ganglion pair (2 mg wet weight) sufficed in preparing the P2 and the subsequent fractions enriched by centrifugation in a Metrizamide density gradient. Qualitative electron microscopy demonstrated the presence of intact synaptosomes. Cholinergic function was demonstrated by Na^+-dependent uptake of ^{3}H-choline, by apparent synthesis of ^{3}H-acetylcholine (^{3}H-ACh) from the ^{3}H-choline, and by stimulated release of ^{3}H-ACh. The uptake of ^{3}H-choline at a concentration of 1 μM was reduced more than 90% at 4°C and by 80% in the presence of 136 mM LiCl/24 mM NaCl at 34°C.

To study ^{3}H-ACh release, the synaptosomal preparation was preincubated with 1 μM ^{3}H-choline for 15 min and then perifused on a Uniflo filter (0.45 μm pore) with media containing 20 μM eserine. Thus, the ^{3}H-ACh was synthesized by the synaptosomal preparation. ^{3}H-ACh and ^{3}H-choline were separated by the cholinekinase-ion pair method. In perifusates ^{3}H-ACh accounted for only 5-10% of the total ^{3}H-cpm released spontaneously. Exposure to high K^+ (15 mM for 5 min, n = 10) or to veratridine (50 μM for 5 min, n = 7) increased the release of ^{3}H-ACh by 35 and 63%, respectively, and of ^{3}H-choline by 24 and 52%, respectively. The evoked release of both compounds was reduced more than 50% in a low Ca^{++} medium.

A long term potentiation of endogenous ACh release is observed in the intact ganglion after brief preganglionic tetany (Briggs, McAfee and McCaman, J. Physiol. 363:181, 1985). To determine if such a potentiation could be observed in the synaptosomes, we homogenized ganglia 15 min after tetany (10 Hz for 60 sec), with the contralateral non-stimulated ganglion as control. Neither ^{3}H-choline uptake nor the release of ^{3}H-ACh or ^{3}H-choline was increased in the synaptosomal preparations from the stimulated ganglion (n = 5). However, when ganglia were homogenized 1 min after tetany, the synaptosomal preparation increased ^{3}H-choline uptake by 155% (n = 4) and increased K^+-evoked release of ^{3}H-ACh and ^{3}H-choline by 74 and 83%, respectively (n = 4). (Supported in part by NIH grants: NS 23272 and 18966).

373.11 NICOTINIC MODULATION OF GABAERGIC TRANSMISSION IN THE HIPPOCAMPUS. S. Wonnacott*1, L. Fryer*1, G. G. Lunt*1, R. K. Freund2, D. A. Jungschaffer*2, and A. C. Collins2 (SPON: V. G. Erwin). 1Dept. of Biochemistry, University of Bath, Bath BA2 7AY, U. K. and 2Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309 U. S. A.

Nicotine-induced seizure susceptibility in different mouse strains is governed by genetic factors, and some correlation with numbers of [^{125}I] α-bungarotoxin binding sites in the hippocampus has been demonstrated (Miner et. al., IPET, 231 (1984) 545). We have explored the possible mechanisms underlying the convulsive property of nicotine by investigating its interaction with GABA systems in the hippocampus.

Electrophysiological recording of population spikes from CA1 pyramidal cells in mouse hippocampal slices indicates that nicotine (800 uM) produces an increase in the population spike amplitude and the appearance of multiple population spikes. These effects are abolished by the addition of the GABA uptake inhibitor nipecotic acid (5 mM). We interpret the nicotine-enhanced excitability as the result of a reduction in synaptic GABA levels following nicotine-induced release of the transmitter.

To test this hypothesis, we have examined the effect of nicotine on [^{3}H]GABA release from hippocampal synaptosomes. Rat synaptosomes preloaded with radiolabelled transmitter were perfused with Kreb's bicarbonate buffer into which pulses (100 ul) of nicotine were introduced. This agonist resulted in a dose-dependent release of discrete peaks of [^{3}H]GABA over the concentration range 5-100 uM. The nicotine-induced release is prevented by the nicotinic antagonist dihydro-ß-erythroidine (10 uM), and agents thought to interact with the ion channel at peripheral nicotinic receptors also diminished the nicotine-evoked [^{3}H]GABA release. α-Bungarotoxin (10^{-7}M) however, failed to have any effect, even after long exposure. Thus it appears that nicotine can act at presynaptic nicotinic receptors on GABAergic nerve terminals in the hippocampus to release the inhibitory neurotransmitter, and this may subsequently lead to a depletion of GABA and removal of inhibition, resulting in seizure activity.

(Supported by a grant from R. J. Reynolds, Inc.)

373.12 ACTIVATION OF $GABA_B$ RECEPTORS INDUCES PRESYNAPTIC INHIBITION AT MUSCLE SPINDLE AFFERENT-MOTONEURON SYNAPSES IN THE FROG SPINAL CORD. Y-Y. Peng and E. Frank. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh Med. School, Pittsburgh, PA 15261.

Synaptic transmission between muscle spindle afferents and motoneurons can be inhibited by stimulating other primary sensory afferents. Although GABA is known to mediate this inhibition, its mechanism of action has been difficult to determine. In this study, pharmacological agents were used to dissect the functional roles of the $GABA_A$ and $GABA_B$ receptors in this inhibition in the bullfrog. Quantal analysis using a deconvolution technique was used to study whether the inhibition caused by $GABA_B$ receptor activation was pre- or post-synaptic.

Baclofen, a specific $GABA_B$ receptor agonist, was used to study the effects of activation of the $GABA_B$ receptors on the monosynaptic EPSP evoked by stimulating triceps muscle afferents. Extracellular recording from the ventral root and intracellular recording from motoneurons showed that baclofen (5μM) reversibly reduced the peak amplitude of the EPSP by 40% without altering its falling phase. A similar result was obtained for the intracellularly recorded unitary EPSP's evoked by activating single triceps muscle spindle afferents. This suggests that activation of $GABA_B$ receptors inhibits the EPSP either postsynaptically on remote motoneuronal dendrites or presynaptically at sensory terminals. Quantal analysis was carried out to distinguish between these alternatives since numerical analysis of the fluctuations of the unitary EPSP showed that transmission is quantal. Baclofen (5μM) lowered the quantal content but did not alter the amplitude of the EPSP evoked by a single quantum. Thus the inhibition produced by activation of $GABA_B$ receptors is entirely presynaptic.

In contrast, activation of $GABA_A$ receptors (by 100μM muscimol, which is primarily a $GABA_A$ receptor agonist) shortened the falling phase of both unitary and composite triceps EPSP's, as indicated by a 66% reduction in EPSP halfwidth, suggesting a large increase in the conductance of the motoneuronal membrane. Furthermore, this effect was reversibly blocked by bicuculline (100μM), a specific competitive $GABA_A$ antagonist. Primary afferent depolarization (PAD) of sensory axons is also produced by the activation of $GABA_A$, not $GABA_B$, receptors. 50 μM bicuculline produced a reversible 78% reduction in the peak amplitude of the PAD recorded intra-axonally in muscle afferents evoked by stimulating individual brachial muscle or cutaneous nerves. At 100 μM it reversibly blocked more than 90% of the dorsal root potential evoked by stimulating adjacent dorsal roots. Thus the same type of receptors that produce GABA-mediated PAD, whose role in synaptic inhibition is currently under study in our laboratory, also produce a postsynaptic effect. Activation of $GABA_B$ receptors, on the other hand, does not evoke PAD but causes presynaptic inhibition.

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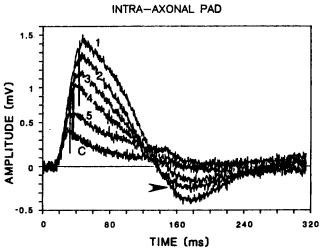
373.13 POSTTETANIC CHANGES OF PRIMARY AFFERENT DEPOLARIZATION AFTER INTRA-AXONAL TETANIZATION OF GROUP IA AFFERENTS. A. Lev-Tov, D. E. R. Meyers, and R. E. Burke. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20892 and Hebrew University Medical School, Jerusalem, Israel (A.L.T.).

In a previous study (J. Neurophysiol. 50:413, 1983), we reported that primary afferent depolarization (PAD) produced in medial gastrocnemius (MG) group Ia afferents by 4 group I volleys in the posterior biceps-semitendinosus (PBSt) nerve was markedly enhanced following prolonged, high frequency (20s, 500 Hz) tetanization of the whole MG nerve. The augmented PAD had increased time to peak and was accompanied by hyperpolarizing undershoot that decayed more rapidly than the enhanced PAD. These effects could not be explained simply by the well-known post-tetanic afferent hyperpolarization; it seemed possible that whole nerve tetanization may activate other segmental pathways or change extracellular ionic concentrations. We have re-examined these phenomena in pentobarbital-anesthetized cats, with intra-axonal recording and tetanization of individual, functionally identified triceps surae group Ia afferents. The figure below illustrates superimposed average records (8 sweeps each) of the transmembrane potential (extracellular fields subtracted) in a soleus group Ia afferent, produced by 4 PBSt volleys (200 Hz, 2xT for group I) before (C) and after (1-5) intra-axonal tetanization (500 Hz, 20 s). Immediately after the tetanus, phasic PAD was markedly enhanced, had prolonged time to peak, and was followed by hyperpolarizing undershoot (arrow), which disappeared more rapidly than the enhanced PAD. These results are qualitatively and quantitatively identical to those obtained using whole nerve tetanization. We conclude that the dramatic changes in PAD must be due to mechanisms intrinsic to the tetanized afferents and not to extrinsic factors. In other experiments, application of a series of short trains (500 Hz, 1s), repeated at 5s intervals after the 20s tetanus, maintained the augmented PAD and hyperpolarizing undershoot for several minutes. We are using this paradigm to study the effect of the post-tetanic changes in PAD on Ia synaptic transmission to motoneurons.



374.1 SIMULATION OF THERMAL EFFECTS ON FOUR MEMBRANE CHANNELS IN HIPPOCAMPAL PYRAMIDAL CELLS. M. P. Thomas and J. M. Horowitz. Dept. of Animal Physiology, University of California, Davis, CA 95616.

We are developing a model for hippocampal pyramidal neurons in hibernators (e.g., hamsters) and nonhibernators (e.g., rats) comprised of four types of ion channels. The model is an extension of one presented by Traub (Neuroscience 7:1233-1242, 1982) to include the effects of temperature. A preliminary version of this model (Fed. Proc. 45:408, 1986) covered basic equations for the four types of channels. This study focuses on channel parameters that should be modified to extend the scope of the model to cover wider changes in temperature.

Following Traub, we have simulated channels using equations of the Hodgkin-Huxley form to describe membrane currents. In addition to voltage-gated sodium channels and delayed rectifier potassium channels, a voltage-gated calcium channel and a calcium-activated potassium channel corresponding to I_{AHP} were included. Rate constants used to describe the activation and inactivation of the four channel types were multiplied by a function that defined the temperature sensitivity of the channel kinetics. The model matched intracellular waveforms well over a range of temperature from 35°C to 40°C. Single spikes were generated at low stimulus currents and bursts of action potentials were produced with higher currents.

At temperatures below 35°C, low stimulus currents evoked a burst of action potentials rather than single spikes. Since in vitro experiments show that single spikes can be evoked at these temperatures, we examined waveforms of individual channel currents at the time of maximum repolarization following the first spike of the burst, time T_{mr}. At T_{mr} for 32°C, ICa was -112.4 uA/cm^2 and I_K was 109.6 uA/cm^2, while at T_{mr} for 37°C, I_{Ca} was -92.6 uA/cm^2 and I_K was 90.3 uA/cm^2. Thus at time T_{mr} there was a slightly larger net inward current at 32°C compared to 37°C. It appears that at 32°C the increased duration of the first spike resulted in activation of g_{Ca} such that the added inward current contributed to the subsequent generation of a burst. When calcium channels are blocked (by setting g_{Ca} to zero) only a single spike is generated at 32°C. To extend the model to simulate data at temperatures below 35°C additional channel types can be included and/or differential temperature sensitivity can be assigned to individual channel types. (Supported by NASA grant NAG2-341.)

374.2 **EXTRACELLULAR UNIT ANALYSIS OF SPIKE DISCHARGE IN SOMATA AND DENDRITES OF CA1 HIPPOCAMPAL PYRAMIDAL CELLS.** R.W. Turner, D.E.R. Meyers and J.L. Barker. Lab of Neurophysiology, Bldg 36, Rm 2C02, NIH, Bethesda MD.

Laminar profile analysis of extracellular field potentials in the CA1 region has shown that the shortest latency population spike is evoked in stratum (st) pyramidale, suggesting that spike discharge in hippocampal pyramidal cells (HPCs) originates in the cell body region. The spike subsequently conducts in a retrograde fashion through the dendritic arborization.* The present study examined the properties of over 400 HPC extracellular single units in st pyramidale (SP), st oriens (SO) and st radiatum (SR) in the rat hippocampal slice to further assess the point of origin for spike discharge. HPC units could be identified on the basis of discharge characteristics, evoked by alvear antidromic stimulation or orthodromic activation via st oriens or st radiatum. The most common single unit response (approx. 95%) in somatic and dendritic regions was a biphasic large positive-small negative (PN) waveform, distinct from the "giant" PN units indicative of cell membrane damage. Other waveforms included small triphasic fiber potentials and small positive-large negative (N) units.

HPC units were superimposed on the population spike of anti- and orthodromic field potentials at all levels of the cell axis. The shortest latency for PN units at threshold intensity was thus found in the region of st pyramidale. Subtraction of underlying field potentials revealed that positive and negative components of PN units were largest in the cell body region, exhibiting a sharp decline in SO and at approx. 130um into SR, similar to that of HPC intradendritic spikes. The properties of HPC units support results of field potential and intracellular analyses suggesting that spike discharge originates in the region of the cell body layer, leading to a retrograde spike invasion of dendritic arborizations.*

Local application of TTX to HPC dendrites selectively blocks the negative phase of SO and alvear-evoked dendritic population spikes, suggesting that the retrogradely conducted depolarization activates voltage-dependent Na^+ conductance in dendritic membrane. The more infrequently encountered N units may in fact be indicative of focal recordings from TTX-sensitive sites. The response of somatic and dendritic single units to TTX application is presently under investigation.

*Turner RW, Richardson TL & JJ Miller. Intracellular analysis of somatic and dendritic spike discharge in CA1 hippoocampal pyramidal cells. IUPS Vancouver 1986.

374.3 **MODELLING OF STRENGTH-DURATION AND CURRENT-FREQUENCY CURVES OF HIPPOCAMPAL GRANULE CELLS OBTAINED FROM INTRACELLULAR RECORDING.** **G.L.F. Yuen and D. Durand. Applied Neural Control Laboratory, Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.**

Data from intracellular studies of the hippocampal granule cell were fitted to a previously developed Hodgkin-Huxley type computer model for this neuron with a sodium and a potassium conductance (see Yuen and Durand, Soc Neurosci Abstr 235.6, 1986). With minor adjustments in the cable and active properties, the model can generate action potentials that have proper threshold voltage, maximum rate of rise, peak amplitude, spike duration and EPSP responses. The shape of the postspike potential also resembles that in the intracellular recordings and reflects a fairly weak potassium conductance. The model at this stage exhibits a tendency to fire repetitively especially with dendritic activation. Since vertebrate neurons tend to have more divergent sodium inactivation (h) and potassium (n) conductance characteristics and these properties have been implicated in controlling repetitive firing in neurons (Jack et al., Electric current flow in excitable cells, 1975), we proceed to further compare this aspect of the model to the intracellular data. Furthermore, for a point-polarized cable such as the granule neuron, the Hodgkin-Huxley equations with proper active parameters should adequately describe its strength-duration relationship (Noble and Stein, J Physiol 187:129,1966), approximating the predictions by Hill's equation. An acceptable fit between the model and the observed strength-duration curve would indicate the overall validity of the active properties in the model, even though voltage clamp data is not available for computing these neurons' behavior.

Preliminary comparisons indicate some deviation of the current model from the data at long pulse durations for the strength-duration curve, although further data analysis and model adjustments are necessary to support this observation. Inadequate data is currently available for evaluating the simulated current-frequency relationship. However, the model exhibits a convex current-frequency curve extending from a firing frequency of 70 to 370 Hz as the current was increased from (repetitive firing) rheobase to 20 times rheobase for a duration of 250 ms. Beyond this current magnitude, the model exhibits damped oscillations aborting the rhythmic firing pattern. The steady-state spike amplitude also decreases noticeably with increasing current magnitude. Further intracellular studies will focus on testing these specific predictions so as to refine the active properties of the model. The resulting model will subsequently be applied to evaluate the effects of ethanol on the active conductances.

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374.4 CHARACTERISTICS OF CA3 HIPPOCAMPAL PYRAMIDAL CELLS ACUTELY DISSOCIATED FROM IMMATURE RATS. C.N. Allen, R.J. Brady, K.L. Smith*, J.W. Swann and D.O. Carpenter. Wadsworth Laboratories, NYS Department of Health and School of Public Health, University at Albany, Albany, NY 12201.

Hippocampal pyramidal cell neurons are an important component of a variety of physiological and pathological processes including learning, memory and epilepsy. An understanding of the neurotransmitter and ionic current mechanisms which control pyramidal cell excitability is therefore important. Single channel recording using the patch clamp technique is one approach which can be used to record the activity of ionic currents which underlie neuronal function. Toward this end Kay and Wong dispersed CA1 pyramidal neurons and studied the ionic currents controlling cellular excitability using the whole cell patch clamp technique (Kay and Wong, J. Neurosci. Meth. 16:227-239, 1986). We now describe a modification of this technique for the isolation of CA3 pyramidal cell neurons from 12 to 15 days old rats. Hippocampal minislices containing only the CA3 pyramidal cell subfield were incubated for 1.5 hr with trypsin at 37°C, washed with fresh PIPES buffered saline for 1 hr and titurated using a Pasteur pipette. This procedure yields isolated pyramidal-shaped neurons with variable lengths of dendrites attached. Giga-ohm seals were obtained using standard techniques, the membrane ruptured with gentle suction and the membrane response recorded in the whole cell configuration. The CA3 neurons had resting membrane potentials of -50 to -60 mV and fired action potentials spontaneously. The action potentials had an amplitudes of 60-80 mV and overshoots to +40 mV when held at -70 mV. The isolated neurons fired a bursts of action potentials in response to a depolarizing pulse of current. This action potential firing pattern is similar to that observed from slices of CA3 neurons recorded from animals of the same age. Spontaneous membrane potential fluctuations were present which often reached the threshold to fire single or a burst of action potentials. The ionic mechanism underlying these spontaneous events is currently being investigated. The neurons have a membrane time constant of about 25-30 msec and resistances of 100-150 Mohm. (Supported by NIH Grants NS23807 to D.O.C., NS18309 to J.W.S. and NS23071 to R.J.B.)

374.5 ELECTROPHYSIOLOGICAL EFFECTS OF COCAINE IN THE IN VITRO RAT HIPPOCAMPUS. J.L. Tyma, W.R. Proctor, and T.V. Dunwiddie, Dept. of Pharmacology, Univ. of Colo. Hlth. Sci. Ctr., and Veterans Admin. Med. Research Service, Denver, CO.

Cocaine and other local anesthetic agents are known to produce central nervous system excitation which can result in generalized seizure activity. Previous studies have demonstrated that systemic injections of cocaine reduce the threshold for stimulation-induced afterdischarges in hippocampus (Lesse and Collins, Biochem. Behav. 13:695). In the present study, we have examined the electrophysiological effects of cocaine in the in vitro hippocampus in order to determine whether the proconvulsant effects of cocaine reflect an intrinsic hippocampal action of this drug.

Rat hippocampal slices were prepared and recorded from using standard electrophysiological methods, and drugs were applied via addition to the superfusion medium. The evoked presynaptic fiber spike, field excitatory postsynaptic potential (epsp) and population spike responses in the CA1 region were reduced by superfusion with cocaine (threshold ca. 5 uM). The rate of interictal spiking (IIS) induced by adding penicillin G (1500 units/ml) and increased potassium (8 mM) to the perfusion medium was also reduced by similar concentrations of cocaine. Cocaine-induced inhibition of IIS ranged from 17% (5uM; n=5) to 100% (100uM; n=3).

Intracellular recordings showed that perfusion with cocaine (3-100uM) did not consistently alter either resting membrane potential or input impedance of CA1 pyramidal neurons. The epsps recorded following stimulation of stratum radiatum were not markedly affected by 3-30uM cocaine, but were reduced by 30% by 100uM. Recurrent ipsps evoked by stimulation of the alveus were more sensitive to cocaine; 30uM cocaine reduced the initial chloride-dependent component of the ipsp by 20%, while the potassium component was reduced by 30%. The greater sensitivity of ipsps to cocaine may be due to the fact that the antidromic population spike evoked by alvear stimulation was approximately 2X more sensitive to cocaine than was the presynaptic fiber spike evoked by stratum radiatum stimulation, suggesting that the axons of the CA1 cells are more sensitive to the local anesthetic effect of cocaine than are the Schaffer and commissural fibers.

These results demonstrate that cocaine (10-100uM) has well-defined local anesthetic actions on hippocampal neurons, but does not have a proconvulsant effect upon interictal spiking within this range of concentrations. Experiments using intracellular recording did not suggest that cocaine has a differential depressant effect upon hippocampal interneurons.
Supported by the Veterans Admin. Medical Research Service.

374.6 IN VITRO EXCITABILITY OF HIPPOCAMPAL NEURONS FROM EPILEPTIC (TOTTERING) MICE. G. Kostopoulos and C.Psarropoulou* (SPON: A. MITSACOS). Dept. of Physiology, University of Patras, Medical School, Patra, 26110 Greece.

In order to elucidate the neuronal mechanisms underlying epilepsy in the tottering mutant,we prepared hippocampal slices from tg/tg (n=7) and phenotypicaly normal (tg/+ or +/+) mice (n=10) and maintained them in a complete submersion chamber (one slice from each mouse). Stimulating str. radiatum by stepwise increasing current (I, 200 μsec, 0-300 μA) we recorded at str. pyramidale of CA_1 the respective field EPSP slopes and population spikes (POP) amplitudes.Using a computer we derived from these data the sigmoidal curves: EPSP/I, POP/I and POP/EPSP and compared them in the two groups of slices. The POP/EPSP curves from the epileptic slices had signifficantly higher maximal slopes (8.5 ± 2.9 - mean ± SD - vs. 5.2 ± 1.6, $p<0.05$) possibly indicating a more homogeneous firing threshold of pyramidal neurons as compared to normal. On the average the POP_{50} corresponded to smaller EPSP in the epileptic as compared to normal slices (0.46 ± 0.3 mV/msec vs. 0.72 ± 0.3 mV/msec) but this difference did not reach statistical signifficance. Exposure to perfusing medium containing mildly elevated potassium concentration (from 3.25 mM to 5.25 mM) shifted all three curves (EPSP/I, POP/I and EPSP/POP) to the left in both groups of slices. However this effect was signifficantly less pronounced in the epileptic slices. The I for $EPSP_{50}$ was decreased by elevated $[K^+]$ by 4.7% ± 3.1% in epileptic as compared to 14.7% ± 9.4% in normal slices. The area under the POP/I curve was increased by 24.4% ± 10.7% in epileptic and by 117.3%±40.5% in normal slices ($p<0.001$). Finally in the postsynaptic excitability curves (POP/EPSP) the EPSP for POP_{50} was decreased by 15.8% ± 5.6% in epileptic as opposed to 49% ± 19.1% in normal slices ($p<0.01$). The demonstrated decreased responsiveness to elevated $[K^+]$ can contribute to a protection from nonsynaptic spread of hyperexcitability during seizures. It could be interpreted as being either part of the epileptogenic mechanism or an accomodation which developed after repeated seizure episodes.

374.7 INTRACELLULAR RESPONSES OF HIPPOCAMPAL NEURONS IN ZERO-MAGNESIUM. L.S. Jones, D.V. Lewis, and R.D. Andrew, Dept. of Pediatric Neur., Duke Univ. Med. Ctr., Durham, NC 27710, and Dept. of Anatomy, Queen's Univ. Kingston, Ontario, Canada K7L 3N6.

Previous work has shown that exposing hippocampal slices to magnesium-free artificial cerebrospinal fluid (0-Mg ACSF) can activate ictal-like events, in addition to spontaneous and triggered interictal bursts, particularly in young rats (26-36 days). The ictaform activity begins within 10-20 min of 0-Mg perfusion and continues for up to 40 min before giving way to interictal bursting. In this study, the effect of 0-Mg on cells from CA1 and CA3 of both young and adult rats were examined.

Hippocampal slices (400 u) were prepared from adult (40-43 days) and young male, Sprague-Dawley rats. 0-Mg ACSF consisted of ACSF with magnesium salts simply removed ($[Mg^{++}]$ about 10 uM).

Perfusion with 0-Mg produced bursting in all neurons studied within ten min of being washed on. Most neurons were not bursting spontaneously in ACSF, but would proceed to burst with as many as 8 spikes per burst. Burst frequency ranged from 0.6 to 3.3 Hz in 0-Mg. Prior to 0-Mg treatment, all cells exhibited prolonged after-hyperpolarization following either spontaneous bursts or bursts produced by antidromic or orthodromic stimulation. This post-burst hyperpolarization decreased in most cells during exposure to 0-Mg; however, the membrane potential hyperpolarized with 0-Mg in most cells also, therefore it is unclear as yet if recurrent circuitry is affected. Response thresholds to extracellular stimulation were determined by stimulating in stratum radiatum until a burst was produced. In the absence of extracellular magnesium, less current was necessary to trigger a burst than in ACSF. The thresholds usually returned to pre-0-Mg levels when the slice was washed with ACSF. This corresponded to the observation that the 0-Mg-evoked spontaneous bursting slowed and often stopped when the slice was returned to ACSF.
Supported in part by NINCDS NS22170, Canadian MRC grant MA7884, and the Queen's University Principal's Development Fund.

374.8 MODELLING DIVERSE HIPPOCAMPAL PYRAMIDAL CELL CURRENTS WITH HODGKIN-HUXLEY-LIKE MECHANISMS SUGGEST DESCRIPTIONS OF PUTATIVE Na+ CURRENTS AND ROLE OF Ca++ MEDIATED K+ CURRENTS IN THE REGULATION OF REPETITIVE FIRING. Lyle J. Borg-Graham* (SPON: N. Grzywacz) Center for Biological Information Processing, Massachusetts Institute of Technology, Cambridge, MA 02139.

Over the past decade a wide variety of non-linear conductances have been described with varying degrees of quantitative success for hippocampal pyramidal cells (HPCs). The difficulty in obtaining complete kinetic data for these conductances results from the small size and extended electrotonic structure of these cells, limitations of the single electrode clamp technique, and the diverse cell population and preparations used in experiments. Modelling the electrical behavior of HPCs with computer simulations is one method of integrating data from a variety of sources in order to develop a consistent description for this cell type. The sparseness of the data, however, requires various assumptions regarding mechanisms underlying the conductance of ion channels. In the model described here, each conductance is assumed to be mediated by one or more two-state gating particles which, in turn, are either voltage- or calcium-dependent. The voltage-dependent gating particles are based on a single-barrier model similar to that proposed by Hodgkin and Huxley. The parameters of each particle type (order, valence, symmetry, base rates, and voltage mid-points) are adjusted in order to reproduce the data (voltage clamp or current clamp) for the current in question. In addition, simple two-state calcium-mediated gating particles and a simple model of intercellular calcium accumulation and diffusion are constructed based on first principles in order to reproduce the relevant data.

The model presently includes descriptions of eleven somatic currents mediated by non-linear conductances. These include three putative sodium currents -- I-Na-trig, I-Na-rep, and I-Na-tail; six potassium currents that have been reported in the literature -- I-DR (Delayed Rectifier), I-A, I-C, I-AHP (After-Hyperpolarization), I-M, and I-Q; and two calcium currents, also reported previously -- I-Ca and I-CaS. The electrotonic structure of the hippocampal pyramidal cell is modelled with a soma/short-cable approximation, and the dendrites are assumed to be linear.

Model simulations qualitatively or quantitatively reproduce a wide range of somatic electrical behavior in HPCs. Present results include the suggestion of multiple sodium currents in these cells and possible mechanisms by which both I-AHP and I-C can mediate repetitive firing in response to changes in the concentration of intracellular calcium. In addition, the model has been used to suggest experimental protocols designed to test the validity of simulation results.

374.9 CHANGES IN EXTRACELLULAR $[K^+]$ AND $[Ca^{2+}]$ EVOKED BY ANOXIA IN RAT HIPPOCAMPAL SLICES. M. E. Morris, K. Krnjević and J. Leblond. Departments of Pharmacology, University of Toronto, Toronto and Anaesthesia Research, McGill University, Montreal, Canada, H3G 1Y6.

O_2 lack leads to translocations of ions in anoxia-sensitive tissues which reflect metabolic imbalance and changes in membrane permeability and/or ion pump activity: the definition and manipulation of underlying mechanisms may provide methods to enhance anoxic tolerance. Ion-sensitive microelectrodes have been used to record extracellular concentrations of potassium ($[K^+]_o$) and calcium ($[Ca^{2+}]_o$) in the CA1 pyramidal cell layer of isolated rat hippocampal slices during brief periods of anoxia (95% N_2/CO_2 for 2-4 min). Reversible increases of 0.5-1.0 mM $[K^+]_o$ were typically evoked; although similar changes were observed at different depths (50-300 µm), simultaneous recordings in different slices from the same hippocampus showed marked differences in rates of rise of $[K^+]_o$ and susceptibility to spreading depression (SD)-like increases of $\geq$20 mM. Stimulus-evoked increases in $[K^+]_o$ were inversely related to temperature; but anoxic-induced accumulations at 23° were small and delayed, in contrast to the more rapid onset of larger, more sustained changes at 33°. The time course of the $\Delta[K^+]_o$ during anoxia closely resembled that of large membrane hyperpolarizations ($\leq$20 mV) and falls in resistance ($\leq$75%) recorded in cells $\leq$50 µm away and of sustained outward currents seen with single-electrode voltage clamp (SEVC) (V_h = -50 to -70 mV) (previously shown to be Cl^--independent, sensitive to Cs and TEA and presumed due to an ↑ in K^+ conductance (G_K) (*Krnjević & Leblond, J. Physiol. 382:791,1987*)). Secondary post-anoxic hyperpolarizations -- believed to reflect electrogenic ion transport -- were recorded during recovery while $[K^+]_o$ levels were still elevated. In association with SD-evoked paroxsymal large ↑s in K^+ membrane and tissue potentials were markedly depolarized, and there was an early, large transient and smaller and prolonged inward current during SEVC (with 0.5 µM TTX, 3 mM CsCl, V_h = -50 mV). $[Ca^{2+}]_o$ changes evoked by anoxia were small ($\leq$ 0.1-0.5 mM) and variable and consistently began before the onset of hyperpolarization. At low temperature either a ↓ or an initial ↑ followed by ↓ was observed; at 33° the falls were either attenuated or replaced by a fluctuating, sustained ↑ in $[Ca^{+}]_o$. Concomitant with changes in $[Ca^{2+}]_o$ and $[K^+]_o$ large, brief inward Ca-inward currents (which were evoked in the presence of TTX, Cs and TEA (10 mM) by 30 mV depolarizations from V_h = -70 mV) were rapidly blocked by N_2 and restored on re-oxygenation. The relatively small and variable changes in $[Ca^{2+}]_o$ during anoxia are clearly insufficient to explain the marked diminution of Ca-inward current which was observed. An anoxic-induced accumulation of intracellular $[Ca^{2+}]$ may however be responsible for both inactivation of Ca-channels and the production of G_K-mediated hyperpolarization.

(Supported by The Medical Research Council of Canada)

374.10 K^+ AT CONCENTRATIONS REACHED IN THE EXTRACELLULAR SPACE DURING NEURONAL ACTIVITY PROMOTES A Ca^{++}-DEPENDENT GLYCOGEN HYDROLYSIS IN MOUSE CEREBRAL CORTEX. P.R. Hof and P.J. Magistretti. Dept of Pharmacology, CMU, 1211 - GENEVA 4, Switzerland.

The concentration of K^+ in the extracellular space increases during neuronal activity from basal levels ranging between 2.5 and 3.5 mM, to 5 and 12 mM. This increase in $[K^+]_o$ is accompanied by a marked decrease in $[Ca^{++}]_o$. During pathological conditions such as spreading depression, hypoxia or ischemia $[K^+]_o$ can reach even higher levels, up to 30 to 40 mM. The hydrolysis of glycogen elicited by various neuroactive agents such as norepinephrine, serotonin, histamine, vasoactive intestinal peptide and adenosine in mouse cerebral cortical slices has been previously described (see Magistretti *et al.*, J. Neurosci., 6:2558, 1986). In view of the fact that adenosine, like K^+, is released in the extracellular space during neuronal activity, we have examined the effect of K^+ on ^{3}H-glycogen levels. K^+ stimulates in a time- and concentration-dependent manner the hydrolysis of ^{3}H-glycogen. Over 60 % of the maximal effect is reached within 30 sec and the EC_{50} for the glycogenolytic action of K^+ is 11 mM. A significant ^{3}H-glycogen hydrolysis (12 to 55 % of basal ^{3}H-glycogen levels) occurs at 5 to 12 mM $[K^+]_o$. The K^+-evoked glycogenolysis is Ca^{++}-dependent, and is blocked by Ca^{++}-channel blockers such as Co^{++} at 5 mM, Ni^{++} at 0.1 mM and Mn^{++} at 1 mM but not by Cd^{++} at 20 µM. This type of pharmacological profile has been suggested to indicate the activation of voltage-sensitive Ca^{++} channels of the T subtype. We also examined the effect of various K^+-channel blockers. Ba^{++}, Cs^+ at 1 mM and TEA at 10 mM produced a small glycogenolytic effect when tested alone; TEA potentiated the ^{3}H-glycogen hydrolysis elicited by K^+ 10 mM, whereas Ba^{++} and Cs^+ were without effect. Interestingly, blockade of K^+ channels of the A-type by 4-aminopyridine at 1 mM strongly enhances the glycogenolysis evoked by K^+ at low concentrations (5-10 mM). In contrast, apamin at 1 nM and 1 µM did not influence the K^+-evoked glycogenolysis. The effect of TTX, a selective Na^+-channel blocker, was also examined. At 2 µM, TTX did not significantly influence the glycogenolytic response of K^+ up to 8 mM, but gradually shifted to the right the concentration-response curve of higher K^+ concentrations, indicating that the contribution of the voltage-sensitive Na^+ channels in membrane depolarization, and in the subsequent entry of Ca^{++}, can be estimated to commence at 8 mM $[K^+]_o$.

These observations further delineate the role of K^+ in intercellular communication and in the coupling between neuronal activity and energy metabolism. They suggest that K^+, released from active neurons, could mobilize energy substrates in regions of increased neuronal activity where the $[K^+]_o$ can reach values up to 10-12 mM; these are concentrations at which K^+ promotes a significant glycogenolysis.

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374.11 INTRACELLULAR INJECTION OF PHORBOL ESTERS INCREASES EXCITABILITY OF NEURONS OF THE MOTOR CORTEX OF AWAKE CATS. M. B. Szente, A. Baranyi, and C. D. Woody, Mental Retardation Res. Ctr., Brain Res. Inst., Depts. of Anatomy and Psychiatry, UCLA Med. Ctr., Los Angeles, CA 90024

Protein kinase C (PKC) is present in high concentrations in neurons of the neocortex, but as yet there is no direct evidence that this enzyme can influence the excitability of neocortical neurons in vivo. In this study electrophysiological effects of two intracellularly injected phorbol esters (PhEs) which activate PKC, phorbol 12,13-dibutyrate and phorbol 12-myristate, 13-acetate, were investigated in neurons of the motor cortex of awake cats. In order to study direct, postsynaptic actions, we applied PhE (1-10 uM PhE dissolved in 1 M potassium citrate containing 50 ug/ml phosphatidyl serine) into the recorded cells by pressure injection.

Signs of increased neuronal excitability were observed in each of 65 cells injected with PhE. Enhancement of excitatory background synaptic activity resulted in an elevated rate of spontaneous firing. The number of spikes evoked by depolarizing constant current pulses gradually increased. The latency of the first action potential produced by delivery of the depolarizing current pulses decreased as did the threshold level of current needed for spike initiation. The slow afterhyperpolarization (AHP) following action potentials and current-induced depolarizations decreased. In some neurons the increase in background firing activity resulted in burst generation. PhE also increased the peak amplitude of action potentials (but not their duration) and the amplitude of fast AHPs following action potentials.

All changes occurred within 2-8 min after injection and lasted for 50 min or longer. Neither increases in input resistance nor depolarizations of the resting membrane potential sufficient to account for these excitability changes were found.

Control injections (n=15 cells) of 4 alpha-phorbol 12,13-didecanoate, which does not activate PKC, failed to induce changes in neuronal excitability.

The results show that activation of PKC by PhE can increase the postsynaptic excitability of neurons of the motor cortex of awake cats. (Supported in part by AFOSR F49620-85-C-0100 and HD 05958.)

374.12 INTRACELLULAR INJECTION OF PHORBOL ESTERS INDUCES LONG-TERM CHANGES OF POSTSYNAPTIC RESPONSES IN VOLTAGE-CLAMPED CELLS OF THE MOTOR CORTEX OF AWAKE CATS. A. Baranyi, M. B. Szente, and C. D. Woody, Mental Retardation Res. Ctr., Brain Res. Inst., Depts. of Anatomy and Psychiatry, UCLA Med. Ctr., Los Angeles, CA 90024

It is not known how protein kinase C (PKC) regulates long-term changes of excitatory/inhibitory postsynaptic potentials (EPSP/IPSP) and their currents (EPSC/IPSC) in neurons of the neocortex. Accordingly, we investigated effects of phorbol esters on postsynaptic responses of neurons in the motor cortex of awake cats.

Intracellular pressure injections of 1-10 uM phorbol 12,13-dibutyrate (PdIB) dissolved in 1 M potassium citrate containing 50 ug/ml phosphatidyl serine induced long-term, reciprocal changes in EPSPs and IPSPs, without proportionate changes in resting potential and input resistance. As measured in 16 identified fast PT neurons, the amplitudes and decay time constants of monosynaptic VL EPSPs increased from 5.8±1.3 to 13.9±2.4 mV, and 9.2±0.6 to 13.1±1.1 ms, respectively (Student's two-tailed t-test, $p<0.005$). The amplitudes and decay time constants of recurrent PT IPSPs decreased from 7.4±1.9 to 2.5±2.1 mV and 74.0±16 to 24.0±6.2 ms ($p<0.01$).

Corresponding changes in EPSCs and IPSCs were measured by single electrode voltage-clamp technique. The peak currents and slope conductances of VL EPSCs increased from 0.86±0.11 to 1.49±0.36 nA ($p<0.01$) and 15.8±2.9 to 26.1±3.3 nS ($p<0.005$). The peak currents and slope conductances of PT IPSCs decreased from 1.01±0.20 to 0.34±0.18 nA ($p<0.02$) and 37.2±4.0 to 13.6±2.2 nS ($p<0.005$). The synaptic reversal potentials did not change after injections, indicating that the ion specificity properties of contributing ion channels were not influenced by PdIB. The effects began within 2-8 min after injection and lasted for 50 min or longer. PdIB suppressed voltage-dependent outward currents (thought to be I_A and $I_K(Ca)$ since they were also reduced by 3-aminopyridine and apamine, respectively) and facilitated an inward tail current following depolarizing command pulses. Control injections of 4-alpha - phorbol 12,13-didecanoate, which does not activate PKC, failed to induce changes in the measured electrophysiological parameters (n=5 fast PT cells).

The results demonstrate that activation of PKC induces sustained changes in synaptic responses of in vivo neocortical neurons through modifications of postsynaptic ion channel conductivities. Since these PKC-induced postsynaptic changes last for 50 min or longer, they could support changes in synaptic efficacy underlying learning and memory in the motor cortex. (Supported in part by AFOSR F49620-85-C-0100 and HD 05958.)

374.13 A SLOW, SODIUM-DEPENDENT K^+ CONDUCTANCE IN LAYER V NEURONS FROM CAT SENSORIMOTOR CORTEX. P.C. Schwindt, W.J. Spain*, R.C. Foehring and W.E. Crill. Dept. Physiol. Biophys., Univ. of Washington Sch. of Med., Seattle WA 98195

Slow outward ionic currents of large neurons from layer V of cat sensorimotor cortex were studied in an *in vitro* brain slice preparation using current clamp and single microelectrode voltage clamp. Following sustained repetitive firing evoked by constant injected current, these neurons exhibit a slow afterhyperpolarization (sAHP) lasting up to 30 s. The sAHP reduces excitability for seconds, and the corresponding slow outward current contributes to a slow adaptation of firing rate. Only the initial 1-3 s of the sAHP (the early sAHP) is reduced by Ca^{2+} channel blockade, whereas the late sAHP (the last 3-30 s duration) is unaffected. Corresponding slow relaxations of outward current follow depolarizing voltage clamp steps in normal perfusate. Like the late sAHP, the slow outward current relaxations are not affected by Ca^{2+} channel blockade. They are abolished by TTX, however, indicating that they depend on Na^+ entry through voltage gated channels. Spikes are not necessary to evoke the slow Na^+-dependent outward current ($I_{K(Na)}$); it is evoked by small depolarizing voltage steps that activate the persistent Na^+ current, I_{NaP}. By comparing current records obtained before and after blockade of $I_{K(Na)}$, its onset can be detected about 100 ms after a step depolarization. The following observations suggest that $I_{K(Na)}$ results from an increase of K^+ conductance rather than from stimulation of an electrogenic Na^+ pump: Outward current amplitude varies with membrane potential as expected for a K^+ current and is reduced in raised $[K^+]_o$. In addition, $I_{K(Na)}$ is blocked by muscarine (5-10 μM) and norepinepherine (10-50 μM), and it is reduced by TEA (5-10 mM). The mechanism by which Na^+ entry increases K^+ conductance is under investigation.

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374.14 INTRACELLULAR INJECTION OF CALCIUM CHELATORS INDUCES BURST GENERATION IN NEOCORTICAL NEURONS. M.J.Gutnick and A. Friedman*, Physiology Unit, Faculty of Health Sciences, Corob Center for Medical Sciences, Ben-Gurion University, Beersheva, Israel.

In intracellular experiments, evidence of voltage-dependent Ca^{++} conductance may be readily obtained in most cortical neurons. Since Ca^{++} influx and consequent intracellular Ca^{++} accumulation can affect membrane excitability through several mechanisms, we examined the effect of intracellular injection of Ca^{++} chelators on electroresponsive properties of neurons in coronal slices of guinea pig parietal cortex maintained in vitro (36°C). Forty eight neurons from all cortical depths were impaled with electrodes that contained EGTA or BAPTA (0.4 M) and K-Acetate (0.2 M) (pH 7). In 83% of these, spike broadening and prominent afterdepolarization appeared within a few minutes of impalement. As this tendancy progressed, most neurons became bursters. That is, they responded to a brief, depolarizing pulse by generating an all-or-none train of Na^+-dependent action potentials superimposed on a slow envelope of depolarization. Addition of 3 mM Mn^{++} to the bath eliminated chelator-induced bursting, yet caused spike broadening in neurons not injected with chelator.

Previous studies have revealed that a small population of burster neurons is normally present at a subpial depth of 0.9-1.3 mm in this preparation. Our data, which indicate that burst generation can be induced in most neocortical neurons regardless of layer, suggest that bursts may be mediated by a Ca^{++} current and that when intracellular Ca^{++} is allowed to accumulate, their generation is prevented by activation of a Ca^{++}-dependent K^+ conductance.

374.15 **FIRING ADAPTATION AND LONG LASTING AFTERHYPERPOLARIZATIONS (AHP) IN HUMAN NEURONS IN THE DEEP LAYERS OF EPILEPTOGENIC NEOCORTEX MAINTAINED "IN VITRO".** M Avoli. MNI & Dept of Neurol & Neurosurg McGill Univ.

Neurons in the deep layers of slices of temporal neocortex removed from epileptic patients were studied with conventional intracellular recording and stimulation techniques. Intracellular injections of lucifer yellow revealed that they were large spiny pyramidal neurons. In over 30 neurons repetitive firing of fast action potentials was evoked by depolarizing square pulses of intracellular current. In the major- ity of the cells the firing rate decreased smoothly over a few tens of ms and (eventually) reached a steady level. Prolonging further the depolarizing pulse a late and more pronounced adaptation appeared. This late phenomenon was capable of blocking action potential generation. A long lasting (up to 4s) AHP followed at the end of the depolarizing square pulse and displayed two distinct components. The early one appeared as a fast undershoot upon termination of the depolarizing pulse, was not clearly related to the amount of injected current and was not affected by changing the resting membrane potential. Conversely the amplitude of the late component was dependent upon the amount of current injected and displayed an equilibrium potential 15-30mV negative to rest. This value and the fact that the AHP could be recorded with KCl filled microelectrodes suggested that it was caused by an increased conductance to K+. Bath application of Cd++ or Co++ decreased and eventually blocked the late component but not the early phase of the AHP. These changes, which are suggestive for a Ca++ dependent K+ conduct- ance as mechanism underlying the late component of the AHP, were accompanied by a marked decrease of firing adaptation during the step of depolarizing current. These electrophysiological properties are compared with, and appear indeed similar to those recorded in cat, rat or guinea pig neurons located in the deep layers of neocortical slices maintained "in vitro". Furthermore the values of the parameters measured in the present experiments are distributed in a unimodal manner suggesting that "in vitro", minimal or no difference might exist among cells located in slices of neocortex displaying different degrees of epileptogenicity "in situ"

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375.1 ELECTROPERMEABILIZATION AS A MEANS OF GAINING ACCESS TO THE CYTOSOL OF INDIVIDUAL CELLS UNDER PATCH CLAMP. D.G. Owen* and W. Piotrowski* (SPON: F.M. Scalzo). MRC Neuropharmacology Group, The School of Pharmacy and Sandoz Institute for Medical Research, London, UK.

The permeability of the plasmalemma to ions and small molecules is increased following transient high intensity electrical fields, thus providing 'access' to the cytosol (Knight, D.E. and Scrutton, M.C., Biochem. J., 234:497, 1986). This facilitates modification of the intracellular milieu and the study of 2nd messenger-mediated signal transduction mechanisms. A major advantage of electropermeabilization over other methods (eg. use of detergents) is that permeabilization of the plasmalemma is highly localized and intracellular organelles are spared. We have applied the method to single selected cells which have been patch clamped for electrophysiological recordings. Bipolar permeabilizing electrodes were made of teflon-insulated platinum/iridium wire (120μ diam.) with a terminal gap of 120μ. The electrode was positioned straddling single cultured rat hippocampal neurones or cultured neuroblastoma/glioma hybrid cells (NG108cc15). Gigohm seals (5-10Gohm) were made between the cell and a patch electrode before permeabilization, in either extracellular or intracellular types of solution. Brief (10us) electrical pulses of 3-5 kV/cm intensity were sufficient to permeabilize the cell as verified by changes in membrane potential and conductance or the accumulation of Trypan Blue, applied to the cell by pressure ejection from a nearby pipette. No change in pipette seal resistance was associated with these events. In extracellular bathing solutions, field intensities of ca. 3kV/cm caused transient depolarizations of 10-20mV, cells resealing over periods of 10s to 1min. Stronger field intensities caused larger increases in permeability which became irreversible. On-cell patch clamp recordings were made from NG108cc15 cells using an intracellular bathing solution. In previously quiescent patches, two types of K^+ channels (14pS non-bursty channel and 100pS bursty channel which opened less often) were recorded following a stimulus of 4kV/cm (10us) with simultaneous application of 3uM Ca^{2+} but not with either Ca^{2+} or pulse alone. This method of permeabilizing cells has proved to be simple, rapid and reproducible and should find wide application in the investigation of the role of intracellular signalling in the regulation of membrane currents.

375.2 A NEW APPROACH TO THE MODELING OF SINGLE CHANNEL CURRENTS AND THE POSSIBLE ROLE OF DIFFUSIVE PROCESSES IN DETERMINING CONDUCTANCE CHANGES IN ION CHANNELS. G.L. Millhauser and R.E. Oswald, Department of Pharmacology, New York State College of Veterinary Medicine, Cornell University, Ithaca, N.Y., 14853.

The kinetics of whole cell and single channel data are usually modeled by assuming that there are several open and closed channel conformations and that the channel protein undergoes random transitions among these conformational states. In single channel experiments the number of closed states, for example, can be estimated by counting the number of exponentials in the closed time histogram. It is usually agreed that kinetic schemes should be as simple as possible, and simplicity refers to minimizing the number of states in the kinetic scheme. This view of gating kinetics has existed since long before the discovery of the patch clamp experiment.

We support the idea that the simplest model is preferable, but we interpret the concept of simplicity in a different way. Instead of taking this concept to mean the least number of kinetic states, we interpret the concept of simplicity as meaning the highest degree of symmetry. The number of adjustable parameters remains small but the number of kinetic states can become quite large. We use the concept of high symmetry to mean that there is a relationship among the rate constants in the kinetic scheme. Thus, no single rate constant can be varied independently of the others.

The specific model that we have pursued is one with all of the rate constants being equal. We studied the closed time distribution of a model with one open state and a variable number of closed states. The basic qualitative characteristic of this model is that the longer a channel is in a closed state the less likely a transition out of that state becomes. The single channel current shows burst-like character such as that seen in a number of systems including the nicotinic acetylcholine receptor.

A recent observation by other workers in the field suggests that there is a fractal scaling of the rate constants in ion channel kinetic schemes. The fractal dimension, D, can vary between one and two. We have numerically calculated the closed time histogram for our model and fit the data to the distribution from the fractal scheme. We find that the fit is excellent and, furthermore, we find that D can be related to the number of closed channel states. When there is one state, D is equal to one; as the number of states becomes infinite, D asymptotically approaches two. Our model also provides an excellent fit to distributions from the brief closures of gramicidin channels and to the open time distribution of the acetylcholine receptor. In both cases the number of states is large (>10).

The most important aspect of this model is that it suggests a physical basis for the ocurrence of bursts, brief closures, fractal scaling and related phenomena. Although a large number of states may seem improbable, it is actually quite consistent with recent molecular dynamics calculations that show proteins actually sample thousands of energy minima as they undergoe random fluctuations. When the number of states is large the protein motion is more like diffusion and less like random kinetic transitions between a small number of well defined protein conformations. We can calculate diffusion constants from our model and we find that fits to real data yield diffusion constants that are completely consistent with processes that occur in membranes.

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375.3 Is the acetylcholinesterase level in Rat and Mouse muscle cells cultures related to the level of free cytoplasmic Ca^{2+} ?

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The role of the calcium ion Ca^{2+} as an intracellular control agent in a variety of physiological processes is well established. In vertebrate skeletal muscle fibers Ca^{2} is involved in muscle contraction, modulation of membrane permeability and regulation of metabolic activity.

Recently it has been suggested that the ionic fluxes through the membranes regulated the level of 2 cholinergic macromolecules the acetylcholine receptors and the A_{12} acetylcholinesterase (AChE) form, the presumed synaptic form of the enzyme. Muscle cells paralysed by veratridin which maintains the Na^{+} channel in the open state showed a dramatic increase in total AChE and in the level of A_{12} form. The effect of veratridin on AChE was blocked in the presence of agents that block Ca^{2+} permeability suggesting that Ca^{2} is involved in this effect.

To verify that the level of AChE synthetized by the muscle cells is related, in a some way to the level of free cytoplasmic Ca^{2}, we analysed the variations of Ca^{2} levels in normal Rat muscle cells treated by agents which modify the ionic permeabilities.

Mainly they are 2 sources for increased muscle Ca^{2} during action potentials and contractions : Ca^{2} flux through membrane ions channels and release from the sarcoplasmic reticulum.

In the mutant mouse, muscular dysgenesis (mdg), the muscle cells did not contract but possess normal membrane properties. This inability to contract could be related to a selective lack of functional slow Ca^{2} channels which may be involved in the excitation contraction coupling. We determined the concentration of free Ca^{2} in these non-contracting muscles (mdg) and analyzed its modulation by different drugs.

The levels of intracellular free Ca^{2} were determined by spectrofluorometry using the Ca^{2} fluorescent indicators : Quin 2 and INDO 1.

375.4 COMPUTER STUDIES OF NEGATIVE FEEDBACK IN NEURONS, Michael Mascagni* (SPON: J. Rinzel). Courant Institute of Mathematical Sciences, New York University, New York, New York 10012.

We study the problem of negative feedback in the nervous system by numerically solving a mathematical model describing a neuron in the presence of feedback. The equations solved model a neuron, composed of a dendritic cylinder attached to a cylindrical axon, in which the axon is synaptically connected back onto the dendrite. The nominal input to this system is a constant current injected into the dendrite. This nominal input can then be modulated by the synaptic feedback current. By varying the synaptic efficacy of this feedback connection one can compare a neuron without feedback to one subject to different amounts of negative feedback.

The mathematical model describing our axon is the Hodgkin-Huxley model for the dynamic electrical behavior of the giant axon of the squid *Loligo* (Hodgkin, A. L., and Huxley, A. F., J. Physiol., **116**: 497, 1952). Our dendritic model is merely the linear cable equations with cable properties chosen to be compatible with the resting values of the Hodgkin-Huxley axon. Our model for the synapse was chosen to be a postsynaptic current injection with a fixed time course that is triggered by the arrival of an action potential at the presynaptic membrane. Evaluation of the behavior of the neuron involved the measurement of the frequency of repetitive firing of the axon as a function of constant background dendritic current injection. This function was computed for various values of the feedback parameters, including parameters corresponding to no feedback.

Studies carried out without feedback showed repetitive firing over a relatively narrow range of currents with firing rates close to that limited by the axon's refractory period. These studies also showed a temperature dependence in the onset of repetitive firing. This temperature dependence is manifested in an hysteresis phenomenon that is insignificant at low temperature (6.3 C°) and dramatic at high temperature (18.6 C°). With negative feedback the range of currents where repetitive firing is observed is substantially increased. The neuron also fires at rates lower than without feedback, leading to a larger relative dynamic range. Also the region of approximately linear current versus frequency response is greatly magnified. This observed phenomenology is consistent with the electrical engineer's use of negative feedback to linearize and stabilize the response of a nonlinear, high-gain amplifier.

375.5 MEMBRANE POTENTIAL AND INTERBURST INTERVAL OF APLYSIA BURSTING PACEMAKER NEURONS ARE MODULATED BY WEAK ELECTRIC FIELD STIMULATION AT HIGH FREQUENCY. A.R. Sheppard, A.J. Godwin*, S.M. Bawin and W.R. Adey. Departments of Physiology and Neurosurgery, Loma Linda University, and Research Service, VAMC, Loma Linda, CA 92357.

We exposed isolated Aplysia abdominal ganglia to 60-Hz electric fields of 10 mV/cm rms. The goal of this study was to evaluate the interaction of extracellular fields with repetitively firing neurons. Fields at this level and frequency do not directly entrain firing of the Aplysia neurons. We examined R15 cells (n=11) and bursters of the left upper quadrant (n=13) at a fixed temperature between 17 and 21 C. Intracellular recordings were begun after stability was reached following recovery from impalement. Following a one hour observation period, electric fields were applied for 20 min along the rostral-caudal axis of the ganglion by a pair of Ag-AgCl electrodes energized by a constant current amplifier. Field stimulation produced changes of 2 to 8 mV in the maximum hyperpolarization recorded during the interburst interval. In correlation, there were shifts in the duration of the interburst interval. The gradual (usually minutes) onset and reversal of field effects varied from cell to cell. Among the various cells, both hyperpolarizing and depolarizing shifts were found. Extreme responses included depolarizations which changed bursting patterns to beating patterns, and hyperpolarizations which inhibited all firing activity for several minutes.

Our results may be generalized to suggest that the extracellular fields generated by the high frequency firing of a group of neurons could influence the much slower firing rate of nearby pacemaker cells and perhaps modulate physiological functions such as neurosecretion. (Supported by the Department of Energy.)

375.6 VOLTAGE CLAMP OF IONIC CURRENTS IN LEECH HEART MUSCLE CELLS. K.J. Thompson and R.L. Calabrese, Dept. Biology, Emory Univ., Atlanta, GA 30322.

The central nervous system of the leech, Hirudo medicinalis, contains a pattern generator for heartbeat (Thompson and Stent, 1976). This central pattern generator paces the activity of heart motor neurons. Isolated hearts are capable of producing intrinsic rhythmic myogenic contractions and rhythmic activity in heart motor neurons entrains this activity. After enzymatic dissociation of the hearts, a myogenic polarization rhythm and associated contractions can occur in individual isolated muscle cells (Maranto and Calabrese, 1984).

The present study was undertaken to determine the ionic basis of the electrical activity of the heart cells. In current clamp, brief depolarizing pulses elicit both action potentials and slow plateau-like potentials in isolated cells. The action potentials are abolished in Na^+-free salines, but are present in Co^{++}- and Mn^{++}-containing salines. The plateau-like potentials persist in Na^+-free saline, are enhanced in Ba^{++} (10 mM) saline, and are absent in Co^{++} (10 mM) and Mn^{++} (5 mM) salines.

For voltage clamp studies the cells were suspended in a low melting point agarose solution. Under two-electrode voltage clamp, large net outward currents flow in response to depolarizing steps from near rest (-65 mV). One outward current exhibits time- and voltage-dependent inactivation at potentials more depolarized than -40 mV, similar to A-current. The other predominant outward current does not inactivate and appears similar to the delayed rectifier. With hyperpolarizing steps the cells show low leakage current, corresponding to a measured input resistance of approximately 100 megohms.

In salines with TEA substituted for Na^+, depolarizing steps elicit a small inward current. In Ba^{++} (40 mM) TEA solution a large, non-inactivating inward current is observed which is blocked by the addition of Co^{++}, suggesting that Ba^{++} flows through Ca^{++} channels.

These results suggest that sodium dependent action potentials, calcium plateaus, and potassium currents contribute to the electrical activity of heart cells.

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375.7 IDENTIFICATION AND CHARACTERIZATION OF IONIC CURRENTS IN HEART INTERNEURONS OF THE LEECH USING SINGLE-ELECTRODE VOLTAGE CLAMP. J.D. Angstadt and R.L. Calabrese. Dept. of Biology, Emory University, Atlanta, GA 30322.

Heartbeat in the leech, Hirudo medicinalis, is controlled by a central pattern generator composed of a set of identified interneurons (HN cells). Spontaneous activity in HN cells consists of repetitive bursts of action potentials terminated by barrages of IPSPs. This bursting pattern depends in part on the intrinsic cellular properties of the HN cells (Arbas and Calabrese, 1987). Current clamp studies have demonstrated that HN cells actively escape from inhibition via the combined actions of a Na-dependent depolarizing "sag" in membrane potential and a depolarization-activated Ca-dependent plateau potential.

We have investigated the intrinsic ionic currents of HN interneurons directly using the switching single microelectrode voltage clamp technique. A slow, non-inactivating inward current produced by hyperpolarizing voltage steps from a holding potential near -40 mV is described which can account for the membrane potential "sag" observed in current clamp. This current persists in salines containing 10 mM Co^{++}, but is not observed in Na-free saline. A second inward current (I_{Ca}) was found which persists in Na-free saline and is blocked by addition of 10 mM Co^{++} to the saline. The Ca^{++} current appears to be completely inactivated at holding potentials more depolarized than -50 mV. A slow outward current ($I_{K(V)}$) similar to the delayed rectifier is also observed. This current exhibits little inactivation over a time course of 100-200 ms and is blocked in salines containing 40 mM Ba^{++}. Finally, a fourth current, which resembles molluscan A-current (I_A) has been identified. This rapidly developing outward current, unlike $I_{K(V)}$, exhibits significant inactivation over a 100-200 ms time course. Like I_{Ca}, the transient outward current appears to be nearly completely inactivated at holding potentials more depolarized than -50 mV. Since HN cells typically oscillate around a membrane potential of -50 mV, the voltage sensitivities of these membrane currents are consistent with the hypothesis that they contribute significantly to the pattern of activity observed in normally bursting HN interneurons.

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375.8 DIFFERENT ACTIONS OF BARBITURATES AND BENZODIAZEPINES ON NA- AND K-CONDUCTANCES IN INVERTEBRATE AND VERTEBRATE NEURONS. A.L. Kleinhaus, J.Johansen, J. Yang & C.F. Zorumski, Dept. Neurol. Yale U. Sch. Med. 333 Cedar St. New Haven, CT. 06510 and Depts. Anat. & Neurob. & Psych. Wash. U. Sch. Med. 660 S. Euclid, St. Louis, MO 63110.

It has previously been reported that benzodiazepines (BZ) and barbiturates (BA) in micromolar concentrations inhibit Ca-dependent action potentials in vertebrate neurons in culture (MacDonald & McLean Adv.Neurol. 44:1986) as well as in identified leech neurons (Johansen et al. PNAS 82:1985; Johansen & Kleinhaus Brain Res.376:1986). The effective concentrations for this effect were similar in the mammalian and invertebrate neurons suggesting that the underlying mechanisms may be related.

In this study, we compare the effects of μM concentrations of Medazepam (MDZ) and Methohexital (MTX) on the voltage-gated Na and K conductances of Retzius cells in the leech *Macrobdella* and chick dorsal root cells (DRG) in culture. Under current clamp conditions, MDZ and MTX prolonged the Na-dependent action potential of leech neurons without reducing their maximum rate of depolarization. These prolonged action potentials were identical to those recorded in the same neurons in the absence of outward currents *i.e.* in Ca-free Ringer's solution containing Mn, tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP). They consisted of an initial spike followed by a plateau lasting several hundreds of milliseconds. Both components were Na-dependent and TTX-resistant, while the plateau was selectively blocked by saxitoxin (STX) suggesting that it originated from the flow of Na through a conductance different from that underlying the spike potential (Kleinhaus & Johansen, Soc. Neurosci. Abstr.1986). Similarly, the plateau of the MTX-prolonged action potential was abolished by 50 μM STX. These results suggest that the drugs' actions resulted from a block of K-conductances. This hypothesis was substantianted by voltage-clamp experiments showing that MTX (100 -1000 μM) reduced both I_K and I_A in the Retzius cell, essentially mimicking the combined effects of TEA and 4-AP (Johansen & Kleinhaus, J. Neurophys. 56:1986).

In contrast, voltage-clamp experiments showed that in DRG cells MDZ and MTX decreased the amplitude of Na,Ca and K currents equally. This lack of specificity for a particular ionic species in the vertebrate cells suggests that in widely phylogenetically distant species the K-conductances may be very similar while significant differences may exist among their Na-conductances. Furthermore, the results suggest that the modulation of ionic conductances by BZ and BA (at μM conc.) are mediated via a common mechanism which may be important for the drugs' sedative and anesthetic actions.

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375.9 DEVELOPMENT OF IONIC CURRENTS IN VARIOUS CELL LINEAGES OF THE ASCIDIAN, Boltenia villosa. L. Simoncini*, M.L. Block and W.J. Moody. Department of Zoology, University of Washington, Seattle, Washington 98195.

We are using the whole-cell voltage-clamp technique to trace the development of ionic currents in surgically isolated blastomeres of different cell lineages in the ascidian Boltenia villosa. Ascidians, which are primitive chordates, are characterized by a mosaic form of development, with very early determination of cell lineages. Boltenia was chosen because the oocyte contains orange pigment granules which partition preferentially into muscle-lineage blastomeres and enable them to be identified at all stages of development. Other cell types can be identified by pigmentation, size, and position in the embryo.

The unfertilized oocyte contains three major voltage-dependent ionic currents: a transient inward Na current, an inward Ca current, and an inwardly rectifying K current. After fertilization the Na current begins to decrease, and by the 2-cell stage is essentially absent. The Na current remains absent through at least gastrulation in all blastomeres. Ca and inwardly rectifying K current densities remain constant at oocyte levels through the 8-cell stage in all blastomeres, suggesting that these channels are added to the embryo in parallel with new membrane. This process continues for the inward rectifier through gastrulation. Ca current density appears to be lower in gastrula-stage cells than in the oocyte, but this could be explained by more efficient perfusion of these smaller cells by pipet solutions. After gastrulation, during the period of neural tube closure and tailbud extention, we see for the first time the appearance of other types of currents and differences in the electrical properties of blastomeres of different cell lineages.

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375.10 PROPERTIES OF SODIUM AND CALCIUM CURRENTS IN NEURONS OF THE JELLYFISH CYANEA CAPILLATA (COELENTERATA; SCYPHOZOA). Peter A. V. Anderson. Whitney Lab. and Depts. of Physiology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086.

Recent work on the structure and composition of Na^+ channels suggests that there has been considerable conservation of the Na^+ channel through evolution. Since coelenterates are the most primitive animals to possess a nervous system and to produce Na^+-dependent action potentials they may provide useful information on the properties of the "primitive" Na^+ channel. To this end, neurons in the motor nerve net of the jellyfish Cyanea were voltage clamped using the whole-cell configuration of the patch clamp technique. Outward current was blocked by a combination of internal Cs^+ and TEA^+ and external 4-AP. A space clamp was achieved by selective axotomy of the neurons.

Inward current in these cells is dominated by a fast, transient current that activates close to -10 mV, reaches peak amplitude around +15 mV and, in 390 mM Na^+, has a mean reversal potential of +62.9 ±0.9 mV (s.e.m.). This current activates with a time constant of 0.5 to 0.8 ms, reaching peak amplitude within 1 ms and inactivates more slowly, with a time constant of 1.2 to 3.7 ms. Inactivation, which is rarely complete, is voltage-dependent; in prepulse experiments, V_h, the potential at which the current is half maximally inactivated, was found to be -15 mV.

This inward current is a Na^+ current. It is abolished by the absence of external Na^+ and its reversal potential is dependent on $[Na^+]_o$. It is, however, completely insensitive to TTX (0.1 mM), STX (10 μM), veratridine (10 μM), BTX (2 μM), Leiurus venom (1 μM) and GIIIA (1 μM) but blocked, in a dose-dependent way, by Cd^{++} (5 mM), verapamil (2 μM), lidocaine (1 mM) and W7 (100 μM), a calmodulin antagonist which blocks Ca^{++} currents in Paramecium. Thus its pharmacology is somewhat reminiscent of that of Ca^{++} currents.

A second inward current is recorded in Na^+-free saline when $[Ca^{++}]_o$ is elevated. This Ca^{++} current activates more slowly, reaching peak amplitude in 2-3 ms, and inactivates in a voltage-dependent manner (V_h = +15 mV), with a time constant of 7 ms. The current/voltage relationships of this current are very different from those of the Na^+ current. In 95mM Ca^{++}, 0 Na^+, it activates at 0 to +10 mV, reaches peak amplitude at +40 mV and reverses around +90 mV. This current is insensitive to lidocaine (1 mM) and W7 (100 μM) but blocked by verapamil. Its properties are consistent with those of the presumed Ca^{++} current underlying synaptic transmission in these cells.

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BASAL GANGLIA II

376.1 EXCITATORY AMINO ACID MECHANISMS IN BASAL GANGLIA STRUCTURES REGULATE LIMBIC SEIZURE ACTIVITY. S. Patel, G. De Sarro, M.H. Millan, B.S. Meldrum (SPON: A.G. Chapman). Department of Neurology, Institute of Psychiatry, London, SE5 8AF, U.K.

We have used focal injections of excitatory amino acid agonists and antagonists to study the role of the basal ganglia nuclei in both the development and spread of limbic seizures induced by pilocarpine.

Male Wistar rats, implanted at the level of the substantia nigra pars reticulata (SNr), entopeduncular nucleus (EP), lateral habenula (LH), mediodorsal thalamus (MD) or pedunculopontine nucleus (PPN) were injected with N-methyl-D-aspartate (NMDA, 500pmol-12.5 nmol), kainate (KA, 20-500 pmol), Y-D-glutamylaminomethylsulphonate (GAMS, 0.02-40 nmol) or 2-amino-7-phosphonoheptanoate (APH, 5pmol-1nmol). 15 min later, the animals received either a subconvulsant (150 mg/kg, ip) or a convulsant dose of pilocarpine (380 mg/kg, ip). Behaviour was monitored for 3h.

APH (a selective NMDA antagonist) protected against pilocarpine-induced seizures in a dose-related manner. Susceptibility to the protective effect of APH varied with the brain region studied. Mimimal effective doses were:- EP 5pmol; LH and MD 10pmol; PPN 100pmol; SNr 1nmol. GAMS (a preferential KA antagonist) injected into either SNr or EP suppressed pilocarpine-induced seizures. SNr is more sensitive to the protective action of GAMS (1 nmol for total seizure suppression) than the EP (40 nmol). Behavioural and EEG signs of motor limbic seizure activity were evident after injections of NMDA and KA into EP prior to a subconvulsant dose of pilocarpine (150mg/kg, ip). High doses of NMDA (12.5 nmol, n=8) and KA (500 pmol, n=4) resulted in motor limbic seizures in all animals tested.

Thus excitatory activity within SNr and EP can initiate limbic seizures. Decreased excitation prevents the development of limbic seizures. These structures apparently also propagate the motor components of limbic seizures to the brainstem and spinal cord via PPN. The anatomical connections of LH and MD may provide a means of interaction between the motor and limbic systems for seizure regulation.

376.2 GLUCOSE USE IN THE DORSAL NUCLEUS ACCUMBENS SHELL (AN AREA HIGH IN MU-OPIOID RECEPTORS) INCREASES DURING KAINIC ACID-INDUCED LIMBIC SEIZURES BUT NOT DURING BICUCULLINE SEIZURES . L. Churchill, R. Cross,* R.P. Dilts, T. Pazdernik,* F. Samson, S. Nelson and P.W. Kalivas. Washington State Univ. and Univ. of Kansas Med. Ctr.

The dorsal nucleus accumbens shell has unique Nissl staining properties (Paxinos & Watson atlas, Plate 15, 1987), a high density of opioid receptors (Herkenham & Pert, Nature 291: 415, 1981) and efferent connections that differ from the lateral nucleus accumbens core (Groenwegen and Russchen, J Comp Neurol 223: 347, 1984). Mu-opioid receptor autoradiography was done using ^{125}I-Tyr-D-Ala-Gly-MePhe-Gly(ol)(DAGO) at 0.2 nM for 60 min at room temperature (displacement by 1 µM naloxone). Glucose use was assessed by the 2-deoxyglucose (2DG) method. Seizures were induced with kainic acid (12 mg/kg, i.p.) or bicuculline (0.6 mg/kg, i.v.). 2DG autoradiography was performed at 2 and 3.5 hr after injection of kainic acid, which corresponded to two different phases of seizure activity, or immediately after bicuculline, which corresponded to the active seizure phase. At 2 hr after kainic acid, rats were in repetitive limbic seizures, whereas at 3.5 hr after kainic acid, rats were engaged in searching and circling activity. During the repetitive seizures (2 hr), glucose use increased in the dorsal nucleus accumbens shell within similar boundaries to the high density of mu-opioid receptor binding. During the searching and circling behavior (3.5 hr), glucose use increased throughout the nucleus accumbens shell as well as within its efferent connections: lateral septum, bed nucleus of stria terminalis, paratenial and paraventricular thalamus, ventral tegmental area and pedunculopontine tegmental nucleus. During tonic seizures induced by bicuculline, a small region of the dorsal nucleus accumbens shell did not increase in glucose use, whereas the rest of the nucleus accumbens rather uniformly increased. A relationship of the GABA-receptive and mu-opioid receptive cells within the dorsal nucleus accumbens shell may be involved within the mesolimbic locomotor circuit, which includes the nucleus accumbens shell, ventral pallidum, ventral tegmental area and pedunculopontine tegmental nucleus. The increases in glucose use within the mesolimbic locomotor circuit during limbic motor seizures may involve mu-opioid receptors.

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376.3 COLCHICINE INJECTIONS INTO SUBSTANTIA NIGRA ACCELERATE KAINATE-INDUCED SEIZURE ONSET BUT ELIMINATE WET DOG SHAKES AND ATTENUATE HYPERSALIVATION. L. Grimes, C. Mitchell, P. Lee, and J. Hong. Toxicology Curr., UNC-CH, Chapel Hill, NC 27514 and Lab. of Behavioral and Neurological Toxicology, NIEHS/NIH, Research Triangle Park, NC 27709

Electrolytic lesions of the substantia nigra (SN) or those produced by local infusions of kainic acid (KA) or N-methyl-D-aspartate have been reported to attenuate seizure components induced by bicuculline, electroconvulsive shock (ECS) or kindling (Garant, D. and K. Gale, Brain Res., 273:156, 1983; McNamara, J. et al., J. Neurosci., 4:2410, 1984. In the present studies, the effects of colchicine (COL) (2.5 ug/site) injections into substantia nigra on seizure components induced by systemic KA and ECS were observed. Fifty-two male Fischer-344 rats were used in these studies. Three weeks after bilateral injections of COL or saline 2.0 mm lateral to midline into SN, rats were given 65 mA (duration =1.0 sec, pulses=50, pulse width=1.0 msec) of current through ear clips. Four days later, these rats were given 75 mA of current. Duration of forelimb and hindlimb tonic extension were measured. The duration of forelimb tonus was significantly reduced at 65 mA but not 75 mA in COL-injected rats. Duration of hindlimb tonus was significantly reduced at both levels of current in COL-treated rats. Two weeks after bilateral injections of COL or SAL 2.5 mm lateral to midline into SN, rats were injected with KA (8 mg/kg, sc) and were observed for 2.5 hr. In COL-treated rats, KA-induced wet dog shakes (WDS) were eliminated and hypersalivation was remarkably attenuated. Other seizure components did not differ from controls. This study was repeated using injections 2.0 mm lateral from midline. In treated rats, WDS were eliminated and hypersalivation attenuated as before, but the latency to onset of intense facial and forelimb clonus was significantly decreased. These studies indicate that COL lesions of SN produced effects on ECS threshold similar to those reported for other lesioning methods. However, its effects on KA-induced behaviors indicate that SN may be important for the relay of certain behaviors (WDS and hypersalivation) but that its more medial portions may have a gating or inhibitory effect on KA-induced seizure onset.

376.4 FETAL STRIATAL GRAFTS IN THE LESIONED RAT NEOSTRIATUM EXHIBIT IMMUNOREACTIVE GABA AND ENKEPHALIN NEURONS AND NADPH-DIAPHORASE POSITIVE CELLS. R.C. Roberts, A.W. Deckel and M. DiFiglia. Dept. of Neurology, Harvard Medical School and Mass. General Hospital, Boston, MA 02114 (RCR & MD) and Dept. of Psychiatry, Univ. of New Jersey, Newark, N.J. 07103 (AWD).

Previous anatomical, behavioral and biochemical studies have shown that fetal striatal cells transplanted into the neostriatum of the adult rat differentiate and partially restore motor dysfunction and some neurotransmitter deficits caused by excitotoxic lesions in the host caudate nucleus. The present study was undertaken to characterize the neurons within the transplants using immunocytochemical methods. Eight adult rats received intrastriatal unilateral or bilateral injections of 0.6 ul of a 250 mM or a 250nM solution of quinolinic acid. One week following the injections, tissue grafts of 17 day fetal striata were injected into the lesioned caudates. Six to ten weeks following the transplants tissue from the neostriatum was processed for the localization of immunoreactive GABA, enkephalin and NADPH-diaphorase activity. A separate series through each caudate was stained with cresyl violet. Omission of the primary antibody to GABA or enkephalin or preabsorption of the primary antibody with the appropriate antigen abolished all staining.

Results showed that in all rats immunoreactive GABA, enkephalin and NADPH-d positive neurons, processes and punctate structures were present within the grafts. GABA immunoreactive somata in the grafts were of medium size (14.7 um, n=30 neurons) and were relatively homogeneously distributed. Enkephalin immunoreactive neurons in the grafts were also of medium size (14 um, n=30 neurons) and were often distributed in clusters. NADPH-d positive neurons in the grafts were approximately 11.4 um in diameter (n=40 neurons). In ten 40 um thick sections selected from 3 different grafts GABA, enkephalin and diaphorase positive neurons ranged from 4-43%, 1-23% and 1.4-6.7% respectively of total grafted neurons per section as determined from counts of adjacent Nissl stained sections. These findings provide an anatomical basis for the functional recovery reported in other studies of fetal striatal transplants in rat caudate nucleus lesioned with excitatory amino acids. Supported by grants to MD from NIH (NS 16367) and the Hereditary Disease Foundation.

376.5 THE QUINOLINIC ACID MODEL OF HUNTINGTON'S DISEASE: DOSE DEPENDENT EFFECTS ON BEHAVIOR. M. Giordano, S.F. Calderon*, A.B. Norman and P.R. Sanberg, Laboratory of Behavioral Neuroscience, Departments of Psychiatry, Neurosurgery, Psychology and Physiology, University of Cincinnati College of Medicine Cincinnati, Ohio 45267-0559.

Rats which receive striatal injections of kainic acid show many of the anatomical, biochemical and behavioral abnormalities seen in patients with Huntington's disease (HD) (Sanberg and Coyle, CRC Crit. Rev. Neurobiol., 1, 1-44, 1984). Quinolinic acid (QA), an endogenous metabolite with excitotoxic properties has recently been shown to cause anatomical and biochemical changes in rats that are more consistent to those in HD (i.e., Beal et al., Nature 321, 168-171, 1986). Thus, the following study was performed to ascertain the behavioral changes of QA-induced striatal lesions in rats.

Male Sprague-Dawley rats (200-300 g) were assigned to one of four groups. Animals received bilateral striatal stereotaxic injections of QA (75nmol, 150nmol, or 225nmol in phosphate buffered saline, or vehicle alone) under pentobarbital anesthesia. Post-operative weights were monitored daily for two weeks. At two and four weeks post-lesion spontaneous nocturnal activity was monitored using Digiscan-16 monitors (Omnitech Electronics, Inc., Columbus, Ohio).

The 150nmol and 225nmol lesioned rats demonstrated aphagia and adipsia resulting in marked weight loss during the immediate post-operative period (the highest dose group needed to be fed intragastrically for a few days and in spite of this, about a third of the rats died). By four weeks all groups showed similar body weights. At both two and four weeks post-lesion, locomotor activity differences between the QA lesion groups and controls were evident and reached significance with respect to ambulation, rearing and stereotyped behaviors. At two weeks only the 150nmol lesioned group was significantly hyperactive compared to controls. By four weeks both the 150nmol and 225nmol lesion groups were hyperactive relative to both controls and the 75nmol lesioned rats in all three parameters analyzed, with the 225nmole rats tending to show the greatest activity. Interestingly, the 75nmol lesion group was slightly less active relative to controls, reaching significance on some activity measures (i.e., total horizontal activity and total number of vertical movements).

These results demonstrated that QA-induced striatal lesions produce behavioral changes similar to other excitotoxic striatal lesions in rats. Neurochemically, striatal QA lesions mimic the neurochemical pathology in HD to a greater extent than do other excitotoxins. The fact that these rats also demonstrated locomotor abnormalities supports the use of this model for basic studies on the pathogenesis and treatment of HD.

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376.6 THE IMMUNOHISTOCHEMICAL LOCALIZATION OF A CERULOPLASMIN LIKE SUBSTANCE IN THE HUMAN CENTRAL NERVOUS SYSTEM. L. R. Edelstein, Dept. of Med., SUNY at Stony Brook, and F. J. Denaro, Dept. of Peds/Inf. Dis., UCSD Med. CT., San Diego, Ca.

Ceruloplasmin (CP) is a protein found in the alpha 2 globulin fraction of human serum. Research has revealed the multifunctional properties of this enzyme. Evidence supports its role in : 1). Copper metabolism and transport, 2). The mobilization and oxidation of iron, 3). It is a serum anti-oxidant, 4). It is a modulator of inflammatory response, and 5). There is evidence that CP can regulate the concentration of biogenic amines (noradrenaline and serotonin). Clinical and biochemical studies have associated CP with a number of neurologic diseases. Parkinson's disease and Huntington's disease are two diseases in which CP is believed to play a part. Further evidance for CP involvement in CNS diseases is deduced from animal experiments. It has been found by use of tritiated CP that cells of the CNS can sequester CP from the blood. While there has been extensive biochemical and clinical research on CP, there has been no immunocytochemical studies of CP in the brain.

In the present study, human autopsy material was examined by immunohistochemistry for evidence of CP staining cells. Tissue which was obtained at time of autopsy was fixed in 10% NBF. It was then paraffin embedded and sectioned. The ABC method or the PAP method was used for the immunocytochemical detection of CP. The CP primary anti-sera was obtained from DAKO and was used at dilutions of 1:500 and 1:1000. CP from Sigma was used in competion controls.

Briefly, the results show : 1. Cortex - negative. 2. Caudate and Putamen - no cells showed staining but the neuropile showed a much higher level of staining than other areas of the brain. 3. Substantia nigra - discretely staining cells and their processes were found in this nucleus. 4. Pontine N. - discretely staining cells were found in this nucleus.

Both the suggested functions of CP and the anatomical locations in which CP has been found in this study, support the theory that CP may be involved in such neurologic diseases as Parkinson's and Huntingtons's disease. In view of this, on going research is not only directed at investigating the distribution of CP in the normal brain but in various disease states as well.

We thank DAKO for supplying the immunocytochemicals and the UCLA Neurologic Tissue Bank for supplying some of the tissue used in this study.

376.7 DIFFERENTIAL LOSS OF SUBSTANCE-P-CONTAINING AND ENKEPHALIN-CONTAINING STRIATOFUGAL PROJECTIONS IN ADULT-ONSET AND JUVENILE-ONSET HUNTINGTON'S DISEASE.

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Huntington's disease (HD) is characterized by prominent neuron loss in the striatum. Recent evidence (Kowall et al. TINS 10:24-29, 1987) indicates that striatal neuron loss is not uniform and that somatostatin-NPY-containing NADPH diaphorase positive interneurons and possibly cholinergic interneurons are relatively spared. The majority of striatal neurons, however, are projection neurons. These are progressively depleted in HD and show the earliest evidence of abnormality. To determine if there is differential loss of striatal projection neurons we undertook an immunohistochemical study of peptidergic striatofugal projections in HD basal ganglia and substantia nigra obtained at autopsy.

We studied five pathologically verified adult-onset cases of HD, two juvenile-onset cases of HD, and several matched control cases. Antisera directed against substance-P, Leu-enkephalin, Met-enkephalin-Arg-Gly-Leu were utilized and tissue was stained using the PAP technique.

In all control cases the normal pattern of dense fiber staining for enkephalins in the lateral pallidum and for substance-P in the medial pallidum and substantia nigra was observed. In all adult-onset HD specimens marked depletion of enkephalin fiber staining in the lateral pallidum and substance-P fiber staining in the substantia nigra pars reticulata was observed. The substance-P fiber staining in the medial pallidum and substantia nigra pars compacta was relatively preserved. In the juvenile-onset HD specimens both the enkephalin and substance-P fiber staining in the lateral and medial pallidum, respectively, were depleted.

These results indicate differential loss of striatal projection neurons in adult-onset but not juvenile-onset HD. This could aid in the identification of the biologic defect underlying HD and provides additional criteria for the evaluation of current models of HD pathogenesis. In addition, these results help to account for the chorea and oculomotor disturbances seen in adult-onset HD, and the lack of chorea characteristic of juvenile-onset HD.

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376.8 LANGUAGE FUNCTIONS IN HUNTINGTON'S DISEASE. K. Podoll* and J. Noth. Department of Neurology, Alfried Krupp Clinic, Essen, FRG.

A comprehensive language test battery (Aachen Aphasia Test) was administered to 45 patients with early, middle and later stage Huntington's disease (HD) and 20 control subjects. In spontaneous speech, many HD patients exhibited a loss of conversational initiative which markedly contributed to their communicative disability. Dysarthria was a common finding, leading to occasional phonemic paraphasias. Reading skills were found to be impaired mainly as a consequence of dysarthria; some HD patients displayed visual dyslexia. In addition to the characteristic disturbances of writing skills due to the choreatic movement disorder, the writing of HD patients with advanced dementia indicated constructional dysgraphia, characterized by frequent omissions, perseverations and substitutions. HD patients exhibited no evidence of word-finding difficulty or other semantic deficits in spontaneous speech, but there was a marked impairment in visual confrontation naming with a significant rise of naming error rate across the three stages of HD. In most instances, the inproper names referred to an object visually similar to the target object, suggesting visual misperception as the main mechanism of the naming disorder in HD. Syntactical structure of spontaneous speech was typically reduced to short, simple sentence constructions. Verbal stereotypies were only rarely encountered and occurred late in the course of the disease. Tests of language comprehension reflected the general degree of dementia. In conclusion, there are apparently no primary language changes in HD, but a variety of language impairments developes secondary to other neurologic and neuropsychological changes.

376.9 EFFECTS OF SENSORY CUES AND L-DOPA ON GAIT MOVEMENT AND MUSCLE ACTIVATION PATTERNS IN PATIENTS WITH PARKINSON'S DISEASE. R. Lemieux*, C.L. Richards, P.J. Bédard, F. Malouin and M. Cioni*, Neurobiology Laboratory, Faculty of Medicine, Laval University, Quebec, G1K 7P4, and Pharmacology Institute, Medical School, University of Catania, Italy.

We studied the changes in bradykinetic movement and muscle activation patterns during gait in 11 patients with Parkinson's Disease in 2 conditions when "off" L-Dopa: 1. free gait, 2. with visual guidance by placing markers on the walkway or with auditory cues provided by a metronome. Free gait was also studied when "on" L-Dopa. Movements of the hip, knee and ankle in the sagittal plane were recorded by means of an electrogoniometer. Surface electrodes were placed on the quadriceps (Q), hamstrings, triceps surae and tibialis anterior (TA) muscles and the myosignals were fed to a Grass polygraph for amplification, rectification and time-averaging prior to being sent to a PDP 11/23 computer for recording and analysis. To define the gait cycle, signals from electronic footswitches were recorded concomitantly with the angular displacements and muscle activations as the patients walked along an 8 m walkway. At least 10 gait cycles were recorded first "off" L-Dopa (at least 12 hrs), and then consecutively with visual guidance and auditory cues and finally when "on" L-Dopa (1-2 hrs). The main finding was that visual and auditory cues induced changes in the bradykinetic (off L-Dopa) movement and muscle activation patterns which were similar in nature to those observed when "on" L-Dopa. These EMG changes varied among the patients; in some both an increased amplitude and timing modification were observed, while in others mainly the amplitude of the activation was affected. Differential effects were, however, observed among the patients for the gait conditions. The EMG changes were observed throughout the gait cycle and in the four muscles studied but could be found predominantly in proximal or distal muscles. For example in the TA, all the patients showed improvements in the activation when "on" L-Dopa, or with visual guidance, while one did not respond to auditory cues. On the other hand in Q, while all the patients demonstrated some changes with auditory cues, in 3 patients little change was observed with L-Dopa or visual guidance. These results demonstrate that, like L-Dopa, increased sensory inputs are capable of modifying both the timing and amplitude of muscle activations of bradykinetic gait patterns typical of Parkinson's Disease. Whether these modifications are mediated by similar mechanisms remains to be determined. This work was supported by a grant from Laval University.

376.10 CORRELATION BETWEEN HISTOLOGICAL AND ELECTROPHYSIOLOGICAL CHANGES IN OUTPUT NEURONS OF THE BASAL GANGLIA IN A PARKINSONIAN MONKEY. L. Tremblay and M. Filion, Lab. Neurobiol., Laval Univ. and Enfant-Jésus Hosp., Québec, Canada, G1J 1Z4.

We are studying the activity of globus pallidus neurons in monkeys (Macaca fascicularis) rendered parkinsonian by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Two animals (C and D) were studied only in the intact state, two (A and E) only in the parkinsonian state, and one (B) in both the intact and parkinsonian states. Sections of the brain of these animals were stained for Nissl bodies with cresyl violet. In the intact and in two of the three parkinsonian animals, neurons were similar in the internal (GPi) and external (GPe) segments of the globus pallidus and in the subnucleus lateralis of the pars reticulata of the substantia nigra (SNsl). In one parkinsonian animal (E), however, the majority of GPi and SNsl neurons were hyperchromatic and slightly shrunken. In GPe, inversely, the majority of neurons were hypochromatic, globular and vacuolar. Counts of nigral neurons of the compacta-type showed losses of 86%, 99% and 90% in monkeys A, B and E, respectively. This demonstrates that the neurotoxic effects of MPTP were similar in the three parkinsonian monkeys, and certainly not greater in monkey E. However, this last animal was sacrificed 14 months after MPTP, whereas monkeys A and B were sacrificed as early as 5 weeks after MPTP. Therefore, the histological changes can be associated with the long duration of parkinsonism in monkey E. An explanation for these changes is suggested by electrophysiological data from the same animals. The mean firing rate of GPi neurons was higher in parkinsonian (109/s, n=91) than in intact monkeys (77/s, n=83). A few SNsl neurons were recorded in parkinsonian monkeys and also exhibited high firing rates. Conversely, GPe neurons exhibited a lower firing rate in parkinsonian (43/s, n=123) than in intact monkeys (74/s, n=97). In parkinsonian monkeys, a group of neurons with relatively low firing rates were recorded in areas of GPi where neurons were not hyperchromatic. In conclusion, the hyperactivity of GPi and SNsl neurons and the hypoactivity of GPe neurons in parkinsonism are likely to give rise, over a period of several months, to corresponding histological changes. Supported by the MRC of Canada.

376.11 MPTP-INDUCED PARKINSONISM IN THE MOUSE: INSIGHT INTO THE PATHOPHYSIOLOGY OF DOPAMINERGIC CELL DEGENERATION. K.F. Manaye, P. Sonsalla, G. Barnett*, M.D. Baring*, R. Heikkila, and D.C. German. Depts. of Psychiat. and Physiol., U. of Texas Health Sci Cntr., Dallas, TX 75235 and Dept. of Neurol., Rutgers Medical School, Piscataway, N.J. 08854.

1-Methyl-4-(2'-methylphenyl)-1,2,3,6-tetrahydropyridine (2'-CH3-MPTP), a substituted analog of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), has been found to be significantly more toxic to midbrain dopamine (DA)-containing neurons than MPTP in the mouse (Youngster et al. Eur.J.Pharmacol. 122:283, 1986). The purpose of the present experiment was to measure the neurotoxic effects of 2'-CH3-MPTP at both the DA cell body and axon terminal region. In the first experiment we investigated the effect of 2'CH3-MPTP (2 X 20 mg/kg, i.p.) on the pattern of midbrain DA cell loss and levels of forebrain DA in BALB/c mice. After approximately 2 weeks, the animals were sacrificed and 30 um thick sections were cut through the midbrain DA cell complex and stained with an antibody to tyrosine hydroxylase (TH). The location of each TH-containing neuron was mapped on one side of the brain, using computer graphics equipment, in 4 normal and 5 treated mice. There was a significant loss (37%) of midbrain DA neurons in the treated mice (mean $\pm$ SEM = 2976 $\pm$ 241 vs. 4693 $\pm$ 345 cells). In these same mice there was a 47% loss of striatal DA and a 23% loss of nucleus accumbens DA. In the second experiment we measured the effect of 2'CH3-MPTP (2 X 20 mg/kg/day every other day X 3) on DA and DA metabolite levels in the midbrain and in the forebrain (4-5 mice/group). There was a 74 $\pm$ 8% loss of striatal DA, a 72 $\pm$ 6% loss of forebrain DA, but only a 25 $\pm$ 10% loss of midbrain DA. Both DOPAC and HVA levels were comparably affected in the forebrain and midbrain. In conclusion: (1) there was a marked loss of DA neurons within the substantia nigra, and also loss within the ventral tegmental area and retrorubral area as judged by both TH- and Nissl-staining; and (2) there was a much greater loss of DA in the forebrain than in the midbrain. These data are consistent with the hypothesis that the neurotoxicity begins initially at the level of the axon terminal. Research supported by the NIH (MH-30546, NS-21752), Biological Humanics Foundation and Dallas Area Parkinsonism Society.

376.12 ANATOMICAL SPECIFICITY OF THE EFFECTS OF L-DOPA IN MPTP-INDUCED HEMIPARKINSONISM. L.J. Porrino[1,2], E. Palombo[2]*, K.S. Bankiewicz[3], and I.J. Kopin[3]. [1]Addiction Research Center, NIDA, Baltimore, MD 21224 and [2]Lab. Cerebral Metabolism, NIMH; [3]Surgical Neurology Branch, NINCDS, Bethesda, MD 20892.

L-DOPA treatment ameliorates the clinical signs that accompany the hemiparkinsonism produced by injection of MPTP into one carotid artery in rhesus monkeys. L-DOPA also reverses the direction of spontaneous circling occurring in this syndrome. Local cerebral glucose utilization (LCGU) was determined in 5 MPTP-induced hemiparkinsonian monkeys treated with L-DOPA (200 mg L-DOPA/20 mg carbidopa) approximately 30 min prior to the 2-(^{14}C)deoxyglucose procedure for the measurement of LCGU. The behavioral response to L-DOPA, including reversal of rotational direction, and elimination of contralateral bradykinesia, rigidity and tremor, was maximal in 2 monkeys, intermediate in 2 and virtually absent in one other. The pattern of LCGU changes in the L-DOPA-unresponsive monkey was identical to that seen in untreated hemiparkinsonian monkeys. In those animals responding to L-DOPA, LCGU was increased ipsilaterally in the substantia nigra reticulata, subthalamic nucleus, and medial globus pallidus, and decreased in lateral habenula. L-DOPA treatment did not reverse the increase in LCGU in the lateral globus pallidus seen in untreated hemiparkinsonian monkeys. In addition to these alterations common to all responding monkeys, in those animals displaying maximal response to L-DOPA, intense increases in LCGU were found in anatomically discrete loci within the ipsilateral basal ganglia and thalamus: (1) the medial one third of the substantia nigra reticulata throughout its rostrocaudal extent, (2) a highly circumscribed region within the most ventromedial portion of the subthalamic nucleus, (3) the reticular nucleus of the thalamus, and (4) a region within the medial globus pallidus adjacent to the dorsal medial medullary lamina.

On the basis of these data, there appears to be a strong correlation between the degree of behavioral repsonse to L-DOPA in hemiparkinsonian monkeys and the presence of a distinctive pattern of anatomically specific alterations in glucose utilization.

376.13 MPTP-INDUCED DOPAMINE DEPLETIONS ALTER SENSORY PROCESSING IN THE CAUDATE NUCLEUS IN THE AWAKE CAT. J.S. Schneider and C.H. Markham. Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Over the past several years, we have shown the basal ganglia's (BG) sensory processing capabilities to be quite complex. We have demonstrated that assessment of sensory processing capabilities of the BG can be a very sensitive measure of the functional integrity of this system. The present study has investigated whether disturbance of nigrostriatal dopaminergic activity results in defects in sensory processing in caudate nucleus (CD) neurons concomitant with sensorimotor behavioral disturbances.

Two hundred-six cells were recorded from the CD of 2 normal adult cats. These baseline recordings served to characterize the sensory responsiveness of CD neurons. Twenty-two percent of CD cells responded to tactile stimulation of the face, while 4% and 8% responded to visual and auditory stimulation, respectively. Tactile responsive cells had mostly small, discrete receptive fields. However, cells with moderate to large receptive fields had the characteristic ability to encode stimulus location on the face relative to the front of the mouth.

These cats were then given 9 injections of MPTP·HCl (5 mg/kg, i.p.) every other day. A total of 255 cells were recorded from the 2 cats during the entire post-MPTP period. In both cats, CD unit sensory responsiveness was comparable to that in the pre-MPTP state during the first 3 post-MPTP recording sessions before signs of sensory or motor impairment were evident. However, over the next 6 recording sessions, only 6% of CD cells in cat #1 and 2% in cat #2 had responses to facial tactile stimulation. Responsive cells had large receptive fields and could not encode stimulus location information. Less than 2% of sampled cells responded to visual or auditory stimulation as well. CD cells in neither animal showed distinct changes in spontaneous activity after MPTP administration. During the period of decreased sensory neuronal responsiveness, animals would no longer orient to visual, tactile, or auditory stimuli, were akinetic, and froze frequently while attempting movement.

Neurochemical analyses showed CD dopamine depletions of 92% and 95% in the 2 cats and tyrosine hydroxylase immunohistochemistry revealed extensive loss of substantia nigra pars compacta neurons and moderate loss of retrorubral neurons. These results demonstrate distinct quantitative and qualitative changes in cat CD neuronal responses to sensory stimulation after MPTP-induced dopamine depletions. These neurophysiological alterations appear to underlie the sensorimotor behavioral disturbances observed in these animals. Supported by USHHS Grant MH41645.

376.14 BROMOCRIPTINE: IN VITRO AND IN VIVO EVIDENCE FOR A SELECTIVE D-2 ACTION. J.M. Trugman, N. Touchet*, and G.F. Wooten. Department of Neurology, University of Virginia, Charlottesville, VA 22908.

Bromocriptine mesylate is a clinically effective antiparkinson agent, yet questions remain regarding its mechanism of action. In addition to being a D-2 dopamine receptor agonist, bromocriptine has been reported to be a D-1 antagonist (Markstein, J. Neural. Transm. 51:39-59, 1981). If true, this D-1 antagonist action would represent a unique property among clinically used antiparkinson drugs. We studied the ability of bromocriptine to compete for D-1 and D-2 receptors using quantitative in vitro autoradiography. The in vivo effects of bromocriptine were studied by ^{14}C-2-deoxyglucose (2-DG) autoradiography in rats with unilateral substantia nigra (SN) lesions.

Serial 20 µm sections through the striatum and substantia nigra pars reticulata (SNr) were incubated in PBS with either 0.3 nM ^{3}H-SCH 23390 (to label the D-1 receptor) or 1.0 nM ^{3}H-spiperone (to label the D-2 receptor) and 11 concentrations of bromocriptine (10^{-12} to 10^{-5} M). Nonspecific binding was defined using 2 µM (+)butaclamol for the D-1 site and 2 µM ADTN for the D-2 site. Bromocriptine potently competed for ^{3}H-spiperone binding in striatum with a K_i value of 2.8 nM. In contrast, bromocriptine did not effectively compete for ^{3}H-SCH 23390 binding in either striatum (K_i=21.6 µM) or SNr (K_i=30.9 µM).

Rats with 6-hydroxydopamine SN lesions were administered bromocriptine (10 mg/kg i.v.), followed one minute later by ^{14}C-2-DG (25 µCi/0.4 ml saline i.v.). Administration of bromocriptine resulted in contralateral rotation beginning immediately after the i.v. injection (mean 4.2 rotations per minute). RCGU remained unchanged in the entopeduncular nucleus and SNr ipsilateral to the lesion, a pattern characteristic of the selective D-2 agonist quinpirole (LY 171555) and differing from the marked RCGU increases produced by L-dopa or the D-1 agonist SKF 38393 (Trugman and Wooten, Brain Res. 379: 264-274, 1986 and Soc. Neurosci. Abs. 12:874, 1986). Bromocriptine lowered RCGU in the lateral habenula bilaterally (+54% ipsilateral to the lesion, +30% contralateral) and increased RCGU in the subthalamic nucleus ipsilateral to the lesion (+21%). Thus, in the entopeduncular nucleus and SNr, two critical basal ganglia outflow nuclei, bromocriptine produces different metabolic effects than does L-dopa.

Based on in vitro binding studies, bromocriptine has high affinity for D-2 receptors (K_i 2.8 nM) and low affinity for D-1 receptors (K_i 21.6 µM) in rat striatum, with a ratio of affinities >1000. In vivo, bromocriptine mimics the RCGU pattern of the selective D-2 agonist quinpirole. Together these studies suggest that bromocriptine is a selective D-2 agonist and does not interact with the D-1 receptor.

376.15 HLA-DR REACTIVE MICROGLIA IN PARKINSON'S DISEASE AND OTHER EXTRAPYRAMIDAL DISORDERS. P.L. McGeer, S. Itagaki* and E.G. McGeer. Kinsmen Lab. of Neurol. Res., Dept. of Psychiatry, Univ. of British Columbia, B.C., Canada, V6T 1W5

HLA-DR is a class II cell surface glycoprotein of the human histocompatibility complex usually expressed on the surface of cells that are simultaneously presenting foreign antigen to T-lymphocytes. Using double immunohistochemical staining for HLA-DR and various neurotransmitter markers, we have examined the relationship between reactive microglia and degenerating neurons in a number of basal ganglia disorders. Using antibodies to tyrosine hydroxylase and HLA-DR, we have examined the dopaminergic nigrostriatal system in Parkinson's disease with and without dementia, parkinsonism-dementia of Guam, and Alzheimer's disease without parkinsonian features. HLA-DR positive microglia were observed in the substantia nigra pars compacta (SNC) of patients with all forms of parkinsonism but not in the SNC of most controls and Alzheimer's cases. Some elderly controls and some Alzheimer's cases showed limited HLA-DR positive staining in the SNC, suggesting an early, presymptomatic stage of parkinsonism. In the various parkinsonian syndromes, HLA-DR positive microglia could be seen phagocytosing dopaminergic neurons and their processes. Positive staining was also observed in the SNC of parkinsonian cases with antibodies to the interleukin-2 receptor. In Huntington's disease (HD), HLA-DR positive microglia could be seen in the region of degenerating somatostatin-containing neurons in the caudate and of substance P-containing fibers in the substantia nigra. In HD, HLA-DR positive microglia were not seen phagocytosing tyrosine hydroxylase-containing neurons of the substantia nigra. These data indicate that an active neuropathological process, mediated by the T-lymphocytic system, is destroying SNC cells in the parkinsonian syndromes and neostriatal cells in HD.

(Supported by grants from the Medical Research Council of Canada and the Alzheimer's Association of B.C.)

376.16 VISUALIZING PRIMATE BASAL GANGLIA WITH MAGNETIC RESONANCE AFTER MANGANESE ADMINISTRATION. M.C. Newland*, J.H. Kordower, T.L. Ceckler*, and B. Weiss. University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Manganese administration enabled the highlighting of regions associated with the extrapyramidal motor system when visualized with magnetic resonance imaging (MRI). This highlighting appeared in a m. fasicularis after inhalation (Newland et al., The Toxicologist, 7:157, 1987). We now replicate and extend this observation using iv exposure and an additional primate species. A cebus monkey and a m. fasicularis were administered 10 mg/kg of Mn (as $MnCl_2$) iv either 2 days or 6 days prior to MRI. T1-weighted images (TR/TE = 450/16) taken 6 days after Mn administration produced highlighting of the globus pallidus, striatum, substantia nigra, and subthalamic nuclei, indicating selective accumulation or retention of Mn in the highlighted regions. Two days after Mn administration only slight contrast was noted. Regional estimates of T1s were employed to quantify the selectivity and clearance of manganese in the extrapyramidal system. In separate experiments the clearance rate of manganese was observed to be faster in the striatum than in the other regions of the extrapyramidal motor system. The brain of an unexposed control monkey was also imaged using identical parameters, but no highlighting appeared. Both the kinetics and dose of systemically administered manganese are important in determining the quality of the images obtained. Although excessive doses of manganese are neurotoxic, it may be possible to exploit its kinetics to enhance contrast at lower doses or to lower its levels in brain with a complexing agent. The utility of this phenomenon in assessing MPTP toxicity and subsequent treatment with adrenal medullary implants in nonhuman primates are under investigation. [Supported by AO5188, ES01248, CA40699, General Electric Corporation, and the John Douglas French Foundation.]

376.17 NIGROTECTAL GABA UTILIZATION RESPONDS TO STRIATAL DOPAMINE RECEPTOR SUPERSENSITIVITY. S. E. Bachus, N. Hayman* & K. Gale. Dept. Pharmacology, Georgetown Univ. Sch. Medicine & Dentistry, Washington, D.C. 20007.

Systemic dopamine (DA) agonists have been shown to reduce GABA turnover in the nigrotectal terminal region in deep layers of superior colliculus (SC)[1]. This effect is relayed via substantia nigra and is dependent upon nigral GABA transmission. In the present studies we investigated whether behavioral supersensitivity to DA agonists would be associated with enhanced sensitivity to the apomorphine (APO)-induced decrease in tectal GABA turnover.

Adult male Sprague-Dawley rats received unilateral microinfusions of 6-hydroxydopamine (6-OHDA) or saline into the medial forebrain bundle, under Equithesin anesthesia. Successful lesions resulted in > 95% depletion of striatal DA, a condition associated with marked behavioral supersensitivity to APO. Unsuccessfully lesioned rats were included with sham lesioned rats. At least 2 weeks later, GABA turnover in superficial and deep layers of SC was estimated by measuring the accumulation of GABA 90 min after local microinfusion of the irreversible GABA transaminase inhibitor, gamma-vinyl-GABA.[1] During this 90 min period, rats received 3 s.c. injections of either APO (1 mg/kg) or water.

APO (1 mg/kg) proved subthreshold for reducing GABA turnover in deep layers of SC in sham lesioned rats, as compared to sham lesioned rats injected with water (water:69.41 + 2.33, APO:66.97 $\pm$ 3.98). However, this dose significantly ($p<.005$) reduced, by 19.3%, GABA turnover in deep layers of SC in 6-OHDA lesioned rats, relative to lesioned rats receiving water (water:75.36 + 3.63, APO:60.83 + 2.65). This reduction is comparable to that seen in nonlesioned rats following 3 injections of 3 mg/kg APO.[1]

A reduction in GABA turnover is found also in superficial layers of SC, in response to APO (3 X 3 mg/kg), but this effect is independent of nigral GABA transmission.[1] Thus, we also examined effects of 6-OHDA lesions on responsiveness of GABA turnover in superficial layers of SC, which do not contain nigrotectal GABA terminals, to determine the anatomical specificity of the supersensitivity. The 1 mg/kg dose of APO did not significantly affect GABA turnover in superficial layers of SC in either 6-OHDA lesioned rats (water:81.72 + 5.79, APO:89.67 + 6.44), or sham lesioned rats (water:93.68 $\pm$ 6.57, APO:85.15 $\pm$ 5.57).

We conclude that nigrotectal GABA transmission is responsive to alterations in striatal DA receptor sensitivity. Moreover, this effect is specific to the target region of GABAergic tectal afferents from substantia nigra, consistent with mediation via nigral relays. It should prove interesting to explore whether other treatments which affect DA receptor function, such as chronic neuroleptic exposure, also shift responsiveness of nigrotectal GABA projections. [1]Melis & Gale, J.P.E.T. 226:425, 1983)

376.18 ENHANCED SUSCEPTIBILITY OF DOPAMINE NEURONS TO DEPOLARIZATION BLOCK AFTER PARTIAL DOPAMINE LESIONS. J.R. Hollerman and A.A. Grace, Depts. of Behavioral Neuroscience & Psychiatry, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

Acute administration of haloperidol (HAL) to rats is believed to activate nigral dopamine (DA) neurons via striatonigral feedback pathways. In contrast, repeated HAL treatment leads to a time-dependent inactivation of DA cell firing as a result of this maintained depolarization. This depolarization block (DB; Bunney & Grace, 1978) can be induced in acute preparations, but extreme depolarizing conditions, such as the extended iontophoresis of excitatory neurotransmitters or intracellular injection of depolarizing current (Grace & Bunney, 1986). Nonetheless, acute administration of HAL alone, even at high doses, will not induce DA cell DB. We now report that acute HAL administration can induce DB if the DA system is first compromised by partial lesioning of nigrostriatal DA afferents.

Albino male rats, following DMI and pargyline pretreatment, received bilateral i.c.v. injections of 200 ug 6-hydroxydopamine (6-HDA) or vehicle. Behavioral assessments of akinesia were made 4-8 days and 4-6 weeks postlesion, followed by extracellular recordings from identified nigral DA neurons. The effects of HAL on DA cell activity were examined in each group by administering HAL i.v. in increasing doses, with maximal doses of 5 mg/kg. In control or vehicle-injected rats, HAL increased DA cell firing rates and induced burst firing, but did not induce DB. In contrast, after behavioral recovery following striatal DA lesions of 80% or more, HAL in doses of 0.1 to 0.2 mg/kg i.v. also caused DA cells to increase firing rates, with a progressive reduction in spike amplitude and prolongation of spike duration, until there was complete cessation of spontaneous spike activity. The absence of cell activity after HAL was not due to the cell drifting away from the electrode, since DB was always preceded by lengthened spike duration and an increase in firing frequencies. Furthermore, administration of the DA agonist apomorphine, which normally inhibits DA cells, caused these inactivated cells to begin firing at fast rates. Subsequent doses of apomorphine then produced their typical inhibition of DA cell firing.

Previous studies in our group have shown that, after recovery from 6-HDA lesions, akinesis could be reinduced by administering HAL at doses subthreshold to those required to induce akinesia in nonlesioned rats (Snyder et al, 1985). The experiments reported here suggest that this enhanced lability to DA antagonists is due at least in part to the constraints placed on DA neurons secondary to the compensations in activity required for behavioral recovery. Indeed, DA neurons partially compensate for DA loss after lesions by increased levels of activity in the remaining DA neurons (Hollerman et al, 1986). However, as a consequence of this compensatory mechanism, the DA neuronal population is compromised in its ability to respond to acute stressors. This is reflected in the enhanced susceptibility of DA cells to DB after partial DA lesions. This finding may have particular relevance to clinical pathologies, such as in the stress-induced exacerbation of Parkinson's disease symptoms. (Supported by NS19608)

376.19 ALTERATIONS IN SUBCORTICAL DOPAMINERGIC FUNCTION FOLLOWING DOPAMINE DEPLETION IN THE MEDIAL PREFRONTAL CORTEX. D. L. Rosin*, A. Y. Deutch, and R. H. Roth (SPON: E.F.S. Kaufman). Depts. of Pharmacology and Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06510.

Behavioral studies suggest that the different forebrain dopamine (DA) projections subserve different functions. However, there is a growing body of literature which suggests that there may be a functional interdependence of the mesocortical, mesolimbic, and nigrostriatal DA systems. In particular, 6-hydroxydopamine (6-OHDA) lesions of the prefrontal cortex (PFC) have been reported to result in an increase in DA turnover in the striatum. DA in the PFC appears to hold cortical projection neurons under tonic inhibition; disruption of such a regulatory control over corticostriatal glutamatergic projections may be expected to have profound consequences for striatal DA function. We have therefore attempted to characterize the effect of selective DA depletion in the PFC on various biochemical indices of subcortical DA function. Adult male rats received 10 ug 6-OHDA in 2.5 ul ascorbate-saline into the PFC, 30 min after pretreatment with desmethylimipramine (25 mg/kg, ip) to minimize concurrent depletion of the cortical noradrenergic innervation. Rats were sacrificed two weeks later and striatal levels of DA and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were measured. In a second study, in vivo tyrosine hydroxylation was estimated by measuring DOPA accumulation after decarboxylase inhibition in both lesioned and vehicle-injected controls. In neither experiment was striatal DA function altered, as reflected by alterations in DOPAC, DOPAC/DA, or DOPA accumulation. A third experiment was therefore performed in which in vitro tyrosine hydroxylase activity was measured in tissue from lesioned and control animals, and from control and lesioned animals treated with haloperidol (100 ug/kg) in order to submaximally activate TH. TH activity was assayed at pH 7.0 under two different cofactor concentrations (0.1 mM and 1.0 mM). Furthermore, in this experiment the medial strip of the striatum (i.e., that region of the striatum which receives projections from the PFC) was dissected separately from the lateral (non-PFC recipient zone) striatum; the nuc. accumbens was also removed. TH activity at both cofactor concentrations was markedly increased by haloperidol treatment. However, DA depletion in the PFC did not result in an increase in TH activity in either the medial or lateral striatum, nor in the nuc. accumbens. These data therefore suggest that specific 6-OHDA lesions of the PFC do not result in significant alterations in striatal or mesolimbic DA function. These studies may suggest that disturbances of affect in schizophrenic reactions are not secondary to primary dysfunction of the PFC. (Supported in part by MH-14092, the American Parkinson's Disease Association, Tourette Syndrome Assn., Scottish Rite Schizophrenia Research Program, and Pharmaceutical Manufacturer's Association).

GLIA IV

377.1 α-AMINOISOBUTYRIC ACID TRANSPORT IN ASTROCYTE MONOLAYER CULTURES. N. Brookes. Dept. of Pharmacol. and Exptl. Therap., Univ. of Maryland Sch. of Med., Baltimore, MD 21201.

Glia possess high-affinity, Na-dependent transporters for a number of neurotransmitters. The function of these systems in relation to neurotransmission have attracted much interest. By contrast, the systems responsible for transport of non-neurotransmitter amino acids in glia have received little attention. α-Aminoisobutyric acid (AIB) is a non-metabolized, synthetic amino acid often used to study neutral amino acid transport. Stöcklin et al. (Soc. Neurosci. Abstr. 11: 394, 1985) reported evidence that AIB is taken up in rat brain astrocytes by a single, Na-dependent system. The present study shows that transport of AIB in mouse brain astrocytes is predominantly independent of Na.

Primary cell suspensions derived from cerebral hemispheres of newborn mice were plated in 35mm collagen-coated plastic dishes and grown to confluence (2-3 weeks) in medium supplemented with 15% fetal calf serum. The monolayer cultures were washed and equilibrated in Tris-buffered (20mM) salts solution (pH 7.0) for 10 min at 34.5°C before addition of ^{14}C-labeled AIB. Na-free solutions were choline-substituted. Na-independent uptake was substantial and consistent in all cultures, whereas Na-dependent uptake was negligible in most batches and variable in a few. The Na-dependent component was not increased by treatment with insulin 0.1 μM and glucagon 0.1 μM or by removal of amino acids for 3 hr before uptake measurement. (Amino acid deprivation and hormone treatment stimulate the ubiquitous A system for neutral amino acid transport; Christensen, Biochim. Biophys. Act. 779: 255,1984).

The initial rate of ^{14}C-AIB uptake in Na-free solution was linear for 20-30 min at 0.2 mM AIB, and for 10 min at 5 mM AIB. A concentration curve was constructed in the range 0.05-10 mM AIB, using 10 min incubations to determine initial rate. These data closely approximate the theoretical curve for a single saturable process with K_m of 5.5 mM and V_{max} of the order of 50 nmol/min/mg protein, similar to the kinetic parameters for Na-dependent uptake found by Stöcklin et al. However, not only were the present kinetic parameters determined in Na-free solution, but ^{14}C-AIB uptake (0.2-5 mM AIB) was 90% inhibited by 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid (BCH, 10 mM), a selective inhibitor of Na-independent L systems. Uptake appeared unusually concentrative for an L system. An astrocyte culture with a cell water content not exceeding 0.5 μL was able to clear 10 μL of incubation solution (0.2 mM AIB) in 10 min. Whether the marked contrast with the results of Stöcklin et al. is based on differences in species, cell type or conditions of culture and uptake remains to be determined. (Supported by MIH grant ES03928).

377.2 ADENOSINE UPTAKE BY LRM55 ASTROGLIAL CELL. W. Shain and V. Madelian. Lab. of Neurotoxicology & Nervous System Disorders, Wadsworth Center for Labs and Research, New York State Department of Health, Albany, NY 12201.

Neurons and glia respond to exogenously applied adenosine. We have previously demonstrated that stimulation of adenosine receptors on LRM55 astroglial cells results in activation of adenylate cyclase and subsequent release of taurine. Analysis of intracellular adenine nucleotides after long-term exposure of cells to adenosine (=>30 min) indicated a significant accumulation of adenosine. Examination of the kinetics of adenosine uptake into LRM55 astroglial cells revealed that the apparent Michaelis constant (K_m) for uptake decreased with the length of exposure to adenosine (K_m > 1200 uM at 0.5 min, = 254 uM at 10 min, = 46 uM at 30 min). Adenosine uptake was independent of adenine uptake since at equimolar concentrations adenine did not inhibit adenosine uptake. When tested at equimolar concentrations adenosine uptake was not significantly inhibited by other nucleosides (thymidine, uridine, inosine, guanidine, and hypoxanthine). However, equimolar concentrations of AMP, ADP, and ATP inhibited uptake by approximately 30%. cAMP did not inhibit uptake. Of the adenosine receptor analogs tested, only cyclopentyl-, cyclohexyl-, and chloro-adenosine significantly inhibited uptake. This inhibition was dose-dependent but inhibition at equimolar concentrations did not exceed 30%. Uptake was inhibited by the adenosine uptake inhibitors dipyridamole (EC_{50} = 5 uM) and papaverine (EC_{45} = 100 uM). The phosphodiesterase inhibitors RO 20-1724 and isobutylmethylxanthine (IBMX) did not inhibit uptake. Since IBMX is also an adenosine receptor antagonist, the latter result indicates a differential selectivity of adenosine receptors and the recognition site for the uptake process. Adenosine uptake was not effected by changes in extracellular Na^+ concentrations. Thus, while adenosine uptake in LRM55 astroglial cells appears to be kinetically different from that reported in synaptosomes, inhibition of uptake by dipyridamole and papaverine is similar to that in synaptosomes. This work was partially supported by grants NS21219 and AA07155.

377.3 **STUDIES ON ASTROGLIAL BINDING OF A POTENT NATURAL MITOGEN: EPIDERMAL GROWTH FACTOR.** K. Huff, L. Ibric*, and L. Schultz*. Neurology Research Labs, Childrens Hospital of Los Angeles, Univ. of Southern California School of Medicine, Los Angeles, CA, 90027.

Astrocytes proliferate as a part of normal brain development, many disease processes such as infection, stroke, and trauma, and neoplastic transformation in gliomas. Epidermal Growth Factor (EGF) is a proliferation signal, is transcribed from an highly conserved base sequence involved in specification of nervous system ontogenic cell lineage, is found immunohistologically in the brain, and is a ligand for a receptor which is the product of a proto-oncogene highly expressed in gliomas. We have studied the response of astrocytes in tissue culture to EGF and demonstrated stability despite several weeks in culture witnout serum, but changes occur with culture density, and with basic fibroblast growth factor (FGF) pretreatment but not dibutaryl cAMP pretreatment. We have further studied the transduction of the EGF signal by means of binding studies using 125-Iodine- labelled EGF.

Astrocytes have a single type high affinity binding site for EGF with a dissociation constant (Kd) of 2.0 x 10-9 not competed for by FGF, Nerve Growth Factor (NGF), or Multiplication Stimulating Activity (MSA II). In a competitive binding assay, insulin at 1 microgram/ml concentration and triiodothyronine (T3) and thyroxine (T4) may have slightly enhanced EGF binding, although at a lower concentration (50 ng/ml) insulin did not show this effect. Lowering the assay temperature increased the number of binding sites. Binding in meningeal cells, apparently similar to fibroblasts, and in C3H 10T1/2 cells, a fibroblast line, was significantly less than astrocyte-EGF binding in our culture conditions. Pretreatment of the astrocytes with lectins concanavalin A and phytohemagglutinin increased EGF binding in a dose dependent way. Pretreatment with Vicia villosa and tunicamycin reduced EGF binding. FGF caused a 70% reduction in specific EGF binding when the cells were pretreated for 18 hours and this inhibition was not duplicated by NGF, Insulin, MSA II, T3, T4, or dexamethasone pretreatment and was FGF dose dependent. The FGF-induced EGF binding inhibition was not as great as EGF down regulation however. This binding inhibition remained at the same ratio at either high or low temperature.

Our data is in support of specific saturable EGF binding sites on astrocytes with little likelihood of fibroblast contamination influencing the culture results. Temperature effects may suggest receptor internal processing. The lectin and tunicamycin effects may imply a glycosylated region of the receptor containing N-acetyl glucosamine and galactoseamine residues. Insulin may be affecting EGF binding through a steric interaction on the cell surface. FGF pretreatment may be producing a heterologous down regulation of the EGF receptor.

377.4 LOCALIZATION OF OUABAIN BINDING SITES IN RABBIT CEREBRAL CORTEX AND HIPPOCAMPUS BY QUANTITATIVE AUTORADIOGRAPHY. M.C. Antonelli*, W.R. Anderson*, D.G. Baskin and W.L. Stahl. V.A. Medical Center and University of Washington School of Medicine, Seattle, WA 98108.

The role of different isoenzymic forms of the Na,K-ATPase in potassium homeostasis during normal development and in neurological disorders remains controversial. Two forms of the enzyme, with different affinities for ouabain, have been identified by biochemical studies and each may function more effectively at different concentrations of K^+ in the extracellular space. However, relatively little is known about regional and cellular distribution and in situ characteristics of these Na,K-ATPases in nervous tissue. The aim of the present study is to establish distribution and ouabain binding characteristics of Na,K-ATPases in brain by quantitative autoradiography (QAR). Quantitative analysis of ouabain binding was done with a microcomputer digital imaging system. For these studies 10 µm cryostat sections were incubated from 4°C to 37°C. Superior autoradiographs were obtained by longer incubations of tissue sections at low temperature and by addition of EDTA to incubation media; both may minimize endogenous protease activity. Maximum binding was achieved within four days at 4°C and within 1.5 hr at 26°C. Maximum binding of [^{3}H]-ouabain to high affinity sites was stimulated in the presence of a) Mg^{2+},P_i or b) Na^+, Mg^{2+} and ATP(nucleotide conditions) and binding was inhibited by N-ethylmaleimide and erythrosin B. High affinity binding (Kd 100nM) was completely blocked under nucleotide conditions by less than 1 mM K^+ and at [^{3}H]-ouabain concentrations of 1-500 nM non-specific binding was nil.

QAR measurements in cerebral cortex showed that the highest concentration of high affinity ouabain binding sites was found in the pyramidal/granular layers, laminae known to have the highest Na,K-ATPase activity in cerebral cortex by both biochemical and histochemical methods (Stahl, Neurochem. Int. 8:449-476, 1986). On the other hand a relatively uniform distribution of high affinity ouabain binding sites was found in adult rabbit hippocampus, a result which differs from histochemical studies. An examination of the distribution and properties of both high and low affinity ouabain binding sites in regions of the hippocampus may help to resolve this apparent discrepancy and to delineate the role of different forms of the Na,K-ATPase in K^+ homeostasis. (Supported by the Veterans Administration and NIH grant NS 20482; M.C.A. was supported by a fellowship from CONICET, Argentina)

377.5 DIFFERENTIATION OF CULTURED RAT ASTROCYTES: EFFECTS OF GROWTH CONDITIONS. E.T. Browning, D.U. Panek*, S.E. Farinelli*, and W.J. Nicklas (SPON: H.E Lowndes) Depts. of Pharmacology and Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

During the past several years it has become possible to obtain nearly pure cultures of astrocytes from neonatal rodent brain as determined by the presence in nearly every cell of the astrocytic marker, glial fibrillary acidic protein (GFAP) (McCarthy and deVellis, J. Cyc. Nuc. Res., 4:15-26,1978; J. Cell Biol, 85:890-902, 1980). Monoamine oxidase-B (MAO-B) and glutamine synthetase (GS), two enzymes that reflect specialized astrocytic functions, are enriched in astrocytes of brain tissue. By culturing in basal medium Eagle (BME) containing Hank's salts we have obtained monolayer cultures which are virtually exclusively flat, tightly adherent cells bearing few if any processes and which express GFAP. Examination of these cultures for MAO-B and GS revealed very low levels of activity, approximately 10-20% of those of adult rat brain. Transfer of such cultures to Dulbecco's modified Eagle's medium (DMEM) at the time of subculture resulted in increases in MAO-B and GS activity of 20- and 10-fold, respectively. Culture in DMEM from the outset resulted in similarly increased activities of MAO-B and GS. These activities were comparable to or exceeded those of adult rat brain. In addition, the cultures grown in DMEM throughout contained cells expressing an increased variety of morphologies essentially all of which were positive for GFAP by immunofluorescence. Of the several differences in composition between these two media, increased bicarbonate concentration and the associated elevation of pH was a major factor producing increased specific activities of MAO-B and GS. Cultures grown in DMEM accumulated cellular protein more rapidly than did those grown in BME of comparable bicarbonate concentration. DMEM grown cultures continued to increase their specific activities of MAO-B for up to 60 days in culture to levels approximately 4-fold higher than adult rat brain. Treatment of such cultures with dibutyryl cAMP produced a further doubling of MAO-B activity. These observations suggest that growth conditions play a substantial role in determining the state of differentiation of astrocytes in culture and that monitoring the presence of macromolecules associated with specialized functions in addition to monitoring GFAP is important in characterizing astrocytic cultures. (Supported by NSF Grant BNS 84-06889 and USPHS Grant NS 17360).

377.6 **METABOLISM OF MPTP AND SEVERAL ANALOGS BY CULTURED ASTROCYTES.** D.U. Panek*, E.T. Browning, P.K. Sonsalla and R.E. Heikkila, Depts. of Pharmacology and Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) produces a selective destruction of the dopaminergic cells of the substantia nigra in primates and mice resulting in a syndrome which resembles Parkinson's disease. A necessary step in the toxicity of MPTP is its biotransformation by monoamine oxidase-B (MAO-B) to a dihydropyridinium intermediate ($MPDP^+$) which is further oxidized to the pyridinium species (MPP^+). Since only MAO-A has been found within dopaminergic neurons, the bioactivation of MPTP by MAO-B is thought to occur outside of the neuron, perhaps in astrocytes. The purpose of the present studies was to investigate the metabolism of MPTP and several MPTP analogs by MAO within cultured astrocytes. Most recent work has focused on the formation only of the more stable MPP^+. However, we have utilized reverse phase HPLC with diode-array UV detection to measure the levels of both $MPDP^+$ and MPP^+ produced by astrocytes.

Astrocytes were obtained from 1 to 3 day old rat cortex and grown in Dulbecco's modified Eagle's medium. Cultures were passed once at 10-14 days and assayed 4 to 8 weeks later. Intact cell cultures expressed about 8 fold higher MAO-B than MAO-A activity when using [^{14}C]-benzylamine and [^{3}H]-serotonin as substrates. Five cm^2 cultures were incubated with various concentrations of MPTP in a bicarbonate buffered balanced salt solution for 2 hours, the medium was acidified and analyzed by HPLC. Greater than 95% of the total $MPDP^+$ and MPP^+ formed was measured in the medium. Preincubation for 20 min with 0.1 µM deprenyl, a concentration selective for inhibiting MAO-B, almost completely blocked the metabolism of MPTP. In contrast, preincubation with 0.1 µM clorgyline, a concentration selective for inhibiting MAO-A, had no significant effect. When cultures were similarly incubated with 2'methyl-MPTP, an analog which has greater dopaminergic neurotoxicity than MPTP in mice, the 2'methyl-$MPDP^+$ and 2'methyl-MPP^+ metabolites were formed. The metabolism of 2'methyl-MPTP was blocked partially by preincubation with either deprenyl or clorgyline. Complete inhibition was observed only in the presence of both inhibitors. The metabolism of other MPTP-analogs will be presented. These data demonstrate that intact astrocytes convert MPTP to $MPDP^+$ primarily via MAO-B but that both forms of the enzyme are involved in the metabolism of 2'methyl-MPTP. Furthermore, the metabolites were almost exclusively present in the surrounding medium. These findings support a role for astrocytes in the neurotoxicity of MPTP-like substances. (Supported by NSF grant BNS 84-06889 and NIH grant NS21752.)

377.7 GLUCOCORTICOIDS REGULATE THE CONCENTRATION OF GLIAL FIBRILLARY ACIDIC PROTEIN IN THE RAT CNS. J.P. O'Callaghan[1], R.E. Brinton[2], M.D. Browning[3] and B.S. McEwen[2]. U.S. Environmental Protection Agency, Research Triangle Park, NC 27711[1] and Laboratories of Neuroendocrinology[2] and Molecular and Cellular Neuroscience[3], The Rockefeller University, New York, NY 10021.

The molecular basis for steroid action in brain appears to involve an alteration in the synthesis of specific proteins, many of which are localized to specific neuronal or glial cell types. One cell-type specific protein in brain that appears to be subject to glucocorticoid control is glial fibrillary acidic protein (GFAP), the major intermediate filament protein of astrocytes. Previously, we reported that the concentration of GFAP in hippocampus (HIP) and cortex (CTX) was affected by exogenous corticosterone (CORT) and adrenalectomy (ADX) (O'Callaghan and Miller, Neurosci Abs. 12: 1282, 1986). We now report the time course of these effects in various regions of the CNS. In addition, CORT supplements were used to assess reversibility of ADX effects on GFAP. Finally, other key nervous system proteins were assayed to determine the generality of the observed effects on GFAP.

Adult male rats were treated with CORT (50 mg/kg/day) for 12 days, adrenalectomized or adrenalectomized and given CORT supplements (50 mg/kg/day). GFAP and other CNS proteins were assayed by solid-phase RIA (Brock and O'Callaghan, J. Neurosci. 7:931, 1987). Twenty-four hours after the last dose of CORT, the concentration of GFAP was decreased by 15-25% in HIP, CTX, striatum, hypothalamus and cerebellum in comparison to vehicle controls. In all regions, GFAP values returned to control levels by 3 weeks post-CORT. In contrast to these results, ADX caused a time-dependent increase in the concentration of GFAP; by 3 weeks post ADX increases of 44% and 58% over intact controls were observed in HIP and CTX, respectively. Daily CORT supplements following ADX reduced GFAP to below control values after 5 days. In rats receiving CORT alone, ADX alone or ADX plus CORT, the concentration of the following proteins did not differ from corresponding controls in any brain region: the neurofilament triplet protein, NF-200; the microfilament protein, actin; the microtubule protein, tubulin; and the synaptic vesicle proteins, synapsin I and p38. The data indicate that the astrocyte protein, GFAP, is under extrinsic control of the adrenal gland. Because this phenomena is observed throughout the CNS and does not appear to involve major neuron-specific proteins, it is most likely mediated through low-affinity type II steroid receptors present in glial cells.

377.8 EFFECTS OF EXTRACELLULAR POTASSIUM ON DNA SYNTHESIS AND GLIAL FIBRILLARY ACIDIC PROTEIN EXPRESSION IN CULTURED NORMAL GLIAL CELLS. K. S. Canady*, F. Ali-Osman*, E. W Rubel (SPON: D. Durham). Departments of Physiology and Biophysics, Neurological Surgery and Otolaryngology, University of Washington School of Medicine, Seattle, WA 98195.

Astroglia are thought to play an important role in the regulation of neuronal excitability by buffering against acute fluctuations in extracellular potassium (K^+) levels. Gliosis, characterized by increased glial proliferation and expression of glial fibrillary acidic protein (GFAP), has been observed in injured and epileptic brain. It has been hypothesized that gliosis is a direct result of the high levels of extracellular K^+ associated with these conditions. In the present study, we examine the effects of varying K^+ levels on synthesis of DNA and protein and on expression of GFAP in cultured glial cells.

Glial cells from the cerebrum and brain stem of 4-day old hatchling chicks were grown in monolayers. Synthesis of DNA and protein was determined by measuring the incorporation of ^{3}H-thymidine and ^{3}H-leucine into cellular DNA and protein, respectively. Cells were plated at a previously determined optimum density in 96-well microtiter plates and treated with potassium chloride (KCl) to achieve final K^+ concentrations of 5-75 mM. After 24-48 hours at 37 °C, cells were treated with ^{3}H-thymidine or ^{3}H-leucine for an additional 24 hours. Cells were then harvested and prepared for liquid scintillation counting. To study the effects of K^+ on GFAP expression, cells were plated on glass slides in medium containing 5-75 mM KCl over 24-72 hours, fixed in cold acetone and processed for immunocytochemistry.

Preliminary results suggest that both ^{3}H-thymidine uptake and GFAP-like immunoreactivity increase in cultured glial cells with higher concentrations of extracellular K^+. These effects are a function of both the K^+ concentration and the duration of exposure of the cells to K^+. We conclude that extracellular K^+ levels may play a key role in the glial reaction observed *in vivo* in conditions of neuronal injury or altered activity levels. Supported by PHS grants NS24522 and GM07108.

377.9 **ASPARTATE AMINOTRANSFERASE PLAYS A SUBSTANTIAL ROLE IN THE OXIDATION OF EXOGENOUS GLUTAMATE IN RAT CORTICAL ASTROCYTE CULTURES.** S.E. Farinelli* and W.J. Nicklas (Spon. C. VanderWende) Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

In addition to their ability to convert glutamate (GLU) to glutamine (GLN) via GLN synthetase (GS), astrocytes metabolize a portion of GLU to 2-oxoglutarate (2-OG) which is subsequently decarboxylated in the citric acid cycle. The present study has been undertaken to determine the relative roles of the enzymes aspartate aminotransferase (AAT) and GLU dehydrogenase (GDH) in the formation of 2-OG from GLU in the astrocyte. Primary astrocyte cultures were prepared essentially according to the method of McCarthy and de Vellis. Following elimination of overlying neurons and oligodendrocytes, the astrocytes were replated at a confluent density onto 25 mm poly-L-lysine coated coverslips and studied within one week. Greater than 95% of the cells in culture stained positively for the astrocytic marker protein, GFA. At this time, the activity of the astrocyte specific enzyme GS was > 60% found in adult rat brain.

To measure GLU oxidation, astrocytes were incubated at 37° in Dulbecco's Modified Eagle's Medium in an airtight apparatus under an atmosphere of 95% O_2/5% CO_2. Thirty minutes after the injection of 50 μM [1-^{14}C]-GLU (1 $\mu C_i/\mu$mole), the medium was acidified with perchloric acid and the liberated $^{14}CO_2$ trapped in a well containing phenethylamine. Because C_1 is the only carbon which is labeled in the GLU, any $^{14}CO_2$ production comes only from the decarboxylation of 2-OG by 2-OG dehydrogenase or from the decarboxylation of GLU by GLU decarboxylase. Since the inclusion of 20 μM rotenone in the assay medium inhibits $^{14}CO_2$ production by >90%, $^{14}CO_2$ production from [1-^{14}C]-GLU can safely be used as an indicator of GLU flux into the citric acid cycle via 2-OG. Addition of the transaminase inhibitors aminooxyacetic acid (0.5-5.0mM) or β-methylene-D,L-aspartate (0.5-5.0mM) to the incubation medium inhibits $^{14}CO_2$ production from GLU by 60-70% suggesting a major role for the transamination pathway. Substantial GDH activity was present in the astrocytes (50% adult rat brain). Previous studies by others indicated that astrocyte cultures prepared from mouse brain appeared to metabolize exogenous GLU solely via GDH even though those cells had substantial AAT activity [Yu, et al.,J. Neurochem.,39:954-960 (1982)].

The data strongly indicate that in rat cortical astrocyte culture, a substantial portion (up to 70%) of 2-OG production from GLU is mediated by the transamination reaction via AAT. This suggests that GLU oxidation by GDH would at most represent 30-40% of the flux from GLU into the citric acid cycle. (Supported in part by USPS grants NS 17360 and NS 21469).

377.10 BRAIN LIPOPROTEINS AND APOLIPOPROTEIN B,E(LDL) RECEPTORS MAY PROVIDE A MECHANISM TO MAINTAIN CHOLESTEROL HOMEOSTASIS IN THE CENTRAL NERVOUS SYSTEM. R.E. Pitas*, J.K. Boyles*, K.H. Weisgraber*, D. Hui*, and R.W. Mahley* (SPON: Y.M. Yao). Gladstone Foundation Laboratories, Univ. of California, San Francisco, CA 94140-0608

The brain of adult mammals derives cholesterol both from de novo synthesis and from the blood, through the blood-brain barrier. Since no net accumulation of cholesterol occurs in the brain, the CNS must have efficient mechanisms to transport cholesterol and maintain cholesterol homeostasis. In plasma these functions are performed by lipoproteins and lipoprotein-receptor interactions. We have previously demonstrated that apolipoprotein (apo-) E is secreted by astrocytes of the brain, and Roheim et al. (Proc. Natl. Acad. Sci. USA [1979] 76, 4646-4649) reported that apo-E is present in cerebrospinal fluid (CSF). Apolipoprotein E-containing lipoproteins are known to interact with apo-B,E(LDL) receptors on cells and to regulate intracellular cholesterol synthesis. The current study was undertaken to determine whether apo-E-containing lipoproteins could provide a mechanism for lipid transport and cholesterol homeostasis within the CNS.

To investigate the mechanism of lipid transport and cholesterol homeostasis within the CNS, the lipoproteins in human and canine CSF were characterized and brain tissue was examined for the presence of apo-B,E(LDL) receptors. Apolipoprotein E (synthesized in the brain) and apo-A-I (a plasma protein not synthesized in the brain) were present in human and canine CSF. Both apolipoproteins are complexed with cholesterol and phospholipid. These lipoproteins had a density of ~1.09 to 1.15 g/ml. In human CSF, the lipoproteins were primarily spherical (~140 A), whereas in canine CSF the lipoproteins were a mixture of discs (200 x 65 A) and spheres (~130 A). The apo-E and apo-A-I in CSF were present on separate populations of lipoproteins in both species. Although the apo-E of CSF was more highly sialylated than plasma apo-E, the apo-E-containing lipoproteins in canine CSF bound to apo-B,E(LDL) receptors with the same affinity as plasma apo-E, as demonstrated by their competition for binding of ^{125}I-LDL to the apo-B,E(LDL) receptors on human fibroblasts. The presence of apo-B,E(LDL) receptors in both rat and monkey brain was demonstrated by immunocytochemistry. Astrocytes abutting on the arachnoid space and pial cells of the arachnoid itself, both of which contact CSF, expressed apo-B,E(LDL) receptors. Relatively few receptors were present in the cells of the grey matter of the cortex. Receptors were more prominent on the astrocytes of white matter and in the cells of the brain stem. Expression of apo-B,E(LDL) receptors by brain cells and the presence of lipoproteins in CSF suggest that the mechanism for lipid transport and cholesterol homeostasis in the CNS is similar to that in other tissues.

377.11 TIME-COURSE OF IN SITU PROTEOLYSIS OF GLIAL FIBRILLARY ACIDIC PROTEIN FOLLOWING TRIMETHYLTIN-INDUCED DAMAGE OF THE RAT CNS. [1]D.M. Niedzwiecki and J.P. O'Callaghan (Spon.: F.E. Gregory). Neurotoxicology Division, Health Efffects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Physical or chemical-induced damage of the central nervous system results in complex cellular responses at the site of injury, one of which is proliferation and hypertrophy of astrocytes. Previously, we demonstrated that the temporal and regional patterns of astrocytic response to chemical-induced injury can be characterized by measuring glial fibrillary acidic protein (GFAP), the major intermediate filament protein of astrocytes (J. Neurosci. 7: 931, 1987). Using the neurotoxic organometal, trimethyltin (TMT), to produce consistent region-specific damage to the adult CNS, we found large dose-dependent increases in GFAP that declined over several months time. Because in situ proteolysis may play a dominant role in the degradation of glial as well as neuronal filaments following injury, the present study examined in situ proteolysis of GFAP following exposure to TMT. Total GFAP immunoreactivity was determined by RIA. Degradation of GFAP was assessed by immunoblot analysis. Light microscopy and RIA of the synaptic vesicle protein, synapsin I were used to monitor neuronal loss following TMT.

Adult male LE rats were killed 4, 7, 14, 21, or 35 days after administration of a single 8 mg/kg (i.v.) dose of TMT or saline vehicle. Hippocampus (HIP), entorhinal cortex (EC) and striatum (ST) were rapidly dissected and homogenized in 95°C 1% SDS or in buffer containing a protease inhibitor cocktail. The latter samples were used to prepare a total particulate fraction and a 150,000 x g supernatant fraction. Samples from TMT-treated rats showed large (150-350%), time-dependent increases in the concentration of GFAP in all regions. In HIP and EC GFAP was elevated at 4 days post TMT, a time point corresponding to the early phases of neuronal damage in these regions. Increases in GFAP in ST were smaller than those in HIP and EC and did not appear until 14 days post TMT, findings consistent with the small degree of neuron damage in this region. Maximal increases in GFAP were observed in HIP, EC and ST by 21 days post TMT. In all preparations, samples obtained from saline treated rats showed only a single 50 K band corresponding to native GFAP. In HIP and EC from TMT-treated rats GFAP-immunoreactive breakdown products were not observed until 2 weeks post dosing, with most fragments appearing in the supernatant fraction. TMT induced increases in GFAP concentration in ST did not result in the appearence of GFAP-immunoreactive breakdown products. The data indicate that injury-induced increases in GFAP do not coincide with the appearance of proteolytic fragments of this protein. In situ proteolysis of GFAP may not be involved in the initiation of glial response to injury but may play a physiological role in the degradation of glial filaments. ([1]NRC Associate)

377.12 ALTERATIONS OF ASTROCYTE MORPHOLOGY AND PROTEIN EXPRESSION IN RAT OPTIC NERVE AFTER CRUSH. G. Stoll* and H.W. Müller (SPON: H.J. Freund). Molecular Neurobiol. Lab., Depart. of Neurology, University of Düsseldorf, F.R.G.

Crushing rat optic nerve (ON) induces a sequence of distinct changes in astroglial morphology and the expression of specific proteins. At the molecular level one of the most prominent effects following ON injury was the rapid disappearance of apolipoprotein E (apo E) from GFAP-positive astroglial cell bodies within 3 days post crush as revealed by immunocytochemical methods (Stoll and Müller, Neurosci.Lett.72:233,1986). Simultaneously, typical reactive astrocyte morphology was expressed and maintained for several weeks in degenerating ON. During this period of time no significant myelin phagocytosis and degradation could be observed. However, between 6 to 8 weeks after lesion the morphology of the GFAP-positive astrocytes was markedly altered by retraction of processes and rounding of the cell body. Numerous intracellular vesicles and myelin inclusions appeared indicating phagocytic activity. Despite these morphological changes into "giant phagocytes" the astroglial marker GFAP (glial fibrillary acid protein was continuosly exprssed within these cells. When acquiring phagocytic properties. however, the astrocytes additionally expressed macrophage-specific antigens. Our results demonstrate molecular, physiological and structural properties shared by astrocytes in the injured ON and macrophages of the immune system indicating significant astroglial plasticity in response to central nervous system injury. Supported by the Deutsche Forschungsgemeinschaft SFB 2oo (C6 and B5)

377.13 HEIGHTENED EXPRESSION OF AN ASTROCYTIC PROTEIN FOLLOWING SPINAL CORD INJURY. S. K. Malhotra[1]*, R. Predy[2], R. Singh[1] and G.D. Das[3]. Departments of Zoology[1] and Pathology[2], University of Alberta, Edmonton, Alberta T6G 2E9, Canada, and Department of Biological Sciences[3], Purdue University, West Lafayette, Indiana.

We are involved in investigations on the role of astrocytes in wound healing following injury to the central nervous system (CNS) and demonstrate that following a laceration-type lesion of the rat spinal cord, the expression of an astrocytic protein (J1-31 antigen) is greatly enhanced in the region of the wound relative to that in the adjacent uninjured region. For this study, laceration-type surgical lesions were made at the mid-lumbar level in spinal cords of Long Evans hooded rats. At intervals following surgery the spinal cords were fixed and the wound site was examined using Golgi-Cox preparations and by immunofluorescence microscopy using antiserum to glial fibrillary acidic protein (GFAP, 50KD), the well-known "marker" for astrocytes. (GFAP is the principal constituent of intermediate filaments in astrocytes.) In addition, we employed a monoclonal antibody (MAb J1-31) which recognizes a protein (J1-31 antigen) of 30KD under reducing conditions for SDS gel electrophoresis (Singh et al., BioSci. Reports, 6:73, 1986). The hybridoma secreting MAb J1-31 was raised using homogenized plaque regions from a multiple sclerosis patient (autopsy sample, Malhotra et al., Microbios. Lett., 26:151, 1984). MAb J1-31 immunostains GFAP positive cells in the CNS (human and rat), but it does not cross-react with GFAP. Also there is evidence that MAb J1-31 does not cross-react with vimentin, another of the intermediate filament core proteins. We have been unable to detect fluorescent staining due to MAb J1-31 outside the CNS or during prenatal CNS development of the rat when vimetin is known to be abundant (Bovolenta et al., Dev. Biol., 102:248, 1984). J1-31 antigen (30KD) appears to be associated with the intermediate filaments of astrocytes as determined by immunoelectron microscopy. Double-label immunofluorescence microscopy performed six months following laceration-type surgical lesion shows that J1-31 antigen is abundant in the region of the glial scar whereas it is barely detectable in the adjacent uninjured region. This enhanced expression may be related to the abundance of intermediate filaments in the reactive astrocytes (Maxwell and Kruger, J. Cell Biol. 25:141, 1965). (Supported by grants awarded by the M.S.I. Foundation and the National Sciences Engineering Research Council of Canada).

377.14 THE GLIAL REACTION IN THE RAT FACIAL NUCLEUS FOLLOWING SELECTIVE NEURONAL DEGENERATION INDUCED BY TOXIC RICIN IN COMPARISON WITH NERVE CRUSH LESIONS. W.J. Streit* and G.W. Kreutzberg. Dept. of Neuromorphology, Max Planck Institute of Psychiatry, D-8033 Martinsried n. Munich, F.R.G. (SPON: M. Reddington)

Following transection of the rat facial nerve, a profound glial reaction characterized by rapid proliferation of microglial cells and a gradual increase in GFAP immunoreactivity of fibrous astrocytes can be observed in the facial nucleus (Streit, W.J. and Kreutzberg, G.W., J. Neurocytol. in press; Graeber, M.B. and Kreutzberg, G.W., J. Neurocytol. 15: 363, 1986). It was shown that resting as well as reactive microglia can be labelled with an α-D-galactose binding lectin-HRP conjugate derived from Griffonia simplicifolia seeds (GSA I-B_4-HRP). The present study was undertaken to examine the glial response after degeneration of facial motor neurons induced by injection of the toxic lectin from Ricinus communis into the facial nerve. Nerve crush lesions were performed on the ricin-injected nerve as well as on the contralateral uninjected side. Within 2-3 days the majority of motor neurons on the ricin-injected side had degenerated, and a conspicuous hypercellularity of non-neuronal cells was apparent. These glial cells were identified by lectin staining to consist for the most part of microglia, and showed high mitotic activity as revealed by the incorporation of tritiated thymidine. By light microscopy the microglial cells were seen to surround dead and dying neurons, often forming clusters of several cells. In the electron microscope enlarged microglia were found to bear the lectin-HRP reaction product on their plasma membrane, and to be actively involved in phagocytosing neuronal debris. In contrast, the contralateral facial nucleus demonstrated no phagocytes, although a similarly high index of dividing microglial cells. Unlike after nerve crush lesions where microglia started to disappear after about 2 weeks, the phagocytic microglia on the ricin-injected side persisted for several weeks. No apparent phagocytic activity of oligodendrocytes was noted. The astrocytic expression of GFAP on the crush side appeared to proceed gradually similar to that described after nerve transection. However, on the ricin-injected side hypertrophic astrocytes displaying thick filament-laden processes were present after 3-4 days where they persisted forming a lasting dense glial scar.

These results show that the expression of GFAP by fibrous astrocytes can be intensified under conditions of neuronal death. Moreover, since no changes in the blood brain barrier could be detected after intravenous injections of HRP, we conclude that resident microglia can be stimulated by neuronal degeneration to transform into macrophages. Thus, our findings do not support the notion that blood-borne macrophages invade the CNS under the experimental conditions employed.

377.15 **INFILTRATION OF MONOCYTES INTO THE DORSAL MOTOR NUCLEUS OF THE VAGUS FOLLOWING AN INTRANEURAL INJECTION OF RICINUS COMMUNIS AGGLUTININ-60 IN RATS.** E.A. Ling and S.K. Leong (Spon : S. Tay). Department of Anatomy, National University of Singapore, Kent Ridge, Singapore 0511.

This study examines the possibility of infiltration of blood monocytes into the dorsal motor nucleus (DMN) of the vagus nerve following an intraneural injection of Ricinus communis agglutinin-60 (RCA-60) in rats. Albino rats ranging between 200-250 gms were each given an intravenous injection of 0.6 ml of carbon suspension of Pelikan India ink (Batch No. C11-1431a) to label the circulating monocytes. The carbon was administered either 4 days before, simultaneously, or 4 days after the administration of 3 ul of RCA-60 in 0.01M phosphate buffer. The RCA-60 was injected into the right vagus nerve at the level where it is crossed by the omohyoid muscle. The animals were all sacrificed by perfusion with 10% neutral formalin 6 days after the injection of RCA-60. The brainstem was then removed, embedded in paraffin and sectioned at 10 um thickness. All sections were stained with Cresyl Fast Violet or Haematoxylin and Eosin.

A variable number of degenerating neurons were observed in the DMN ipsilateral to the side of RCA-60 injection. Destruction of the neurons by the cytotoxic RCA-60 was evidenced by the total vacuolation of their cytoplasm. There was a massive increase in the number of non-neuronal cells when compared with the contralateral DMN. These non-neuronal cells were either closely associated with the blood vessels, the degenerating neuronal somata or in the neuropil. No carbon labelled non-neuronal cells were observed in the DMN in rats given carbon 4 days prior to RCA-60 injection. Occasional labeled cells, however, were observed in rats given RCA-60 and carbon simultaneously. On the other hand, in rats given carbon 4 days after the injection of RCA-60, numerous carbon-labeled cells were observed in the DMN. These carbon-labeled cells were observed in the wall of blood vessels, in the perivascular region or at a satellite position to a degenerating neuron. It is concluded from this study that the non-neuronal carbon-labeled cells in the DMN are derived from circulating monocytes which were earlier labeled by carbon injected intravenously. They could have been attracted by the dying neurons in the DMN killed by the injected RCA-60. It appears that most of the monocytes infiltrated the DMN 4 days after the RCA injection. The absence of carbon-labeled cells in the DMN of rats given carbon prior to RCA injection indicates the complete turnover of carbon-labeled monocytes within the period of 4 days.

NEUROENDOCRINE CONTROLS: PITUITARY VII

378.1 EFFECT OF HEAD-UP TILT ON PLASMA VASOPRESSIN, HEART RATE, AND BLOOD PRESSURE IN ANESTHESIZED RATS. R. Golin*, L. Keil* and W.F. Ganong. Department of Physiology, University of California, San Francisco, CA 94143.

To determine the effect of tilting on vasopressin secretion and the cardiovascular system, male Long Evans rats were anesthetized with inactin to eliminate extraneous stimuli, placed in the supine position, and subjected to 60° head-up tilt for 5 - 30 minutes. Plasma vasopressin increased in a linear fashion with time, from 6.9 ± 3.0 to 17.9 ± 5.9 pg/ml at 5 minutes; from 10.6 ± 2.5 to 28.5 ± 5.3 pg/ml at 15 minutes; and from 7.9 ± 1.3 to 54.2 ± 15.0 pg/ml at 30 minutes. Heart rate tended to increase, but the only statistically significant increase was at 15 min. Blood pressure was variable at 5 and 15 min and there was a decline at 30 min from 93.3 ± 4.0 to 84.6 ± 4.0 mmHg. After bilateral lesions that destroyed 80% or more of both paraventricular nuclei (n=5), tilting produced an increase in plasma vasopressin from 16.8 ± 4.0 to 48.5 ± 11.4 pg/ml in 30 minutes, and there were no significant changes in blood pressure or heart rate. After bilateral vagotomy, 30 minutes of tilting produced an increase in plasma vasopressin from 15.8 ± 2.2 to 30.1 ± 4.3 pg/ml with a significant increase in heart rate and no change in blood pressure. However, after sinoaortic denervation (n=3), plasma vasopressin before tilting was increased to 51.0 ± 8.0, and after 30 minutes of tilting, it fell to 46.3 ± 4.5 pg/ml. In the sinoaortic denervated rats, heart rate did not change and blood pressure fell from 105.8 ± 5.8 to 90.0 ± 8.7 mmHg. The data demonstrates that under these experimental conditions, 60° head-up tilt produces an increase in plasma vasopressin, presumably due to increased vasopressin secretion. The vagally innervated low pressure baroreceptors in the chest play little role in the vasopressin and cardiovascular responses to this stimulus, and they are mediated instead via the arterial baroreceptors in the carotid sinus and aortic arch. The changes in vasopressin secretion occur after lesions of the paraventricular nuclei and hence do not appear to depend on neurons in these nuclei or ascending pathways that end in or pass through them.

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378.2 BRAIN SEROTONIN DEPLETION ALTERS THE VASOPRESSIN SECRETORY RESPONSE TO OSMOTIC STIMULATION AND THE PLASMA LEVELS OF ANGIOTENSIN II TO HYPOVOLEMIA. M.S. Brownfield, J. Gildner*, J. Greathouse*, J. Armstrong*, and L.D. Van de Kar. Dept. of Comp. Biosci., Univ. of Wisc. Sch. Vet. Med., Madison, WI 53706, and Dept. of Pharmacology, Loyola Univ. Sch. Med., Maywood IL 60153.

We have recently used pharmacological methods to provide evidence for a role for central serotonin (5HT) in stimulating vasopressin secretion in the rat via a 5HT2 receptor mechanism (Fed. Proc. 46:976, 1987). This conclusion was based on the demonstration that 5HT1 agonists were without effect while 5HT2 agonists stimulated plasma vasopressin (pAVP) levels which could be prevented by pretreatment with selective 5HT2 receptor antagonists. That the effect might be due to central mechanisms was suggested by the fact that posterior hypothalamic deafferentation blocked p-chloroamphetamine (a 5HT releaser)-induced increase in pAVP that was seen in sham operated rats.

In order to explore the physiological relevance of these findings we challenged rats with normal saline (NS),hypertonic saline (HS; 2 ml 1000 mosmol/kg per 100 g bw, ip), or polyethylene glycol (PEG; 2 ml 300 mg/ml PEG 6000 per 100 g bw, ip) 12 days following intracerebroventricular injection of vehicle (NS with 0.1% ascorbic acid) or 5,7-dihydroxytryptamine (5,7dHT; 200 ug following pretreatment with desipramine, 25 mg/kg). Rats were killed 30 minutes after challenge. RIA was used to measure pAVP and pAII. HPLC was used to evaluate destruction of 5HT neurons.

Serotonin depletion had no effect on the resting levels of either hormone since there was no significant difference between vehicle-NS and 5,7dHT-NS groups. The effect of osmotic challenge on pAVP was inhibited by brain 5HT depletion ($p<0.05$) while there was no effect on pAII. In contrast PEG-induced volume depletion caused significant increase in pAVP that was not affected by 5HT depletion. However, 5HT depletion caused a significant increase ($p<0.05$) in the pAII response to hypovolemia.

These studies suggest that brain 5HT neurons are involved in the osmotic regulation of pAVP by stimulating secretion, but not in its volume regulation. Conversely, a central serotoninergic mechanism appears to play a restrain-ing influence on the volume regulation of pAII.

378.3 AGING AND WATER DEPRIVATION ALTER VASOPRESSIN CONTENT IN LYMPHOID TISSUE AND GUT. Paul F. Aravich*, William F. Silverman, Celia D. Sladek, Suzanne Y. Felten, David L. Felten and John R. Sladek, Jr. Dept. Neurobiology and Anatomy, Univ. of Rochester Med. School, Rochester, NY 14642. (SPON: W. M. Williams).

There is great interest in the effects of aging on various regulatory systems, including those related to the immune system. While vaspopressin (VP) and oxytocin (OT) are the classic hormones of the hypothalamo-neurohypophysial system (HNS), recent evidence suggests that they may be produced in at least 16 other locations, including the thymus and the gut (Endoc Revs '86 7:449; Anat Rec '86 214:5A; Histochem '86 84:401). We now report that aging and water deprivation exert independent and differential effects upon the VP content of certain lymphoid tissues and the gut.

The subjects were young adult (4 mo old), middle aged (i.e., 15 mo) and elderly (25 mo old) male Fischer-344 rats given free access to water or subjected to 72-hrs of water deprivation. Following sacrifice, tissues were collected, analyzed via a specific RIA and saved for immunocytochemical examination. It was found that, relative to protein content, thymic VP content was independently increased by both aging ($p=.047$) and water deprivation ($p=.028$). Thus young adult rats or nondeprived rats had less VP than the older rats or water deprived rats. Because thymic VP expression has been shown to be influenced by the mineralocorticoids but not by the glucocorticoids, the water deprivation effect may be related to aldosterone abnormalities. When thymic VP content was expressed relative to wet weight or relative to the entire gland, VP content also was increased by deprivation ($p<.003$), though age effects no longer occurred. Because of the presence of VP in the thymus, mesenteric lymph nodes were examined. We found that not only is VP-like immunoreactivity present in lymph nodes, but that aging markedly ($p<.001$) reduces its expression. Finally, aging reduced ($p<.001$) the VP-like content of the fundus, which was increased ($p=.039$) by water deprivation. Immunocytochemical examination of the various tissues is in progress, as is an assessment of the effects of the independent variables on OT content.

Vasopressin, like various other peptides, has now been demonstrated to be produced by neural, endocrine and immune tissues. As with many of these peptides, VP exerts specific effects on the immune sytem. Hence, the functional consequences of the aging and water deprivation alterations reported here must be assessed. In view of our recent demonstration that nutrition and exercise exert region specific effects on central VP systems, the impact of various life style factors on the VP content of these peripheral tissues also should be evaluated.

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378.4 BIOCHEMICAL INDICES OF OXYTOCIN AND VASOPRESSIN FUNCTION IN AGED FISHER RATS. W.F. Silverman, P.A. Aravich*, M. Gallagher*, J.R. Sladek, Jr. and C.D. Sladek, Depts. of Neurobiology & Anatomy and Neurology, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642.

Significant differences exist among investigators with regard to the fate of the classical neurohormone arginine-vasopressin (AVP) during senescence in rodents. Most previous studies have examined basal plasma levels of this peptide, and increased (Fliers and Swaab, Peptides 4:77, 1983), decreased (Zbuzek and Wu, Exp. Gerontol. 17:133) and unchanged levels (Sladek et al., Peptides 1:141) have all been reported. Sladek et al., in addition, reported that plasma AVP levels from 30 month old Fisher rats were lower than young controls following 72 hours of water deprivation, a known stimulus for AVP release from the neurohypophysis. The present study examined AVP as well as OXY levels in serum and posterior pituitary under basal and stimulated conditions in Fisher 344 male rats. 4 and 25 month old subjects were housed in metabolic cages and provided food and water *ad libitum*.. Animals in the stimulated group were deprived of water for 72 hours prior to sacrifice. Serum was collected from trunk blood and AVP and OXY extracted following the method of Robertson et al. (J. Clin. Invest. 52:2340, 1973). Also, posterior pituitaries were removed, weighed, homogenized in acetic acid and centrifuged to provide samples for AVP and OXY RIA. The RIA procedure and the antisera used have been previously described (Sladek et al., Am J. Physiol. 250:H443).

Measurement of plasma osmolality and hematocrit indicated a consistant degree of water deprivation stress was present in both age groups ($p<0.01$ and $p< 0.004$ respectively). Serum AVP in the aged rats showed no statistically significant change with respect to young controls under either basal or stimulated conditions. Vasopressin concentrations in the neurohypophysis, however, were reduced ($p<0.04$) relative to their 5 month old counterparts. In contrast, serum OXY levels were significantly higher in the aged subjects ($p<0.04$) compared to 4 month old rats, though no changes were observed in the neurohypophyseal concentrations. The latter finding suggests that clearance of OXY at the level of the kidney tubules is affected in aging rather than central regulatory mechanisms.

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378.5 VASOPRESSIN SECRETION IN AGED RATS: WATER BALANCE AND URINARY VASOPRESSIN MEASUREMENT IN SPRAGUE-DAWLEY AND FISCHER 344 STRAINS. C.J. Phelps, S.W. Carlson*, M.J. Gallagher*, and C.D. Sladek. Departments of Neurobiology and Anatomy and of Neurology, University of Rochester School of Medicine, Rochester, NY 14642

In order to provide physiological baseline values for future experimental procedures, indices of vasopressin secretion were assessed in male Sprague-Dawley and Fischer 344 rats 3 and 20 months of age housed in metabolic cages with food and water ad lib. Daily water intake, urine volume, urine osmolality, and urine vasopressin (VP) levels were measured. Urine samples were prepared for radioimmunoassay (RIA) using a modification of the acetone-ether extraction method of Robertson et al. (J. Clin. Invest. 52:2340, 1973). The RIA for VP was adapted from that developed for tissue determination, using an antiserum produced in conjunction with Arnel Laboratories (Sladek et al., Amer. J. Physiol. 250:H442, 1986); the assay has a sensitivity of 1.0 pg, and oxytocin crossreactivity of 0.02%. Assay aliquots of 50 to 300 ul were adequate for VP detection. Values for individual Sprague-Dawley rats showed similar baseline urinary VP, after housing acclimation (2-9 days), for 30 to 50 days. Sample means over time, with standard errors based on n samples:

	Urine VP Conc. (pg/ml)	24 hr VP Excretion (pg)
rat #4, 20 m.o. (n=10)	8.5 ± 1.1	101.5 ± 17.5
rat #5, 20 m.o. (n=17)	7.9 ± 0.8	214.3 ± 24.4
rat #7, 3 m.o. (n=21)	7.5 ± 0.5	106.3 ± 9.1
rat #8, 3 m.o. (n=21)	10.4 ± 0.8	157.2 ± 16.7

Markedly higher VP (6-10 x baseline) concentrations were recorded in young rats when the animals were initially housed. When new animals were placed in the same room, aged rat #4 showed urine VP concentrations 150-200 pg/ml, while aged rat #5 showed no response. Urine osmolalities showed proportional increases. Preliminary sampling (6 days) of F344 males suggests higher urine VP concentration in this strain: 102.0 ± 19.1 pg/ml for 3 m.o. (n=10); 93.3 ± 18.2 pg/ml for 20 m.o. (n=10), and total VP excretion (627.3 + 15.3 pg/24 hr for 3 m.o.; 361.1 + 28.2 pg/24 hr for 20 m.o). These initial samples indicate significantly lower ($p<0.001$, Student's t-test) total VP secretion in aged versus young 344 males.

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378.6 NICOTINE STIMULATES THE RELEASE OF VASOPRESSIN AND OXYTOCIN FROM SUPRAOPTIC CULTURES. Mariana Morris* and Barbara A. Bennett (Spon: N.Alexander). Department of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

The cholinergic system is known to have potent effects on vasopressin and oxytocin secretion in vivo. The objective of these studies was to investigate the effects of nicotine and high potassium on the release of vasopressin (AVP) and oxytocin (OT) from specific brain regions. An explant culture system was used. Microdissected explants of the supraoptic (SON) and paraventricular (PVN) regions were collected from the brains of male rats (5 weeks of age). The explants were cultured for 2 days and the explants were tested with Earles salt solution containing nicotine (4 x 10-3 M) or K+ (55 mM). There were 4 incubation periods (30 mins): control, nicotine, control and potassium. Media levels of AVP and OT were measured by RIA.

Table. Effect of Nicotine on Media Peptides.

	AVP (pg/well)		OT (pg/well)	
	Control	Nicotine	Control	Nicotine
PVN	16.8±5.5	12.5±3.8	31.1±3.5	39.8±5.2
SON	23.0±1.7	32.0±3.0*	11.8±2.8	29.0±3.6**

* = $p < .05$; ** = $p < .01$

Nicotine produced a significant stimulation of both vasopressin and oxytocin, but only in the SON region. The increase was greater for oxytocin, with release stimulated 2.5 fold. Depolarizing levels of potassium did not produce any change in peptide release in either region. This lack of stimulation by high K+ was also seen using acute cultures of the nuclear regions. This was in contrast to the marked effects observed in the isolated posterior pituitary.

These results show that there is a difference in the neurosecretory regions, the PVN and SON, in terms of both structural organization and secretory control. They suggest that in the isolated system nicotinic receptors still function to activate hormone secretion.

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378.7 OPIOID PEPTIDE DIRECTLY HYPERPOLARIZES NEURONS IN RAT HYPOTHALAMIC SLICES. J.-P. Wuarin* and F. E. Dudek. Dept. of Physiology, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Opiates and opioid peptides have been shown to hyperpolarize neurons in the rat locus coeruleus (North, R. A. and Williams, J. T., J. Physiol. 364: 265, 1985). Several previous studies with extracellular recordings from supraoptic and paraventricular neurons have reported that these substances decrease spontaneous firing in about half of the cells. We have undertaken a preliminary study with intracellular recording to examine the mechanism of action of opioid peptides on these cells.

Using rat hypothalamus slices, we found a hyperpolarization in 4 out of 7 cells recorded from the supraoptic nucleus after bath application of the mu receptor agonist [D-Ala^2, NMe-Phe^4, Gly^5-ol] enkephalin (DAGO, 10^{-6}M). A decrease in input resistance accompanied the hyperpolarization of 4-8 mV (n=2). These effects persisted in tetrodotoxin (1 µg/ml, n=4), which suggests a direct action of the peptide. The recordings were made with KCl-filled electrodes, and the IPSPs reversed polarity within minutes after impalement of a cell. Since the chloride equilibrium potential was presumably positive with respect to resting potential, the persistence of a DAGO-induced hyperpolarization allowed us to rule out an increased chloride conductance.

Two cells recorded in the region of the paraventricular nucleus were also hyperpolarized by DAGO in the presence of tetrodotoxin, and their membrane conductance was also increased.

These results suggest that opioid peptides hyperpolarize some of the neurons in the supraoptic and paraventricular nuclei of the rat hypothalamus. This effect seems to be direct, accompanied by an increase in membrane conductance, not mediated by a chloride current and possibly due to the action of a mu-type receptor.

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378.8 LOW-THRESHOLD CALCIUM SPIKES RECORDED IN THE REGION OF THE RAT HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. J. G. Tasker* and F. E. Dudek (SPON: J. Weber). Dept. of Physiology, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Intracellular electrophysiological studies of the hypothalamus have been performed primarily on neurons of the supraoptic nucleus, whereas relatively little intracellular work has been done on the paraventricular nucleus (PVN). From these studies has emerged a general picture of the electrical properties of magnocellular neuroendocrine cells (MNCs).

We have recorded intracellularly from the PVN region in slices of rat hypothalamus. Eight of 11 cells showed electrical properties different from those characteristic of MNCs. After the fast Na^+ spikes (about 60 mV amplitude, 3 msec duration), these cells showed depolarizing potentials of 20-30 mV amplitude and 100-250 msec duration. These potentials were all-or-none, spike-like events which were seen with suprathreshold depolarizing current pulses or after hyperpolarizing pulses. Steady hyperpolarizing currents (0.1-0.3 nA) were often necessary to detect them. These potentials, but not the faster spikes, persisted in tetrodotoxin (TTX) but were abolished in 0.2 mM Ca^{2+}, 0.5 mM Cd^{2+} and thus appear to be generated by a Ca^{2+} conductance. Hyperpolarizing after-potentials (2-6 mV amplitude, 0.5-3.5 sec duration) were seen after the depolarizing potentials. They were also TTX-resistant and Cd^{2+}-sensitive, and are probably caused by a Ca^{2+}-dependent K^+ conductance.

In sharp contrast to MNCs, spontaneous synaptic activity in these cells was very sparse. Graded EPSPs and synaptically mediated action potentials could be evoked in these cells, as in putative MNCs, by extracellular electrical stimulation medial to the fornix.

Our results corroborate recently acquired evidence from another laboratory (Poulain, P. and Carette, B., Brain Res. Bull., in press) that a non-MNC cell type in or near the PVN can be distinguished on the basis of its electrical properties alone. These large depolarizing potentials have not been reported in hypothalamic MNC's, but resemble the low-threshold Ca^{2+} spikes seen in neurons of other areas of the brain. They appear to be generated by the de-inactivation, at hyperpolarized membrane potentials, of a voltage-dependent Ca^{2+} conductance.

These cells may be parvocellular neurons or perhaps interneurons situated outside of the PVN.

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378.9 α-BUNGAROTOXIN FACILITATES MUSCARINIC CHOLINERGIC STIMULATION OF VASOPRESSIN RELEASE FROM THE RAT HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM IN VITRO. K.M. Michels, R.B. Meeker and J.N. Hayward. Neurobiology Curriculum and Dept. Neurology, University of North Carolina, Chapel Hill, N.C. 27514.

Acetylcholine (ACh) is generally considered to exert direct excitatory control of vasopressin (VP) release via nicotinic cholinergic receptors (nAChR) in the supraoptic nucleus (SON) and via muscarinic cholinergic receptors (mAChR) in the neural lobe (Gregg, 1985, 1986; Sladek, 1983). However, alpha-bungarotoxin (α-BTX), which is the only cholinergic probe that binds selectively and with high affinity to the SON magnocellular neurons (Clarke et al., 1985; Meeker et al.,1986; Michels et al., 1986), is now known to recognize a brain protein which is distinct from the neuronal nAChR (Whiting & Lindstrom, 1987). We therefore examined the possible role of the α-BTX binding site in the cholinergic control of VP secretion in the acutely prepared rat hypothalamo-neurohypophysial system (HNS) in vitro. Following 60 min of perifusion with oxygenated DMEM-H medium at 35°C, stable basal levels of VP release were attained (3-6 pg/min). Subsequent stimulation with ACh (10^{-4}M) for 5 min caused an immediate rise of VP to 9.6 ± 1.2 pg/min with a return to baseline within 5 to 15 min. Atropine (10^{-5}M) blocked this stimulatory effect of ACh but hexamethonium (10^{-4}M) had no effect, suggesting a sole action of ACh at muscarinic cholinergic receptors (mAChR).

The combination of ACh and α-BTX (10^{-6}M) resulted in a potentiation of VP release to a peak level of 44.3 ± 7.1 pg/min and was sustained for more than 25 min. α-BTX alone produced a smaller, delayed VP release peaking at 13.2 ± 1.2 pg/min. Atropine eliminated the potentiating effect of α-BTX, reducing VP release to the level stimulated by α-BTX alone.

In summary: 1) ACh stimulation of VP release, in our acutely prepared rat HNS in vitro, is muscarinic in nature with no evidence for involvement of nAChR; 2) The ability of α-BTX to potentiate the ACh-induced release of VP does not fit a classical nicotinic or muscarinic cholinergic pharmacology, suggesting a unique functional role for the α-BTX binding protein within this hypothalamo-neurohypophysial system (HNS).

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378.10 REGULATION OF VASOPRESSIN SECRETION BY SOMATOSTATIN-28. M.R. Brown. Autonomic Physiology Laboratory, UCSD Medical Center, San Diego, CA 92103.

Somatostatin-28 (SS-28) injected into the lateral cerebroventricle (icv), but not when given intravenously, produces a dose dependent 100 ng-1 µg) elevation of plasma concentrations of vasopressin (AVP). This increase of plasma concentrations of AVP mediates the elevation of mean arterial pressure (MAP) and the lowering of heart rate (HR) observed following the icv administration of SS-28. This conclusion is based on the observation that SS-28 does not increase MAP or decrease HR in Brattleboro rats or in rats pretreated with a vasopressin V_1-receptor antagonist (given systemically). To evaluate what role endogenous somatostatin-like peptides may play in the regulation of vasopressin secretion, experiments have been performed to identify a site of action of SS-28 and to measure stimulus-provoked vasopressin secretion in animals depleted of endogenous SS concentrations. All experiments have been performed in awake, male Sprague-Dawley rats equipped with chronic brain ventricular and/or parenchymal cannulae, and right atrial and femoral artery catheters. AVP was measured by radioimmunoassay. SS-28 has been injected into multiple brain regions in an attempt to localize its site of action. SS-28 (30 ng) when injected into the paraventricular nucleus of the hypothalamus, but not when injected into the anterior or posterior hypothalamus, central nucleus of the amygdala (Ce), nucleus of the solitary tract and lateral ventricle, produced a significant elevation of MAP. Higher doses of SS-28 (1-3 µg) injected into the Ce increased MAP and lowered HR.

The role of endogenous brain SS in the regulation of vasopressin release and MAP was assessed in animals treated with cysteamine. Cysteamine administration (90 mg/kg given subcutaneously 3 hr prior to experiments) depleted brain concentrations of SS-like peptide, as confirmed by radioimmunoassay. This treatment did not significantly alter basal plasma concentrations of vasopressin, MAP, HR or animal behavior. Cysteamine treatment did not change the MAP responses to hemorrhage or to SS-28 or angiotensin-II given icv.

Water deprivation for 24 hr, hypertonic saline and 30% hemorrhage were used in separate experiments as stimuli for AVP secretion. Cysteamine treatment attenuated the elevation of plasma concentrations of AVP following water deprivation and hemorrhage. In contrast, cysteamine pretreatment did not prevent the elevation of plasma AVP levels in animals given hypertonic saline. Icv injection of SS-28 (300 ng) to cysteamine-treated animals receiving 30% hemorrhage resulted in normalization of plasma concentrations of AVP.

These observations are consistent with the hypothesis that a SS-related peptide may participate in the central nervous system regulation of pituitary AVP secretion under some circumstances.

378.11 IMMUNOHISTOCHEMICAL EVIDENCE FOR MODULATION BY ESTROGEN OF NEUROPEPTIDE COEXPRESSION IN THE MAGNOCELLULAR NEUROSECRETORY SYSTEM OF THE FEMALE RAT. P.E. Sawchenko and M.C. Levin*, The Salk Institute, La Jolla, CA 92037.

Double immunohistochemical staining methods were used to assay the effect of gonadal steroids on the expression of coexisting peptides in the magnocellular neurosecretory system of the female rat. It was confirmed in colchicine-treated animals that immunoreactive corticotropin-releasing factor (CRF) and cholecystokinin (CCK) coexist in subsets of oxytocinergic neurons. In addition, dynorphin-immunoreactivity was also detected in a majority of oxytocin containing magnocellular neurons. Also consistent with previous studies, magnocellular vasopressinergic cells were found to display angiotensin II-(AII), dynorphin- and galanin-immunroeactivities. All these results occurred in colchicine-treated ovariectomized (OVX) rats independent of whether or not the animals received replacement injections (10 µg/day for four days) of estradiol benzoate (EB). Comparisons of staining patterns were also carried out between groups of non-colchicine treated OVX rats that received replacement injections of either EB or vehicle. Relative to vehicle-treated controls, animals that received EB replacement showed enhanced staining (increased cell number and staining intensity) for each of the peptides that was found to coexist in vasopressinergic neurons, while staining for those colocalized with oxytocin was not discernably affected. Consistent with this pattern, immunoreactive dynorphin, which was colocalized in both oxytocinergic and vasopressinergic magnocellular neurons in colchicine-treated animals, showed enhanced staining only in magnocellular vasopressinergic neurons in response to EB treatment. Repetition of these experiments in normal animals sacrificed at the estrus, as opposed to the diestrus II, phases of the reproductive cycle yielded a similar pattern of results. The results suggest that circulating gonadal steroids affect peptide expression differentially in oxytocinergic versus vasopressinergic neurons. All peptides examined that could be colocalized in vasopressinergic cells showed evidence of enhanced expression in the presence of estrogen, while none of those colocalized with oxytocin appeared affected. The results in normally cycling rats indicate that this kind of influence may be manifest under normal physiologic conditions. These phenomena provide interesting parallels and contrasts with adrenal steroid influences on the expression of coexisting peptides in an adjoining population of parvocellular neurosecretory neurons.

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378.12 OXYTOCINERGIC NEURONES IN RAT HYPOTHALAMUS: DEXAMETHASONE-REVERSIBLE INCREASE IN THEIR CORTICOTROPIN RELEASING FACTOR-LIKE IMMUNOREACTIVITY IN RESPONSE TO OSMOTIC STIMULATION. J. Dohanics*, K.J. Kovacs* and G.B. Makara* (SPON: A.J. Martinez) Inst. Exptl. Med., Hungarian Acad. Sci., POB 67, H-1450 Budapest, Hungary

Recent results have shown altered corticotropin releasing factor (CRF) content of the neurointermediate lobe (NIL) of the pituitary in response to various manipulations including osmotic stimulation. This study was undertaken to elucidate whether the changes in the NIL are accompanied by changes in CRF-like immunoreactivity of neurosecretory neurones of the hypothalamus in response to osmotic stimulation.

Wistar rats of both sexes were given either tap water ad lib., 2% saline or their access to tap water was limited to 20 min daily. Some rats were adrenalectomized (ADX) or treated with dexamethasone (DEX) for 7 days. Thirty six to 48 hours before perfusion with fixative consisting of buffered formaldehyde and picric acid, animals received 75 µg colchicine icv. Forty µm thick vibratome sections were stained for CRF-like (CRF-LI), arginine vasopressin-like (AVP-LI) and oxtyocin-like (OXY-LI) immunoreactivities using the avidin-biotin-peroxydase complex method. Magnocellular neurones of the paraventricular (PVN), anterior commissural and supraoptic nuclei (SON) showed increased CRF-LI, AVP-LI and OXY-LI in response to both types of osmotic stimulation, while CRF-LI of parvocellular perikarya of the PVN decreased. Increased staining intensities were observed in magnocellular neurones in ADX rats challenged osmotically. Systemic DEX administration, as well as implantation of DEX near the SON, sharply attenuated CRF-LI but not AVP-LI or OXY-LI of magnocellular neurones in osmotically-stimulated rats. The enhanced CRF-LI seemed to coexist with OXY-LI but not with AVP-LI in magnocellular neurones.

The results suggest that osmotic stimulation causes increases in CRF-LI in addition to increases in AVP-LI and OXY-LI of magnocellular neurones presumably due to increased systhesis of these peptides. DEX seems to inhibit synthesis of CRF but not that of AVP and OXY in these cells in response to osmotic stimulation.

378.13 **DIFFERENTIAL LOCALIZATION OF CORTICOTROPIN RELEASING FACTOR IN THE RHESUS MONKEY MEDIAN EMINENCE DURING DEVELOPMENT.** P.C. Goldsmith, K.K. Thind and C.A. Padula*. Dept. of OB/GYN and Repro. Sci., Univ. of Calif., San Francisco, CA 94143.

In order to assess the neurosecretory role of corticotropin releasing factor (CRF) containing nerve terminals in the median eminence (ME) of developing primates, immunostaining for CRF was performed in pre- and postnatal female rhesus macaques. Medial basal hypothalami (MBH) from late fetal (140-160 day) animals were obtained following cesarean section and fixed by immersion in buffered 3% paraformaldehyde/0.2% glutaraldehyde. MBH tissue from colchicine-treated juvenile (7-8 month) females was obtained following perfusion with the same fixative. Vibratome sections (40 microns) in the frontal plane were immunostained with 1:500-1:1,000 rabbit anti-oCRF (oC30, W. Vale) using colloidal gold or PAP, and examined under the light and electron microscopes.

CRF immunopositive (CRF+) staining in juveniles extended throughout the external layer of the ME to the hypophyseal portal capillaries and their loops, even along the ventral infundibulum (INF). Abundant CRF+ nerve terminals were usually prevented from direct access to the parenchymal basal lamina (BL) only by tanycytic processes. In contrast, CRF+ staining in fetal sections was absent or only faintly apparent in the ME zona externa. Some beaded CRF+ axons projected as far as the palisade layer along the ventral INF, but none approached within 50-100 microns of the BL.

Although these results might have reflected the superior structural preservation and poorer immunogold penetration in the fetal than the juvenile ME [Lamberts and Goldsmith, J Histochem Cytochem 34: 389 (1986)], we obtained these same results with PAP, which is much less dependent upon membrane permeability for reliable labeling. Furthermore, CRF+ staining was even more intense with PAP than with colloidal gold, especially in ME nerve terminals in juveniles. This confirms our results, since more CRF was probably lost from the perfusion-fixed ME in juveniles than the immersed fetal ME.

Taken together these results suggest that CRF+ nerve terminals show a differential distribution in the late fetal versus juvenile monkey ME in which CRF+ terminals were more numerous and intensely stained. Although such staining characteristics might also reflect quantitative differences (relative to the conditions at the time tissues were obtained), qualitative dissimilarities in the location of CRF+ terminals were apparent. Whereas CRF+ nerve terminals in the human ME display the adult pattern by mid-gestation, the distinctive localization observed here may signal differences in response to stress in intrauterine versus postnatal life.

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378.14 SIMULTANEOUS DETERMINATION OF CRF AND ACTH RELEASE FROM HYPOTHALAMIC-PITUITARY BLOCKS PERFUSED IN VITRO: RESPONSES TO PHYSIOLOGICAL AND PHARMACOLOGICAL MANIPULATION. J.C. Ritchie, P.K. Liu*, C.B. Nemeroff and M.D. Davis*. Dept. of Psychiatry, Box 3870, Duke Univ. Med. Ctr, Durham, N.C. 27710.

The hypothalamic-pituitary-adrenal axis (HPA) is mobilized during certain external and internal stimuli and is a major component in the homeostatic response to physiological and psychological stresses. A major regulatory factor of ACTH secretion is cortocotropic releasing factor (CRF) which is a 41 amino-acid peptide synthesized by hypothalamic neurons originating within the medial border of the paraventricular nucleus. These neurons send their projections to the hypothalamic median eminence where they terminate within the portal capillary bed. When CRF is released, it enters the vascular network, and is transported to the pituitary where it binds to corticotroph receptors, initiating ACTH release. Other putative hypothalamic-hypophyseal releasing and inhibiting factors may be involved in ACTH regulation, as well. In order to study the dynamic integration between CRF and ACTH in this neuroendocrine axis, we utilized the excised and perfused hypothalamic-hypophyseal block as a model to preserve the functional vascular supply in vitro.

Male Sprague-Dawley rats were sacrificed and the entire hypothalamus with attached pituitary removed and pinned to the bottom of a small chamber. After closing most of the major blood vessels, a small drawn-glass catheter (100 um dia. tip) was inserted into the anterior cerebral artery (a.c.a.) of the Circle of Willis and was used to perfuse and sustain the tissue block with warm, oxygenated media (300 ul/min; Earle's balanced salts + 0.1 % BSA) via a peristaltic pump. The pituitary was inserted into one end of a collection tube through which the exudate from gland was withdrawn. The perfused media was collected over a 10 minute sampling period (3 ml/10 min), then acidified and split into two equal aliquots for separate determinations of CRF- and ACTH- like immunoreactivity (-LI) by radioimmunoassay. At the completion of each experiment, a bolus of india ink was introduced through the catheter to verify patency of the vessels.

Infusion of synthetic human CRF (courtesy of W. Vale) evoked a concentration-dependent release of ACTH-LI, doubling basal values at 20 pM CRF while quadrupling them at 1.0 nM CRF. Elevating the potassium concentration in the perfusion media to 40 mM, elicited a 5-fold increase in CRF and a 6-fold increase in ACTH secretion. In preliminary experiments, electrical stimulation (2 - 30 Hz) of the hypothalamus, via bipolar electrodes placed superficially on the median eminence, initiated a frequency-dependent rise in ACTH secretion, which appeared to be at least partially independent of intrinsic CRF release. We are currently exploring CRF- and non-CRF-mediated ACTH regulation using this model.

378.15 ROLE OF VASOPRESSIN IN THE ACTH RESPONSE TO HYPOTENSION IN NEUROHYPOPHYSECTOMIZED, CONSCIOUS DOGS. H. Raff*, M.M. Skelton*, D.C. Merrill*, and A.W. Cowley, Jr.* (SPON: M. McQuillen). Dept. of Physiology and Medicine, Medical College of Wisconsin, St. Luke's Hospital, Milwaukee, WI 53226.

It is well known that vasopressin (AVP) increases ACTH secretion and potentiates the action of corticotropin-releasing factor (CRF) at the corticotrope. We have previously shown that neurohypophysectomy attenuates the ACTH response to hypotension suggesting that increased release of vasopressin from the posterior pituitary is necessary for normal ACTH responses (AJP 249:R281, 1985). The purpose of the present study was to determine if acute infusion of vasopressin could normalize the ACTH response to hypotension in neurohypophysectomized dogs. Four dogs underwent clamped decreases in blood pressure by a controlled iv infusion of sodium nitroprusside. Then, at least one month after transbuccal neurohypophysectomy, dogs underwent a hypotensive stimulus without (NHX) and with (NHX+AVP) simultaneous acute iv infusion of AVP (6 ng/kg/min). This infusion rate resulted in plasma AVP levels similar to those achieved during hypotension in the intact dog. The results were as follows:

	Mean Art. Pressure (mmHg)			Plasma ACTH (pg/ml)		
	CTL	15 min	45 min	CTL	15 min	45 min
Intact	100±4	70±5*	70±4*	43±6	276±66*#	285±78*#
NHX	99±4	70±3*	70±3*	50±8	83±15*	86±14*
NHX+AVP	94±4	66±2*	66±2*	52±6	173±30*‡	131±27*‡

*diff from control, ‡NHX+AVP > NHX, #intact > NHX+AVP ($p<0.05$)

Neurohypophysectomy had no effect on basal (CTL) mean arterial pressure or ACTH. All three treatment groups exhibited similar degrees of hypotension. Neurohypophysectomy significantly attenuated but did not eliminate the ACTH response to hypotension. Infusion of exogenous AVP during hypotension (NHX+AVP) significantly increased the ACTH response as compared to hypotension alone (NHX) but did not restore the ACTH response to that exhibited in the intact dogs. We conclude that (1) neurohypophysectomy attenuates the ACTH response to hypotension and (2) a component of the ACTH response to hypotension is due to elevated peripheral levels of vasopressin. We hypothesize that stimulation of ACTH secretion during hypotension is in part due to elevated peripheral vasopressin levels acting either by recirculation to the adenohypophysis or by activation of afferent pathways to CRF-ACTH secretion. (Supported by NIH HL36681).

378.16 DIFFERENTIAL EFFECT OF REMOVAL OF THE NEUROINTERMEDIATE PITUITARY LOBE ON OXYTOCIN AND VASOPRESSIN CONTENT IN DISCRETE AREAS OF THE RAT HYPOTHALAMUS. C. A. Johnston, K. D. Fagin*[1] and A. Negro-Vilar. Reprod. Neuroendo. Sect., Lab. Reprod. Dev. Tox., NIEHS, NIH, Research Triangle Park, NC 27709 and [1]AMGEN, Thousand Oaks, CA 91320.

Recently, much evidence has accumulated concerning the possible role for the neurointermediate pituitary lobe (NIL) in the neuroendocrine regulation of anterior pituitary hormone secretion. Although investigations have provided evidence for possible direct influences of the NIL upon anterior pituitary hormone secretion, the question of whether the NIL can influence higher brain components involved in the regulation of anterior pituitary secretion remains virtually unanswered. For example, little is known concerning the ability of the NIL to influence neuropeptide levels in discrete areas of the brain, which may be involved in that regulation. Two neuropeptides which have been implicated in the neuroendocrine regulation of anterior pituitary secretion and which are located in the NIL in considerable amounts are oxytocin (OXY) and arginine vasopressin (AVP). In the present study, we evaluated whether the in vivo content of OXY and AVP in discrete areas of the brain containing cell bodies, fibers and/or terminals of these neuropeptide neurons was affected following surgical removal of the NIL (NIL-X). Adult male rats were subjected to NIL-X or visualization of the NIL (SHAM) using a parapharyngeal approach. Animals were sacrificed by decapitation thirteen days following NIL-X or SHAM operation and the two neuropeptides were measured by specific radioimmunoassays in the median eminence, arcuate nucleus and in the paraventricular nucleus. The concentration of OXY was dramatically increased following NIL-X in the paraventricular nucleus and the median eminence while the level of OXY in the arcuate nucleus was not affected by the removal of the NIL for thirteen days. On the other hand, AVP concentrations in the paraventricular nucleus and median eminence were not significantly altered following NIL-X and were somewhat decreased in the arcuate nucleus compared to SHAM controls. These results demonstrate that removal of the NIL can differentially influence the concentrations of two neuropeptides which have been implicated in the neuroendocrine regulatory control of anterior pituitary hormone secretion, and which share similar characteristics in terms of structure and anatomical distribution. The data also suggests that, in addition to its apparent ability to directly influence anterior pituitary secretion, the possibility that the NIL may also influence this neuroendocrine regulation by interacting at a higher brain level should also be considered.

378.17 THYROID HORMONE REGULATION OF THYROTROPIN-RELEASING HORMONE mRNA IN THE PARAVENTRICULAR NUCLEUS IS INDEPENDENT OF THYROTROPIN. K.J. Koller, R.S. Wolff*, and R.T. Zoeller Laboratory of Cell Biology, National Institute of Mental Health, Bethesda, MD 20892.

Thyroid hormones exert a negative feedback effect on thyrotropin (TSH) secretion from the pituitary. However, while thyrotropin-releasing hormone (TRH) is known to regulate pituitary-thyroid function, the effect that thyroid hormones have on TRH has remained unclear. We have shown previously that TRH mRNA levels in the paraventricular nucleus (PVN) increase after thyroidectomy and decrease after T_3 treatment (Endocrine Soc. Abs., 1987, in press). The influence of TSH on thyroid hormone regulation of TRH, however, is ambiguous.

To examine this question, seventeen male Sprague-Dawley rats were thyroidectomized (TX) and hypophysectomized (HX) and given food and a salt- and glucose-enriched water solution ad. lib. Daily subcutaneous injections of saline (0.9%; n=6), T_3 (20 µg/kg; n=5), or T_3 and TSH (20 µg/kg & 1 U; n=6) were made for twelve days. Sixteen additional animals were sham operated and injected with either saline or T_3 as above. On day thirteen, the animals were weighed and decapitated; trunk blood was collected, and brains were removed and frozen on dry ice. Twelve micron cryostat sections were cut through the PVN of each brain, thaw-mounted onto gelatin-coated slides, and stored at -80°C until hybridization. Sections were then warmed to room temperature, fixed in 4% formaldehyde, dehydrated through a series of ethanol solutions, and delipidated in chloroform. After rehydration, an ^{35}S-labeled 48-base oligonucleotide complementary to the TRH mRNA was applied to each section overnight in a hybridization buffer containing 4x SSC and 50% formamide at 37°C. Optical densities of the film over the PVN was analyzed using a Loats image analysis system, and the results expressed as percent of controls (sham operated, saline injected). The results were as follows (mean ± SEM): Sham-saline: 100 ± 5%; Sham-T_3: 75 ± 4%; TX/HX-saline: 125 ±8 %; TX/HX-T_3 : 65 ± 5%; TX/HX-T_3/TSH: 60 ±5%. These results confirm that thyroid hormones negatively regulate TRH mRNA levels in the PVN and demonstrate that TSH has no effect on this regulation.

378.18 ADRENERGIC INNERVATION OF THYROTROPIN RELEASING HORMONE (TRH) SYNTHESIZING NEURONS OF THE RAT HYPOTHALAMUS. Zs. Liposits*[1], R.M. Lechan[2], W.K. Paull*[1], P. Wu*[3], and I.M.D. Jackson[3]. (SPON: J.D. Dexter). [1]Dept. of Anatomy, University of Missouri-Columbia, Columbia, MO 65212; [2]Endocrine Division, Tufts-New England Med. Center, Boston, MA 02111; [3]Div. of Endocrinology, Brown University, Rhode Island Hospital, Providence, RI 02902.

Thyrotropin releasing hormone synthesizing neurons, residing in the parvocellular subnuclei of the hypothalamic paraventricular nucleus (PVN) (Lechan and Jackson, Endocrinology, 111:55-1982), contribute to the central regulation of the pituitary-thyroid endocrine axis. The central catecholaminergic system has previously been demonstrated (Krulich, L., Neuroendocrinology, 35:139,1982) to influence the thyrotropin secretion. In order to elucidate the possible involvement of the central epinephrine system in the innervation of TRH-immunoreactive (IR) neurons of the PVN, ultrastructural immunocytochemical double labelling studies (Liposits et al., Histochemistry, 85:95, 1986) were performed on the hypothalami of colchicine treated (80µg/100 g. b.w.; 36 h before sacrifice) male rats.

Free and membrane-bound ribosomes, numerous neurosecretory dense core granules (80-130 nm in diameter) were intensely labelled in TRH-IR neurons. Unlabelled axon terminals formed asymmetric synapses with dendrites and cell bodies of TRH-IR neurons. The axons of TRH-synthesizing neurons were also observed to establish asymmetric synaptic specializations with parvocellular neurons of the PVN.

The immunocytochemical dual antigen localization technique revealed that phenylethanolamine-N-methyltransferase (PNMT)-IR, adrenergic axons overlapped TRH-synthesizing paraventricular neurons. Dendrites and perikarya of TRH-IR neurons were heavily surrounded by PNMT-IR axons. The ultrastructural analysis of these juxtaposed elements demonstrated that PNMT-IR axon terminals formed asymmetric synapses with both dendrites and somata of TRH-synthesizing neurons.

These data indicate that the thyrotropin releasing hormone synthesizing neurons of the PVN receive a direct, synaptic input from the central epinephrine system. The results are in harmony with recent pharmacological (Terry, L.C., Neuroendocrinology, 42:102, 1986) and ultrastructural (Shioda et al., Cell Tissue Res., 245:247, 1986) observations related to the role of catecholamines upon the central TRH system. The data also support the concept of a neurotransmitter action of TRH within the neuronal circuits of the PVN. Supported by NIH Research Grants NS 19266 and AM 34540.

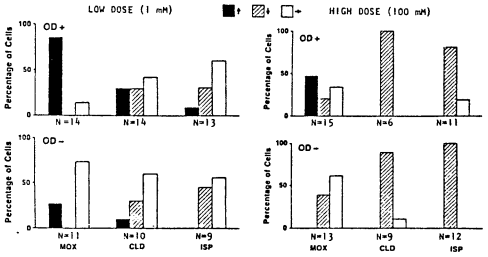
378.19 THREE-DIMENSIONAL (3D) COMPUTER RECONSTRUCTION OF MICROCAPILLARY MODULES IN THE MEDIAN EMINENCE OF THE HYPOTHALAMUS.
L. S. Hibbard, B. J. Dovey-Hartman*, and R. B. Page. The M. S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033.

The capillary plexus in the median eminence forms a humoral connection for the transfer of information between the brain and the anterior pituitary. It is organized in a modular pattern of repeating vascular loops standing on a base of hexagonally arrayed capillaries. Capillary loops may have a simple hairpin shape or may arborize in the internal zone of the median eminence to form complex vascular formations. To establish these modules as independent units it is necessary to identify mechanisms which regulate blood flow into each unit. We have developed a system of computer programs for the 3D reconstruction of capillaries from digitized transmission electron micrographs (TEMs) of thin sections of the rabbit median eminence to ascertain whether the physical means for such control exists (smooth muscle sphincters strategically-located in the loops and arbors). Using digital imaging techniques, capillary lumens are extracted from serial TEMs, mosaics of multiple overlapping images are generated, and the mosaic-images are placed in register. The lumen features are detected by a spatial-domain operator which searches for pixels having high gray levels, low local variance, and contiguity with other pixels having the same properties. The mosaics are assembled using fast Fourier transform (FFT) correlation of the lumen edges. Image alignment is carried out in two stages: coarse alignment is effected by the superposition of image centroids and principal axes, and fine alignment consists of alternating rotational and translational refinements of the coarse alignment, by FFT correlation of the high-frequency image features. In two early studies (Hibbard, et al., Comput. Biomed. Res. 16:411, 1986), bundles of parallel capillaries were observed, with connections, at intervals, between them. Currently, a much larger study is underway, in an attempt to reconstruct an entire capillary loop, with automatic detection of endothelial and smooth muscle cell features. The endothelial cell nuclei give rise to particularly high-valued parallel edges in gradient images derived from smoothed, digitized TEMs. This property, along with the proximity of endothelial nuclei to the lumens (already detected above), is incorporated into a routine which identifies regions which are likely to contain nuclei. Current progress on the reconstruction and the elaboration of cellular features will be presented. (Supported by NSF grant BNS 8506479 and NIH grant NS 15962.)

378.20 TYPES OF ADRENORECEPTORS MEDIATING A1 NORADRENERGIC NEUROTRANSMISSION TO MEDIAL PREOPTIC-MEDIAL SEPTAL NEURONS.
Y.I. Kim, C.A. Dudley and R.L. Moss, Dept. Physiol., UTHSCD, Dallas, TX 75235.

It has been demonstrated that norepinephrine (NE) neurons in the A1 region project to the medial preoptic-medial septal (MPO-MS) area and that electrical stimulation of the A1 region orthodromically alters the electrical activity of MPO-MS neurons. Since the A1-stimulation-bound orthodromic excitation (OD+) and inhibition (OD-) could be mimicked by locally applied NE of low and high doses, respectively, the orthodromic events are presumed to be mediated through different types of adrenoreceptors. Thus, the present study was designed to determine the types of adrenoreceptors involved.

Ovariectomized, estradiol-progesterone-primed Sprague-Dawley rats anesthetized with urethane were used for the study. Multi-barrelled glass micropipettes were used to record single unit activity and to locally apply various adrenergic agonists and antagonists. The effects of the adrenergic agonists (1 or 100 mM in-drug barrel concentration, pressure-ejected) on the activity of MPO-MS neurons that orthodromically responded to A1 stimulation are summarized below.



MOX: methoxamine (alpha-1), CLD: clonidine (alpha-2), ISP: isoproterenol (beta)
↑ : excitation, ↓ : inhibition, → : no effect

A1-stimulation-bound excitatory responses were reliably mimicked only by a low dose of MOX while inhibitory responses were reliably mimicked by a high dose of CLD and ISP. To corroborate the results of the synaptic mimicry study, NE-induced neuronal responses that matched the A1-stimulation-bound responses were subjected to possible blockade by the alpha and beta adrenergic blockers, phentolamine (PT, 0.1 M) and timolol (TM, 0.1 M), respectively. The excitatory effects of NE (0.5 mM in-drug barrel concentration, pressure ejected) on neurons excited by A1 stimulation were blocked by iontophoretically applied PT (5 of 5 cases), but not by TM (8 of 8 cases). On the other hand, the inhibitory effects of NE (50 mM) on neurons inhibited by A1 stimulation were blocked by TM (6 of 9 cases), but not by PT (7 of 7 cases).

It was concluded that the excitatory A1 noradrenergic input to MPO-MS neurons is mediated by alpha-1 adrenoreceptors while the inhibitory A1 input is mediated by beta receptors. Supported by NIH grants NS-10434 and HD-09988.

CHARACTERIZATION OF MUSCARINIC CHOLINERGIC RECEPTORS

379.1 THE ONTOGENY OF CHOLINERGIC MUSCARINIC RECEPTORS IN MOUSE TELENCEPHALON: AN IN VITRO AUTORADIOGRAPHIC STUDY WITH [^{3}H]PIRENZEPINE.
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Recent studies have demonstrated the existence of two distinct populations of muscarinic receptors, M_1 and M_2, in mammalian tissues. Of these, M_1 receptors are generally considered post-synaptic markers of brain cholinergic pathways. Since the cholinergic system has been implicated in the regulation of cortical plasticity, detailed knowledge of M_1 receptor site distribution during ontogeny is essential for understanding cholinergic influences on the development of the cortex. In this investigation, we evaluated the development of M_1 sites in mouse cortex, caudate-putamen, and hippocampus, using in vitro autoradiography with the ligand [^{3}H]pirenzepine.

M_1 muscarinic receptor binding was detected in all areas of the immature cortex as early as E15. At one day after birth (P1), receptor binding was distributed uniformly (10-16 fmol/mg) throughout the entire somatosensory cortex, except in layer 1. Labelling increased rapidly between P5-P15. After P15, the tendency was for higher receptor binding to occur in bands over layers II-IV and over layer VI, which were separated by a band of lower binding over upper layer V. Between P20 and adulthood, total binding decreased by 22-34%. In the caudate-putamen, binding also increased most noticeably between P5-P15 with subsequent 50-60 fmol/mg greater binding in the lateral portions. Islands of higher intensity labelling were also observed from P15 to adulthood, predominantly over the lateral aspect. Total binding decreased by about 30% between P15 and adulthood. In the hippocampus, M_1 receptor density was quite low through P5; the largest increases occurred after P10. The pyramidal cell layer in CA1 showed higher density of binding than the dentate granular cell layer. Labelling in the hippocampal white matter exceeded that in the cellular layers in CA3, CA4, and dentate gyrus. In contrast to the cortical and caudate-putamen regions, [^{3}H]pirenzepine binding in the hippocampus continued to increase into adulthood.

These results demonstrate an early appearance of M_1 receptors in the fetal mouse telencephalon, a rapid increase in receptor density beginning at the end of the first week of post-natal development, and changes in receptor distribution during maturation. Differences between the ontogeny of the M_1 receptor and total muscarinic binding, as measured by the specific binding of [^{3}H]propylbenzilylcholine mustard, suggest differential roles in development supported by PO1 HD19920 and the McKnight Foundation.

379.2 AUTORADIOGRAPHIC STUDIES OF SUBTYPES OF MUSCARINIC RECEPTORS IN THE RAT BRAIN.
W. Lee, S.J. Wall and B.B. Wolfe (Spon: E. Stellar). Dept. Pharmacol., U of PA Med. Sch., Phila., PA 19104

The purposes of the present studies are 1) to characterize the binding assays for muscarinic antagonists ^{3}H-quinuclidinyl benzilate(^{3}H-QNB) and ^{3}H-pirenzepine(^{3}H-PZ) using autoradiographic methods, and 2) to examine ontogeny of subtypes of muscarinic receptors using these methods.

Using 24 μ brain sections, experiments were performed to determine the time required for ^{3}H-QNB to reach equilibrium. The binding reached maximal values only after at least 14 hr of incubation at 23°C in the presence of 0.32 nM of ^{3}H-QNB. The amount of ligand bound remained constant between 14 and 22 hr of incubation. Several possibilities exist to explain why the binding of ^{3}H-QNB plateaus at 14 hr. 1) True equilibrium for the binding of ^{3}H-QNB to muscarinic receptors is reached. 2) Degradation of the ligand occurs and/or 3) Degradation of the binding sites occurs upon prolonged incubation at 23°C. To examine the second possibility, incubation buffers containing ^{3}H-QNB were stored at 4°C or 23°C for 16 hr and were subsequently used to label muscarinic binding sites on tissue sections. There was no significant differences in the amount of specific binding using ^{3}H-QNB stored at the two different temperatures after 4 hr of incubation at 23°C. To examine the third possibility, tissue sections were first stored in incubation buffer at 23°C for various periods of time, then incubated in ^{3}H-QNB containing buffer for 5 hr. Tissue sections which were not exposed to 23°C prior to labelling with ^{3}H-QNB were used as control. The specific binding was 69±7% and 64±3% of control when tissue sections were preincubated 12 hr and 17 hr, respectively. These observations suggested that tissue sections are not stable for extended periods of incubation and that equilibrium for the labelling of muscarinic binding sites by ^{3}H-QNB in 24 μ sections could not be reached before a significant loss of the binding sites occurred. To find out if equilibrium could be obtained using ^{3}H-QNB as a ligand, 6 μ and 12 μ tissue sections were incubated with 1.2 nM of ^{3}H-QNB for various amount of time. For both thicknesses equilibrium was reached within 1 hr and remained stable up to 7 hr of incubation at 23°C. In addition, the same amount of specific binding was obtained when 12 uM tissue sections were labelled with 1.2, 2.2 and 4.2 nM of ^{3}H-QNB for 2 hr at 23°C, indicating that saturation of muscarinic binding sites has been achieved. Thus, 12 μ tissue section incubated in nanomolar concentrations of ^{3}H-QNB for 2 hr at 23°C was adopted as the standard procedure for labelling muscarinic binding sites.

The time course for the binding of ^{3}H-PZ was also examined using 24 μ tissue sections. Equilibrium was reached after 2 hr and remained constant up to 8 hr of incubation in 4.3 nM of ^{3}H-PZ. All subsequent studies were carried out with 12 μ tissue sections incubated in various concentrations of ^{3}H-PZ for 2 hr at 23°C. Scatchard analysis performed on cortex layers 1-3 and 4 yielded K_d values of 3 to 6 nM, in good agreement with K_d values generated from studies using cortical homogenates.

The localization of the changes in muscarinic receptor subtypes with age is being examined currently. (Supported by GM31155)

379.3 REGIONAL DIFFERENCES IN MUSCARINIC RECEPTOR STIMULATED GTP-ASE ACTIVITY IN RAT BRAIN. S. Ghodsi-Hovsepian*, W.S. Messer, Jr. and W. Hoss (SPON: C.L. Hinman). Dept. of Medicinal Chemistry, Univ. of Toledo, College of Pharmacy, Toledo, OH 43606.

The activity of low K_m GTPase associated with the stimulation of muscarinic receptors was measured in several different areas of the rat brain including cerebellum, cortex, hippocampus, medulla, midbrain and striatum. GTPase activity was monitored by determining [^{32}P]-P_i released from [gamma-^{32}P]-GTP essentially as described earlier (P.H. Franklin and W. Hoss, J. Neurochem. 43:1132, 1984). The activity of the enzyme was increased selectively by the cholinergic agonist carbachol and inhibited by the M_1-selective muscarinic receptor antagonist pirenzepine. The relative increase (over the basal level) of low K_m GTPase activity stimulated by 1 mM carbachol was greatest in cortex, hippocampus and medulla, which had increases of >40 %, intermediate in midbrain and striatum and least in cerebellum (18%). Pirenzepine inhibited carbachol-stimulated (but not basal) GTPase activity, displaying differential potencies among the brain regions. Medulla and cortex were the most sensitive to pirenzepine with IC_{50} values of 0.03 and 0.5 μM, respectively, whereas hippocampus and striatum were less sensitive with values of 10 and 4.5 μM. The midbrain, however, was insensitive to inhibition by pirenzepine even at concentrations >10 μM. With the exception of the medulla, there was in general a good correlation between the inhibition of carbachol-stimulated GTPase activity by pirenzepine and the distribution of M_1 muscarinic receptors determined by autoradiographic methods. Areas such as medulla may have small populations of M_1 receptors that are very efficiently coupled to G-proteins. Supported in part by NIH grant DA04068 to W.H.

379.4 FUNCTIONAL STUDIES OF A CEREBRAL CORTICAL M2 MUSCARINIC RECEPTOR. D.J. Anderson and M. McKinney. Abbott Laboratories, North Chicago, IL 60064.

The functional interaction of muscarinic receptors with the adenylate cyclase system was studied in rat neocortex. Dispersed cellular aggregates were prepared from adult rat fronto-parietal cortex by teasing finely minced tissue through Nitex filters. ATP stores in the aggregates were prelabeled with [3H]adenine and the [3H]cAMP formed during incubations was isolated by ion exchange chromatography. Forskolin (FORSK) elevated cAMP levels 10-fold over basal by 15 min with an EC50 value of 10-30 uM. Carbachol (CARB) mediated a dose-dependent inhibition (EC50=1.3 uM) of 10 uM FORSK-elevated cAMP production with maximal inhibition of 25-35%. Oxotremorine was fully efficacious in mediating this response. Atropine completely blocked the response to 10 uM CARB (IC50=18 nM), indicating that the inhibition of cAMP formation in this preparation is mediated by the muscarinic receptor. Pirenzepine, a M1-selective antagonist, blocked the response to 10 uM CARB with low potency (IC50=3.5 uM), indicating that a M2 muscarinic receptor subtype mediates the effect. 4-DAMP methbromide and secoverine were relatively potent blockers of this muscarinic response. The response to 10 uM CARB was not affected by 10 mM EGTA, 50 uM d-tubocurarine, or 100 nM tetrodotoxin. Propylbenzilylcholine mustard (PBCM) alkylated muscarinic receptors in this preparation; a 20 min treatment with 100 nM PBCM completely blocked specific [3H]N-methylscopolamine binding; half-maximal occupancy of PBCM occurred at a concentration of 5 nM. PBCM (10 nM) reduced the maximal CARB response without a large shift of the CARB concentration-response curve, indicating that there is not a large component of "spareness" in the system. Further characterization of this cortical receptor-effector system should elucidate the relationship between adenylate cyclase and other biochemical systems linked to the M2 receptor subtype.

379.5 HIGH AFFINITY PIRENZEPINE BINDING ASSOCIATED WITH PHOSPHOINOSITOL HYDROLYSIS IN INTACT HUMAN NEUROBLASTOMA (SH-SY5Y) CELLs. Lin Mei,* W.R. Roeske, H.I. Yamamura. Department of Pharmacology and Internal Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Muscarinic agonist carbarchol (CCh) stimulates phosphoinositol hydrolysis in intact human neuroblastoma (SH-SY5Y) cells. This model system was further characterized by comparing the inhibition effects of pirenzepine (PZ), a M_1 selective antagonist and AF-DX 116, a M_2 selective antagonist and the effects of phorbol ester treatment.

Accumulated formations of IP_1, IP_2 and IP_3 in the presence of 10 mM LiCl were both agonist concentration-dependent and agonist incubation time-dependent under our conditions. Of these three inositol phosphates, the formation of IP_1 was always the highest. The radioactivities (fmol/1,000 cells) eluted from the anion exchange resin column for IP_1 were 0.06 after 3 min, 0.11 after 10 min, and 1.2 after 60 min incubation. In the following, therefore, phosphoinositol hydrolysis refers to the accumulated formations of IP_1.

The EC_{50} value of CCh stimulated IP_1 formation was 10 uM with the Hill coefficient of the dose-response curve being 0.91. Active phorbol esters PMA and beta-PDD decreased CCh-stimulated IP_1 formation. In the presence of 10 uM PMA and beta-pDD, Emax was reduced to 25 % the effect of CCh alone. This inhibitory effect was not reversed by 10^{-2} M taurine. 10 uM alpha-PDD did not reduce the Emax.

PZ (10^{-4} - 10^{-8} M), AF-DX 116 (10^{-4} - 10^{-6} M) and atropine (3 x 10^{-7} - 10^{-9} M), a classical muscarinic antagonist, all shifted the CCh dose-response curves to the right without reducing the maximal effects of CCh. The pA_2 value from Schild regression analysis of AF-DX 116 inhibition of CCh-stimulated phosphoinositol hydrolysis was 6.5 with the slope being 1.1. The K_I value converted from the pA_2 value was 320 nM which is in agreement with the apparent disociation constants (K_d) from AF-DX 116/[^{3}H](-)QNB inhibition studies (250 nM). However, the slope from the Schild plot of the PZ inhibition over the whole range of concentrations was less than one. Schild analysis revealed that PZ inhibited CCh-stimulation IP_1 formation by interaction with two affinities. The K_I value converted from the high affinity site's pA_2 value was 10 nM, which was in good agreement with the K_d values of PZ from [^{3}H]PZ direct binding, PZ/[^{3}H]PZ and PZ/[^{3}H](-)QNB indirect binding studies. Supported by NIH grants.

379.6 CHARACTERIZATION OF MUSCARINIC RECEPTORS IN THE ANTERIOR PITUITARY CLONE CELL LINE GH_3. M.D. Collado*, P.G. Lysko* and R.C. Henneberry. (SPON: H. deF. Webster). Molecular Neurobiology Section, Laboratory of Molecular Biology, NINCDS, NIH, Bethesda, MD. 20892.

Muscarinic receptors have been described in anterior pituitary homogenates and anterior pituitary primary cultures. Effects of muscarinic agonists on secretion of anterior pituitary hormones have been described as well; muscarinic agonists stimulate thyrotropin and growth hormone and inhibit corticotropin and prolactin secretion. Two different biochemical responses have been associated with stimulation of the different muscarinic receptor subtypes M_1 and M_2. Phosphoinositide hydrolysis is coupled to stimulation of M_1 receptors and stimulatory secretory responses while inhibition of cAMP formation is associated with M_2 receptors and inhibitory secretory responses.

The aim of the present work has been the characterization of muscarinic receptors in a homogeneous population of intact GH_3 cells using the muscarinic antagonist [^{3}H]-Methylscopolamine ([^{3}H]-MSC) and the selective antagonist piranzepine to differentiate M_1 and M_2 subtypes. The effect of muscarinic agonists on phosphoinositide breakdown were also studied to correlate the presence of M_1 with physiological function.

The binding experiments on intact cells were performed at 37°C and pH 7.4 in Krebs-Ringer-Hepes. Binding equilibrium was reached in 15 min and was stable for 1 h. Scatchard analysis showed one binding site for [^{3}H]-MSC with high affinity (K_d 180 pM) and B_{max} 271 fmols/mg protein. Comparing these results with [^{3}H]-MSC binding to GH_3 membranes under the same conditions also showed one high affinity binding site with K_d 230 pM and B_{max} 76 fmols/mg protein.

Competition experiments using different agonists (acetylcholine, carbamylcholine and oxotremorine) and antagonists (imipramine and atropine) to displace [^{3}H]-MSC binding, showed IC 50s in the nM range for atropine and in the μM range for the agonists and imipramine. Using the partially selective antagonist piranzepine in the competition experiments a biphasic curve was obtained, suggesting the presence of two binding sites. To confirm this suggestion the effect of muscarinic agonists acethylcholine and carbamylcholine was tested measuring the levels of inositol phosphates IP, IP2 and IP3 in the presence of Li^+. We found that muscarinic agonists in uM concentrations induce a significant increase in IP3 levels within 20 min., supporting the presence of M_1 binding sites.

379.7 MUSCARINIC RECEPTORS IN TWO CELL LINES THAT HAVE DIFFERENTIALLY COUPLED RECEPTORS. J. Baumgold. Membrane Biochemistry Section, DMNB, NINCDS, Bethesda, MD. 20892

Activation of muscarinic receptors leads to several cellular responses, including inhibition of adenylate cyclase and stimulation of phosphoinositide (PI) turnover. Whether each of these responses is mediated via distinct muscarinic receptor subtypes, or whether a single receptor subtype can mediate any of these responses, is a question that we have addressed in the following work. We identified and studied two cell lines that have muscarinic receptors coupled to separate and distinct effector systems. Activation of muscarinic receptors in NG108-15 cells leads to the inhibition of prostaglandin E1 (PGE1)-stimulated cAMP accumulation. Stimulation of PI turnover could not be detected in these cells. On the other hand, activation of muscarinic receptors in SK-N-SH neuroblastoma cells resulted in stimulation of PI turnover but not in inhibition of cAMP accumulation.

The muscarinic antagonist pirenzepine inhibited the effect of carbachol on both the cAMP accumulation response in NG108-15 cells, and the PI turnover in SK-N-SH cells. The IC50 for the pirenzepine inhibition curves for both of these effects was 2 ± 2 μM, consistent with an M2 receptor subtype. Furthermore, binding studies revealed that both of these cell lines had identical pirenzepine inhibition curves for [^{3}H]-N-methyl-scopolamine binding, with Ki values of 1-3 μM, again consistent with an M2 receptor subtype. In addition, McN-A-343, a selective M1 agonist, failed to either inhibit PGE1-stimulated adenylate cyclase in NG108-15 cells, or to stimulate PI turnover in SK-N-SH cells.

Muscarinic receptors have also been subtyped on the basis of their agonist affinity. In order to determine the agonist affinity state of the receptor for each response, we determined the carbachol dose-response relationship for the appropriate response in each cell type. The dose-response relationship for carbachol stimulation of PI turnover in SK-N-SH cells was sigmoidal and had an IC50 of 8 ± 2 μM, consistent with a high-affinity state of the receptor. Similarly, the dose-response relationship for carbachol inhibition of PGE1-stimulated adenylate cyclase in NG108-15 cells was also sigmoidal and had an IC50 of 3 ± 2 μM, again consistent with an agonist high-affinity state of the receptor.

These results demonstrate that the agonist high-affinity state of the M2 receptor subtype is capable of mediating both adenylate cyclase inhibition and PI turnover.

379.8 [^{3}H]PBCM-LABELLING OF MUSCARINIC CHOLINERGIC RECEPTORS THAT SELECTIVELY COUPLE TO PHOSPHOLIPASE C OR ADENYLATE CYCLASE IN TWO CULTURED CELL LINES. M. Liang*, M. W. Martin* and T. K. Harden (SPON: B. S. Pallotta). Dept. of Pharmacology, Univ. of North Carolina School of Medicine, Chapel Hill, N. C. 27514.

Although both second messenger response systems are fully functional in both cell lines, activation of muscarinic cholinergic receptors (MR) only results in inhibition of adenylate cyclase in NG108-15 neuroblastoma x glioma cells and stimulation of phosphoinositide hydrolysis in 1321N1 human astrocytoma cells. MR on both NG108-15 and 1321N1 cells were covalently labelled by the MR antagonist, [^{3}H]-propylbenzilylcholine mustard hydrochloride ([^{3}H]-PBCM) and the properties of the [^{3}H]-PBCM-labelled species of both cells were compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). 1321N1 and NG108-15 cells each primarily expressed a single [^{3}H]-PBCM-labelled species with an apparent size of approximately 92,000 and 66,000 daltons, respectively. [^{3}H]PBCM-labelling was completely inhibited by 1 μM atropine or by down-regulation of MR by an overnight incubation with carbachol. The apparent size of the [^{3}H]-PBCM-labelled species of both cell lines was not altered by treatment with a series of protease inhibitors or by treatment with dithiothreitol and iodoacetamide. Since MR are glycoproteins, the contribution of carbohydrate groups to the difference in apparent size of the [^{3}H]-PBCM-labelled proteins on 1321N1 and NG108-15 cells was determined by treatment of [^{3}H]-PBCM-labelled membranes with endoglycosidase F (Endo F), an enzyme that removes both complex and high mannose type N-linked carbohydrate chains. Endo F treatment reduced the apparent size of the [^{3}H]-PBCM-labelled species in 1321N1 cells from 92,000 daltons to approximately 77,000 daltons and in NG108-15 cells, from 66,000 daltons to 45,000 daltons. Higher concentrations of Endo F, longer incubation times, or readdition of fresh enzyme did not produce a further reduction in apparent size of the [^{3}H]-PBCM-labelled protein in either cell line. Neuraminidase produced no further reduction of the apparent size of the [^{3}H]-PBCM-labelled species from either cell type after Endo F treatment, suggesting the absence of sialic acid containing 0-linked carbohydrate chains on MR. These results suggest that different MR proteins may be responsible for the different biochemical responses mediated by MR, i.e. inhibition of adenylate cyclase and stimulation of phosphoinositide hydrolysis. (Supported by USPHS grants GM 38213 and NS23019).

379.9 ANTIMUSCARINIC ACTIVITY OF S-ISOBUTYL-ADENOSINE (SIBA) AND ITS ANALOGS WITH RESPECT TO MUSCARINIC RECEPTORS IN RAT BRAIN AND GUINEA PIG ILEUM. R.M. Smejkal*, I.M. Abalis*, Marvin C. Pankaskie*† and P.K. Chiang (SPON: R. T. Borchardt). Walter Reed Army Institute of Research, Washington, DC 20307-5100, and †University of Nebraska, Omaha, NE 68105.

S-Isobutyl-adenosine (SIBA), an attributed inhibitor of transmethylation reactions, and its analogs were tested for their effects on acetylcholine-induced contraction of guinea pig ileum and on the specificity of various ligands with respect to muscarinic receptor subtypes in different regions of rat brain. At 100 μM, SIBA and its analogs caused a 15-20% inhibition of [^{3}H]N-methylscopolamine ([^{3}H]NMS) binding to homogenates of both whole brain and cerebral cortex (rich in M_1 subtype). In comparison, a 20-30% inhibition was observed for SIBA and a subset of the analogs in brain cerebellum (rich in M_2 subtype). At high concentrations, SIBA and most of the analogs tested inhibited the binding of [^{3}H]quinuclidinyl benzoate ([^{3}H]QNB) to the total population of muscarinic receptors. However, at low concentrations, these drugs showed small stimulatory effects on [^{3}H]QNB binding sites. Interestingly, the stimulation of NMS-inaccessible [^{3}H]QNB binding sites (a subpopulation of [^{3}H]QNB sites) to whole brain homogenates was observed in the presence of low concentrations of SIBA and several analogs. The maximal stimulation was about 137% of control at 1 μM, after which stimulation decreased. On the other hand, SIBA and all the analogs tested inhibited the binding of [^{3}H]pirenzepine ([^{3}H]PZ), in a dose-dependent manner with a K_i value within the μM range, to the whole brain and cerebral cortex. The rank order of potency for the inhibition of [^{3}H]PZ binding is N^6-methyl-S-benzyladenosine > N^6-methyl-S-cyclohexyladenosine > N^6-methly-S-phenyladenosine > N^6-N^6-dimethyl-SIBA > SIBA > N^6-methyl-SIBA > S-isopropyl-adenosine > 7-deaza-SIBA. Some of these analogs were tested for their ability to antagonize the acetylcholine-induced contraction of guinea pig ileum, a tissue containing predominantly M_2 subtype receptors. The potency of the analogs was slightly better than that of pirenzepine, but several orders of magnitude lower than atropine. The order of potency for these analogs was opposite that described above: N^6-methyl-SIBA > N^6-methyl-S-phenyl-adenosine > N^6-methyl-S-cyclohexyladenosine > N^6-methyl-S benzyladenosine. These results suggest that SIBA and some of its analogs have differential effects on muscarinic receptor subtypes both in rat brain and in guinea pig ileum. Their actions appear to be more similar to those of pirenzepine than to the other antagonists tested, implying some specificity for the M_1 receptor subtype.

379.10 DIFFERENTIATION OF MUSCARINIC CHOLINERGIC RECEPTOR SUBTYPES IN GUINEA PIG BRAIN: COMPARISON OF RADIOLIGAND AND NEUROPHYSIOLOGICAL DATA. B.G. McCarthy and S.J. Peroutka (SPON: M.K. Floeter). Departments of Neurology and Pharmacology, Stanford University, Stanford, CA 94305.

Radioligand binding studies were used to analyze muscarinic cholinergic receptor subtypes in guinea pig cortex and thalamus. Muscarinic cholinergic receptors were labeled with ^{3}H-quinuclidinyl benzilate (QNB) in the absence or presence of 10^{-5} M carbachol. Specific binding in all experiments was defined as the excess over blanks taken in the presence of 10^{-6} M scopolamine. Muscarinic cholinergic agents that were tested included DMPP, MCN-A-343, metacholine, pilocarpine, propionyl-choline, and suberyldicholine. None of the agents tested significantly distinguished muscarinic cholinergic receptors labeled by ^{3}H-QNB in guinea pig cortex from those in thalamus. The greatest selectivity observed was with pilocarpine, which showed a 4-fold selectivity for thalamic muscarinic cholinergic receptors labeled by ^{3}H-QNB over those in cortex. The presence of 10^{-5} M carbachol (added to theoretically block ^{3}H-QNB binding to M_2 binding sites) did not alter significantly the drug affinities for ^{3}H-QNB labeled receptors.

The data in the present study will be compared to the recent physiological data of McCormick and Prince (PNAS 82:6344-6348, 1985) which suggest that the tested agents are able to differentiate between muscarinic cholinergic receptor subtypes in guinea pig brain. Neurophysiological data indicate that muscarinic cholinergic receptor subtypes can be distinguished in guinea pig brain. However, the differentiation of muscarinic cholinergic receptors into M_1 and M_2 binding site subtypes, based on radioligand data, does not correlate with the pharmacological characteristics of M_1 and M_2 muscarinic cholinergic responses observed neurophysiologically.

379.11 BINDING OF THE PUTATIVE CARDIOSELECTIVE MUSCARINIC ANTAGONIST, [3H]AF-DX 116, TO M2 MUSCARINIC ACETYLCHOLINE RECEPTORS IN RAT CEREBRAL CORTICAL MEMBRANES. M. Watson. Dept. of Pharmacology, University of Medicine and Dentistry of New Jersey- New Jersey Medical School, Newark, N.J. 07103-2757.

Previous data obtained from the use of selective antagonists such as pirenzepine (PZ) and AF-DX 116 (11-2-[[2-[(diethylamino) methyl]-1- piperidinyl] acetyl] -5,11-dihydro-6H-pyrido (2,3-b) (1,4) benzodiazepine-6-one) in many binding and functional assays has lead to the recent subclassification of muscarinic acetylcholine receptors (mAChR) into at least two (M1/M2) subtypes (Watson et al., TIPS Suppl. II: 46, 1986). While PZ has high affinity at M1 mAChRs, the novel "cardioselective" antagonist AF-DX 116 has emerged as a competitive selective inhibitor at M2 mAChRs. AF-DX 116 inhibits binding of the highly specific but non-subtype selective mAChR antagonist [3H](-)quinuclidinylbenzilate ([3H](-)QNB) to freshly prepared cardiac membranes with uniform high affinity (Ki=40nM; Hill=1.0). The recent availability of [3H]AF-DX 116 of high specific activity (62.0 Ci/mmole, New England Nuclear, Boston, MA) now permits the validation of these data and the characterization of [3H]AF-DX 116 binding sites by the direct study of the specific binding of [3H]AF-DX 116 to rat cerebral cortical and cardiac homogenates. A rapid filtration technique was used to separate bound from free ligand, and the filters were presoaked in aqueous polyethylenimine (1hr, 0.1%, Sigma) to minimize filter binding. Atropine sulfate (1uM) was used to define specific tissue binding. [3H]AF-DX 116 (10nM, 25°C) binding to cerebral cortical membranes showed fast association kinetics, reaching steady state in 45 min. Dissociation kinetics were very rapid. Although non-specific binding was relatively high (>50% at Kd), nearly 70% of counts were specific at 10nM. Stereospecificity of [3H]AF-DX 116 labeled mAChR binding sites was revealed by inhibition studies (10nM, 25°C, 2hr) of benzetimide stereoisomers, with dexetimide showing ~500x greater potency than levetimide. Inhibition studies of agonists produced shallow curves (Hill<1), while antagonists generally were steep. The order of potency was determined to be atropine=dexetimide=scopolamine>AF-DX 116>oxotremorine>PZ>>acetylcholine=carbamylcholine>levetimide. A specific regional distribution was seen in the brain, with cerebellum>pons-medulla>hypothalamus>cerebral cortex>hippocampus>corpus striatum. [3H]AF-DX 116 bound to cerebral cortex and heart homogenates, yielding data comparable to indirect studies. High affinity AF-DX 116 binding is seen in the cerebral cortex after 2-site analysis of [3H](-)QNB inhibition data, with 25% of sites showing high (Ki=50nM) affinity and 75% of AF-DX 116 sites showing low (Ki=500nM) affinity. Thus, [3H]AF-DX 116 labels a subclass of mAChRs labeled by [3H](-)QNB, suggesting it may be a useful ligand for M2 mAChR identification and studies of M2 mAChR mechanisms and possible heterogeneity.

379.12 N-Substituted piperidinyl benzilates: affinities for brain muscarinic cholinergic receptors. G.R. Luthin, D.J. Brunswick, B.B. Wolfe, and S.M. Tejani-Butt, Depts. of Psychiatry and Pharmacology, U. of PA. Sch. Med., and Neuropsychopharmacology Unit, V.A. Medical Center, Philadelphia, PA 19104.

The piperidinyl and quinuclidinyl esters of benzilic acid are potent antagonists of muscarinic cholinergic receptor activity. N-substitutions using small-chain alkyl groups have been demonstrated to modulate the binding affinities of these ligands. It was of interest in the present study to clarify further the effects of N-substitutions on binding affinities for some benzilate esters. For these studies, piperidinyl benzilate was chosen as a model compound, as this ligand possesses high affinity for muscarinic receptors, and unlike the quinuclidinyl benzilates does not have a chiral center.

N-Substituted derivatives of 4-piperidinyl benzilate were formed by reaction of the sodium salt of 4-piperidinol with the appropriate alkyl halide, followed by reaction with ethyl benzilate. The affinity of each compound for muscarinic receptors was estimated from its ability to compete for (^{3}H)-QNB binding to membranes prepared from rat forebrain. Measured IC(50) values were corrected to Ki values using the Cheng and Prusoff equation. The Ki values obtained for atropine and QNB under these conditions were approximately 0.5 and 0.02 nM, respectively. Unsubstituted (R = H) piperidinyl benzilate exhibited a Ki value of 2 nM. Addition of a methyl or an ethyl group increased the affinity to 0.5 nM, while isopropyl and n-propyl substitutions decreased the affinity to 10 and 30 nM, respectively. From this, it appeared that modest 1, 2 or 3 carbon alkyl substitutions could alter the potency of piperidinyl benzilate over a 50-fold range of affinity. Further substitutions were used to introduce an aromatic ring one or two methylene groups removed from the nitrogen atom. The following compounds were tested, and the potencies obtained were: R = CH_2-phenyl (benzyl), 0.24 nM; R = CH_2-CH_2-phenyl (phenethyl), p-nitrobenzyl, or p-aminophenethyl, 10 nM; R = p-fluorobenzyl, 150 nM. The ΔG values were calculated for the derivatives as $-RTlnK_a$, where $K_a = 1/K_i$. In the benzyl series, the relative changes in ΔG values associated with p-nitro and p-fluoro substitutions were + 2.23 and + 3.87 kcal/mol, respectively, compared to the benzyl substitution alone. These relative changes in ΔG presumably were not solely the result of an electronic effect on the piperidinyl nitrogen, as the Hammett function did not predict the relative affinity changes. Because this binding region could tolerate rather bulky (eg, benzyl or phenethyl) substitutions as well or better than short-chain alkyl substitutions (eg, propyl), it is not clear that this binding interaction is a stabilization due to simple hydrophobic forces. It can be tentatively concluded that secondary sites of interaction near the piperidinyl nitrogen may exist, and may in fact contribute to the overall binding energy as a result of a specific form of hydrophobic interaction. (Supported by Research Funds from V.A., USPHS MH 36761, NS23006, and GM 31155).

379.13 STUDIES ON THE DIFFERENTIAL INHIBITION OF MUSCARINIC RECEPTOR SUBTYPES BY PYRIDINIUM OXIMES IN RAT BRAIN AND HEART. I.M. Abalis, R.G. Andre and P. K. Chiang. Walter Reed Army Institute of Research, Washington, DC 20307-5100.

The pyridinium oxime 2-PAM, bispyridinium bis-oxime TMB-4 and the H-oximes HI-6 and HS-6 are antidotes for organophosphate poisoning. We investigated the nature of the interaction of these oximes with their binding sites in various regions of rat brain and heart through the use of the muscarinic antagonist ligands [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB), [^{3}H]N-methyl-scopolamine ([^{3}H]NMS) and [^{3}H]pirenzepine ([^{3}H]PZ). All four oximes tested inhibited the binding of [^{3}H]QNB, [^{3}H]NMS and [^{3}H]PZ to rat brain and heart with K_i values in the micromolar range. The rank order of potency is: TMB-4 > HS-6 > 2-PAM > HI-6. All four were more potent inhibitors of the labeled ligands to homogenates of cerebral cortex (an area rich in M_1 subtype receptors) than to homogenates of cerebellum or heart (an area rich in M_2 subtype receptor). Furthermore, these inhibitors were more potent in inhibiting [^{3}H]PZ binding, in comparison to their effects on [^{3}H]QNB or [^{3}H]NMS binding to muscarinic receptors of cerebral cortex. Scatchard analysis of the binding of [^{3}H]NMS, [^{3}H]QNB and [^{3}H]PZ to cerebral cortex in the presence of I_{50} concentrations of each oxime gave lower affinity constants (K_D), but similar or slightly lower B_{max} values with Hill coefficients equal to unity, suggesting competitive inhibition. However, in the presence of I_{50} concentrations of the oximes, a decrease in the number of NMS-inaccessible [^{3}H]QNB binding sites (a subpopulation of [^{3}H]QNB binding sites) was observed in the cerebral cortex and cerebellum; but the binding affinity was not affected. Interestingly, most of the oximes significantly altered the rate of dissociation of the [^{3}H]NMS binding in receptors from cerebellum but had no effect on the dissociation rate in receptors from the cerebral cortex. On the other hand, these oximes failed to change the rate of dissociation of the [^{3}H]PZ binding to the pirenzepine-sensitive M_1 muscarinic receptor subtype in cerebral cortex, also indicative of competitive inhibition. These results showed that all of the oximes have differential effects on muscarinic receptor subtypes and may exert their therapeutic action by blocking the high affinity sites of the central muscarinic receptors and, thus, counteract the effect of excess acetycholine at muscarinic receptor sites.

379.14 ONTOGENY OF PHARMACOLOGICAL SPECIFICITY OF THE CEREBRAL CORTICAL MUSCARINIC RECEPTOR IN THE RAT. D.C. Serbus* and K.E. Light Center for Addiction Studies, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Development of the cortical muscarinic receptor system in the rat is known to be essentially a postnatal phenomenon (Evans et al., **JPET 235(3):612, 1985**; Serbus et al., **Neurotoxicol. 7(2):257, 1986**), but the question of the interaction of the emerging receptors with a variety of pharmacological agents has not been addressed. Male Sprague Dawley rats were reared in litters of ten and cortices were obtained by decapitation on postnatal (PN) days 4, 8, or 20; lactating female rats provided adult cortical tissue. Ten-point (0.1nM to 10uM) competition assays were performed on P_2-level synaptosomal aliquots (10 ug protein/assay) of each cortex to determine the potency of a series of non-radiolabelled ligands for competition with (^{3}H)-quinuclidinyl benzilate (QNB, 0.3nM) for binding to muscarinic receptors. K_i values were calculated (Cheng and Prusoff, **Biochem. Pharmacol. 22(23):3099, 1973**) from ALLFIT-determined IC_{50} estimates obtained by duplicate analysis of replicate cortices.

K_i for Competition with (^{3}H)-QNB Binding (uM)

Age	Oxo	Phentol	Pyril	Curare	Dex ($x10^{-3}$)	Lev
PN4	0.008	0.049	0.117	0.412	0.245	0.014
PN8	0.144	3.37	1.36	3.39	0.214	0.256
PN20	0.219	11.9	2.78	4.12	0.127	0.247
Adult	0.111	2.55	>100	>100	0.200	0.310

(Oxo=oxotremorine; Phentol=phentolamine; Pyril=pyrilamine; Dex=dexetimide; Lev=levetimide)
Neither (+) bicucculine nor methysergide showed measurable competition at 10^{-5}M, regardless of age.

CONCLUSION: Cortical muscarinic receptor pharmacospecificity develops early in postnatal life. As evidenced by a time-dependent increase in K_i for non-muscarinic drugs, the developing muscarinic receptor increasingly refines its ability to screen-out the influence of non-muscarinic drugs. Stereoisomeric discriminative power (the potency ratio of Dex:Lev) was in turn found to develop within the first postnatal week, primarily due to a loss of competitive potency of Lev. We conclude that the postnatal ontogeny of the cortical muscarinic system entails refinement of pharmacological specificity of the emerging receptors as well as the previously demonstrated increases in receptor number, associated enzymes, and coupled processes. Supported by PHS Grant AA06483.

379.15 CHARACTERIZATION OF MUSCARINIC BINDING IN ASTROCYTE CULTURES FROM RAT CEREBRAL CORTEX. D.E. Rosenblatt[1], D. Mash[3], and W.F. White[2], Division on Aging[1] and Department of Neuroscience[2], Harvard Medical School, Boston, MA 02115, Dept. of Neurology[3], University of Miami School of Med., Miami, FL 33136.

Astrocytes have receptors for a number of neurotransmitters. Pharmacological studies suggest the presence of muscarinic receptors on astrocytes (Murphy, S. et al., Br. Res. 364:177, 1986) and Repke and Maderspach (Br. Res. 232:206, 1982) have reported ^{3}H-quinuclidinylbenzilate (QNB) binding on chicken astrocytes. In light of the importance attributed to changes in the cholinergic system in aging and Alzheimer's disease and the frequency with which the rat has been used as a model for both of these conditions, we have undertaken the characterization of cholinergic binding in rat cortical astrocytes.

Primary astrocytes were prepared from one day old rat pups by a modification of the method of McCarthy and DeVellis (JCB 85:890, 1980). Cultures were seeded using one cortex per 150 cm^2 flask in DMEM; Ham's F12 1:1 with 15 per cent fetal calf serum (FCS). Cultures were fed every three days with medium containing 10 per cent FCS and shaken overnight on day seven to remove oligodendrocytes. Secondary cultures were prepared by trypsinization and seeding into six well plates. Cultures were 95 per cent or more pure based on staining for GFAP, fibronectin, and galactocerebroside.

Total muscarinic binding was assayed using ^{3}H-QNB. Pirenzepine and oxotremorine were used to assay for receptor subtypes. Attempts to study binding in whole cells were hampered by cellular trapping of ^{3}H-QNB. Studies were therefore carried out either on saponized secondary cultures (0.05 per cent saponin for 10 min. at room temperature) or membrane preparations from primary cultures.

QNB binding was done in phosphate buffer pH 7.5 at 20°C. Binding was terminated by filtration on Whatman GFC glass filters. Radioactivity on the dried filters was measured by scintillation counting.

QNB binding in membranes and saponized cells gave slightly different results. The KD in the membrane preparation was similar to that for whole brain but the Bmax was much lower. Further characterization of the muscarinic site and elucidation of the sub-type is in progress.

This work was supported by the Brookdale Foundation and the MacArthur Foundation Research Program on Successful Aging.

379.16 AGONISTS AND ANTAGONISTS INTERACTION WITH THE RAT LARGE AIRWAY MUSCARINIC CHOLINERGIC RECEPTOR. K.K. McMahon* (SPON: J. Buggy). Dept. Pharmacol., Univ. South Carolina, School of Medicine, Columbia, SC 29208.

The neurotransmitter acetylcholine causes bronchioconstriction and glandular secretions in large airways by interacting with muscarinic cholinergic receptors on the smooth muscle and gland cells of the respiratory tract. Reported is the characterization of these muscarinic receptors of the large airway by determining the receptors affinities for a variety of agonists and antagonists. The large airways used are from the first to the third biforcations of the airways. The adjoining and peripheral lung tissues were stripped away. Radioligand binding and competition displacement were used to determine the density of receptors, affinities of ligands, and the effect of the additions of Mg^{+2} and guanine nucleotide on affinities of ligands. The radioligand used was [^{3}H]-QNB. The receptor density was 141 fmol/mg protein and the K_D for QNB was 40 pM. The order of affinities of the antagonists tested was: atropine, Ro 2-3773, ipratropium > telenzepine > AF-DX116, hexahydrosiladifenidol, pirenzipine. With the exceptions of ipratropium and Ro 2-3773, the competition curves of the antagonists had Hill coefficients of 1. This suggests that the receptor population was homogeneous with regard to antagonists binding. The order of affinities of the agonists tested was: oxotremorine > acetylcholine, oxotremorine-M > carbachol, RS86 HB > McN-A-343 > AHR 602. Comparisons of binding data in rat large airways, cortex, ileum and heart for several agonists and antagonists were made. All the competition curves of the agonists had Hill coefficients of less than 0.8. This suggests that the receptor population was heterogeneous towards the agonists. Multiple agonist affinity states of the receptor-population were determined by nonlinear regression analysis of these data. The addition of 10 mM Mg^{+2} to the assay mixture causes the affinities of agonists to increase. When the guanine nucleotide, 0.1 mM Gpp(NH)p, was also present in the assay the affinity for all of the agonists was diminished but to differing degrees. The Hill coefficients for the agonists were increased in the presence of Gpp(NH)p. The data suggest that an M_2cardiac type muscarinic receptor population which is coupled to a G protein is present in the rat large airway. (Supported by the American Lung Association, South Carolina Affiliate.)

379.17 CHARACTERIZATION OF MUSCARINIC CHOLINERGIC RECEPTORS IN RABBIT THORACIC AORTA. T. Tsukahara, N.F. Kassell, K. Hongo, H. Ogawa. Dept. of Neurosurgery, Univ. of Virginia Sch. of Med., Charlottesville VA 22908.

Vascular endothelium was shown to release vascular relaxing factors(s) (EDRF) when stimulated by acetylcholine (ACh). This response is considered to be mediated by muscarinic receptors as the relaxing effect of ACh is inhibited by atropine. However, the existence of muscarinic cholinergic receptors on the vascular endothelium is still controversial, since EDRF is not released by ACh from cultured endothelium cells from bovine aorta. An autoradiographic study was performed to characterize the muscarinic receptors on the vascular endothelium of rabbit thoracic aorta, using 3H-propylbenzilyl choline mustard (PrBCM), a potent muscarinic antagonist, which binds specifically and irreversibly to muscarinic receptors. Ten μm cryostat sections taken through rabbit thoracic aorta were mounted on glass slides and incubated with 1 nM and 25 nM 3H-PrBCM. After developing autoradiography on the tissue, the silver grains on the endothelium and the smooth muscle layer were counted and quantified under light microscopy. Total binding was sensitive to pretreatment with 10^{-6}M atropine or 10^{-6} M cold PrBCM. The specific bindings (cold-PrBCM sensitive bindings) were saturable. The saturation time of the bindings was not significantly different between the receptors in the endothelium and those in smooth muscle. The density of the specific binding was higher in the smooth muscle layer than in the endothelium. These results suggest the existence of muscarinic cholinergic receptors on the endothelium of rabbit thoracic aorta and that the affinity to PrBCM of the receptors on the endothelium and

379.18 **IMPAIRED MUSCARINIC TRANSDUCTION IN HAMSTER CARDIOMYOPATHY.** P. Chidiac*, A. Nagy*, M.J. Sole* and J.W. Wells. Departments of Pharmacology and Medicine, Faculty of Medicine, and Faculty of Pharmacy, University of Toronto, Toronto, ON M5S 1A1, Canada.

The antagonist N-[^{3}H]methylscopolamine ([^{3}H]NMS) has been used to label muscarinic receptors in left ventricular homogenates from cardiomyopathic Syrian hamsters (TO strain) during late stages of the disease and from age-matched, random-bred (RB) controls. The inhibitory behavior of carbachol versus the specific binding of 1 nM [^{3}H]NMS was studied in the presence and absence of 0.1 mM GMP-PNP as described by Wong et al. (Biochemistry 25:6995, 1986). Three classes of sites were required for a multi-site model to provide an adequate description of the data acquired with myopathic tissue in the absence or presence of GMP-PNP; data acquired with control preparations required 3 and 2 classes in the absence and presence of nucleotide, respectively.

Simultaneous analyses indicated that four dissociation constants (K) are sufficient for the model to describe all of the data taken together: one each for the sites of highest (log K_1 = -7.72 ± 0.11) and lowest affinity (log K_3 = -4.51 ± 0.05) observed with both strains both with and without GMP-PNP, and one each for the sites of medium affinity with both strains either in the absence (log K_2 = -6.10 ± 0.06) or in the presence (log K_2 = -5.67 ± 0.09) of the nucleotide. The values obtained for the relative capacities of each class (F_S, S = 1, 2, or 3) are listed in the Table. Further simplification involving single values of F_S for both strains caused the variance of residuals to increase significantly ($P < 0.01$) in all cases except F_3 in the absence of nucleotide ($P = 0.73$). The variance also increased significantly in analyses with 12 values of K_S and various arrangements involving fewer values of F_S.

	without GMP-PNP			with GMP-PNP		
STRAIN	F_1	F_2	F_3	F_1	F_2	F_3
RB	0.13	0.65±0.03	0.22±0.02	0.04	0.55±0.04	0.41±0.04
TO	0.30	0.46±0.03	0.23±0.02	0.11	0.31±0.04	0.58±0.04

The activity of GTP-stimulated adenylate cyclase was measured in left ventricular homogenates according to a procedure modified from that of Salomon et al. (Anal. Biochem. 58:541, 1974). The inhibitory potency of carbachol was the same for RB and TO hamsters (log IC_{50} = -5.94 ± 0.04), but maximal inhibition decreased from 31 ± 1% in normal tissue to 16 ± 2% in diseased tissue ($P = 0.0019$). The value of IC_{50} compares favorably with the values of K_2 obtained in the binding assays; moreover, the disease-related decrease in maximal inhibition recalls the corresponding decrease in F_2. The data indicate that hamster cardiomyopathy is accompanied by a defect in G-protein-mediated regulation of adenylate cyclase by muscarinic receptors. (Supported by the Medical Research Council of Canada, the Heart and Stroke Foundation of Ontario, and the US Public Health Service)

380.1 ROLE OF CERVICAL LYMPHATICS IN THE SYSTEMIC HUMORAL IMMUNE RESPONSE TO HUMAN SERUM ALBUMIN (HSA) MICROINFUSED INTO CEREBROSPINAL FLUID (CSF). C. Harling-Berg*, P. Knopf*, K.D. Pettigrew* and H.F. Cserr. Brown University, Providence, RI 02912.

A significant fraction of HSA (13%-50%) infused into brain or CSF drains via the olfactory nerve sheath and cribriform plate into deep cervical lymph, reaching cervical lymph nodes in high concentration (Bradbury & Cserr. In: Exp. Biol. Lymph Circ., Elsevier, 1985,355-394). We have evaluated the role of cervical lymphatic drainage in systemic immunization against antigen leaving the CNS, by examining 1) the effect of cervical lymph obstruction on serum antibody titers to centrally administered HSA and 2) the role of deep and superficial cervical lymph nodes in systemic antibody secretion. Experiments were conducted in 4 groups of male Sprague-Dawley rats: normal, cervical lymph obstructed (CLO), sham operated controls, and inguinal lymph obstructed (ILO). A sterile HSA saline solution (90 μg in 10μl) was infused into lateral ventricular CSF (0.5 μl/min) through a catheter implanted 7 days previously. Lymph flow was obstructed 10 days prior to antigen infusion in CLO rats, by surgical removal of nodes and ligation of large cervical lymph vessels, and in ILO rats, by node removal. Serum HSA antibody titers were measured 14 days post infusion using an enzyme-linked assay. In control rats, HSA infusion into CSF yielded serum titers with a geometric mean of 234 (N=12). Serum titers were significantly reduced in CLO rats (N=11) as compared to normals (P<.02), sham operated controls (P<.01, N=5) and ILO rats (P<.02, N=10) (Smirnov Test). In an additional series of normal CSF-infused rats (N=11), cell culture techniques were used to localize the site of antibody secreting cells. Deep and superficial cervical lymph nodes, inguinal nodes, and spleen were removed aseptically, and cells cultured for 4-5 days. White blood cell (WBC) concentrations of lymph node and spleen cultures for each rat were equal ($1\text{-}2 \times 10^7$ WBC/ml media). 100% of cervical node cultures and 54% of spleen cultures had detectable antibody titers, whereas there was no detectable antibody (ND) at starting dilutions of 1:5 in inguinal node cultures. The range of titers (expressed per 10^7 WBC) for superficial and deep cervical nodes and spleen were 5-94, 5-111, and ND-14, respectively. Results indicate that drainage of brain extracellular fluids into cervical lymphatics plays a significant role in brain-immune system interactions. Supported by USPHS Grant NS-11050.

380.2 INTERLEUKIN-1 IMMUNOREACTIVE STRUCTURES IN PERIPHERAL ORGANS M. Schultzberg*, S.B. Svenson*, A. Undén* and T. Bartfai (SPON: H. Aldskogius). Dept. of Pathology, Karolinska Inst., Huddinge Hosptl; Dept. of Vaccine Production, National Bacteriol. Lab.; Dept. of Biochem., Stockholm University, Stockholm, Sweden.

Interleukin-1 (IL-1) is a 17 kD polypeptide, which is synthesized in macrophages and monocytes upon stimulation by e.g. bacterial endotoxins. Among its biological activities are stimulation of thymocyte proliferation and acting as an endogenous pyrogene. The human and murine IL-1 have several homologies in their amino acid sequence. Antiserum was raised in rabbits to a synthetic peptide corresponding to the amino acid residues 169 to 194 in the murine IL-1 precursor (Lomedico et al.,1984). The antibodies were used in immunohistochemical studies of peripheral organs. Sprague-Dawley rats were perfused with 4% paraformaldehyde containing 0.2% picric acid. Pieces of the gastrointestinal tract, the coeliac-superior mesenteric ganglion complex, urinary bladder, vas deferens, liver, kidney, adrenal gland and the lymphatic organs, thymus, spleen and lymph nodes were dissected out, immersed in fixative and rinsed in 10% sucrose before sectioning and staining. Interleukin-1 immunoreactive structures with a varicose appearance were found to follow the distribution pattern of nerve fibres in these organs. In the gastrointestinal tract, IL-1 immunoreactive fibres were observed in the smooth muscle layers, the myenteric and submucous plexus and in the connective tissue, where they often surrounded blood vessels. A dense network of immunoreactive fibres was also seen in the coeliac ganglion, and particularly numerous fibres could be seen in smooth muscle layers of the urinary bladder and vas deferens. Blood vessels surrounded by IL-1 immunoreactive fibres were found in all organs including the lymphatic organs.
In conclusion, IL-1-like immunoreactivity was found in fibrous structures in several peripheral organs, with a distribution resembling that of nerve fibres. The exact localization of the IL-1 immunoreactive material needs further investigation. It may reside within neurones, but the possibility exists that the antibodies react with IL-1 bound to specific receptors. High-affinity binding sites for IL-1 have recently been demonstrated on e.g. T cells (Dower et al.,1985) and nerve cells (Farrar et al.,1986). The role of neuronal IL-1 or IL-1 receptors on peripheral neurones awaits further studies.

Dower et al. (1985) J.Exp.Med.162:501-515.
Farrar et al. (1986) 16th Ann. Meeting Soc. for Neurosci. 376.10
Lomedico et al. (1984) Nature 312:458-462.

380.3 PROLIFERATION OF BOTH CONNECTIVE TISSUE MAST CELLS (CTMC) AND INTESTINAL MUCOSAL MAST CELLS (IMMC) IN NEONATAL RATS IS STIMULATED BY NERVE GROWTH FACTOR INDEPENDENTLY OF THYMUS. M.D. Coughlin, M.Tomioka*, R.H. Stead*, R.W. Hamill§ and J. Bienenstock*. Depts. of Neurosciences and Pathology, McMaster Univ., Hamilton, Ont., Canada L8N 3Z5, and §Dept. of Neurology, Univ. of Rochester, Rochester, N.Y. 14603

The effects of nerve growth factor (NGF) on mast cell development were studied in neonatal rats. Newborn Lewis rats were treated daily with 0.05, 0.5, and 5.0 ug/g body weight of 2.5S NGF, or with vehicle alone, and sacrificed on day 14 after birth. NGF caused a marked increase in the number of mast cells [CTMC, 3x; IMMC, 10x; Spleen mast cells, 50x; at high dose] and histamine levels, in a dose dependent manner. Alcian blue/Safaranin staining at pH 0.5 differentially colored the hyperplastic mast cell population: tongue, pinna and skin mast cells mostly stained red; and stomack, duodenum, ileum, spleen and liver mast cells stained blue. Berberine sulphate produced fluorescence in the tongue, pinna and skin mast cells only. These cells contained rat mast cell protease (RMCP) type I only; whereas the mucosa of the gut contained only RMCP type II positive cells. Both types of protease were evident in spleen and liver. The experiment was repeated on litters of CR:NIH-RNU (athymic) rats using the middle dose of NGF. Both euthymic and athymic animals exhibited a similar degree of mast cell hyperplasia in the gut and increased histamine content in all tissues, suggesting that the NGF effect was not mediated by T-cells. This contrasts with our recent observation that the hemopoietic colony stimulating activity of NGF depends on the presence of T-cells [Matsuda et al, submitted for publication]. Our present study indicates that NGF affects both CTMC and IMMC in neonatal animals and that the effect is thymus independent. This stimulatory effect of NGF on mast cell growth is consistent with a role for NGF in promoting tissue repair. (Supported by the Medical Research Council of Canada.)

380.4 PEPTIDERGIC INNERVATION OF THE BURSA OF FABRICIUS, THE LYMPHOID ORGAN NECESSARY FOR DEVELOPMENT OF HUMORAL IMMUNOCOMPETENCY IN BIRDS. C.B. Lacey and R. Elde. Dept. of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455

Recent evidence has suggested that the nervous system plays a role in immunoregulation. For example, innervation of the thymus, spleen, and lymph nodes by postganglionic noradrenergic fibres has been demonstrated (Felten *et al.*, J. Neuroimmunol. 10:5, 1985). These findings prompted us to investigate the possible innervation of the bursa of Fabricius, a dorsal diverticulum of the caudalmost portion of the GI tract which is recognized as the site of B-cell development and differentiation in avian species.

The presence of various peptidergic nerve fibres and terminals was investigated using indirect immunofluorescence on the bursa, duodenum, and colon from 5 to 7 day old chicks. The tissues were immersed in Zamboni's fixative overnight and ten micron sections were cut on a cryostat. Series of tissue sections were incubated overnight with the following antisera: rabbit anti-vasoactive intestinal peptide (VIP), rabbit anti-calcitonin gene related peptide (CGRP), rabbit anti-serotonin, rabbit anti-FMRF-NH_2, rabbit anti-met-enkephalin, and goat anti-tyrosine hydroxylase (TH). Sections were then washed in PBS and incubated in fluorescein-labelled goat anti-rabbit IgG or swine anti-goat IgG.

All antisera stained fibres and terminals in the enteric nervous system of the duodenum and colon. Within the bursa of Fabricius, VIP- and CGRP-immunoreactive fibres were observed among the subserosal layers of smooth muscle, as well as within the interplical extensions of smooth muscle. In some cases there appeared to be an association of immunostained nerve fibres with vascular elements. In addition, both types of immunoreactive fibres were identified in the bursal follicles containing developing lymphocytes. CGRP-immunoreactive fibres were noted penetrating into the mucosa and epithelium, often in close association with lymphocytes. Less frequently, FMRF-NH_2- and TH- immunoreactive nerve fibres and terminals were noted within the muscular layers. The remaining antisera failed to reveal specific staining of neural elements in the bursa of Fabricius.

These results suggest that VIP- and CGRP-immunoreactive nerve fibres and terminals may participate in regulation of B-lymphocyte development and differentiation within the bursa.

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380.5 THE LOCALIZATION OF RECEPTOR BINDING SITES FOR SENSORY NEUROPEPTIDES AND SENSORY NERVE FIBERS IN LYMPH NODES. P. Popper, C.R. Mantyh*, S.R.Vigna*, J.E. Maggio and P.W. Mantyh. Center for Ulcer Research and Education; Brain Research Institute, UCLA, Los Angeles, Ca 90024 and Harvard Medical School, Boston Ma 02115

Clinical and experimental findings point towards an interaction between the nervous and immune systems. The two major routes by which the CNS can directly modulate peripheral tissues are via the sensory and the autonomic nervous systems. In the present report we have used immunohistochemistry and quantitative receptor autoradiography to explore the sensory and sympathetic innervation of lymph nodes and to define the sensory neuropeptide receptor binding sites expressed by lymphoid tissue. Canine mesenteric lymph nodes were dissected out and fixed in Bouins fixative, 4% paraformaldehyde for immunohistochemistry or embedded in Tissue Tek and frozen on dry ice. Fixed and unfixed lymph nodes were cut at 30 um on a cryostat and stored at -20°C. Fixed sections were processed for tyrosine hydroxylase (TOH), calcitonin gene related peptide (CGRP), substance K (SK), substance P (SP), somatostatin (SOM), and vasoactive intestinal polypeptide (VIP) using fluorescence or the avidin-biotin technique. Unfixed sections were processed for CGRP, SK, SOM, SP, and VIP receptor autoradiography. CGRP, SP, TOH and VIP-containing fibers were present in lymph nodes in the hilus, capsule, and throughout the medullary and T cell region, while few if any were observed within cortical nodules. These fibers were coarse and varicose in appearance. TOH containing fibers were mostly associated with blood vessels, although these adrenergic fibers were also detected along trabeculae in the medullary cords with no obvious relationship to blood vessels. CGRP, SP and VIP fibers were mainly seen in association with blood vessels although some CGRP fibers were seen leaving the blood vessels and ending in the parenchyma. For the receptor autoradiography, CGRP binding sites were seen in the germinal follicles and on blood vessels and trabeculae in the medullary and T cell regions. SP binding sites were restricted to the germinal follicles and VIP binding sites were found in germinal follicles and along blood vessels in the medulla. These findings suggest that the sensory and sympathetic innervation of lymph nodes regulates blood and lymph flow through the node and that immune cells express a variety of receptors and therefore may be targets for released sensory neuropeptides. Supported by Southern California Arthritis Foundation, The Sloan Foundation and NIH 23970.

380.6 CORTICOSTERONE AND β-ENDORPHIN LEVELS IN PERIPHERAL BLOOD AFTER ANTERIOR HYPOTHALAMIC LESIONS AND ENDOTOXIN ADMINISTRATION. L.T. Chen*, C.P. Phelps, C.L. Chen* and W.I. Li* (SPON:M.F. Nolan) Dept. of Anatomy, Univ. South Florida, Tampa, FL and Department of Reprod., Univ. of Florida, Gainesville, FL

In an effort to better understand mechanisms for hypothalamic modulation of immune function we have measured blood levels of corticosterone (B) and β-endorphin (End) at intervals after brain damage with and without acute immune challenge (endotoxin-LPS, 0.5 mg/100g bw iv). The anterior hypothalamic area (AHA) was approached stereotaxically in anesthetized adult male rats with a triangular-shaped (2.0x2.5mm) knife. In order to produce AHA lesions (AHAL) the knife was lowered in a median sagittal plane and rotated bilaterally in the AHA 1.0mm from the midline. Sham (Sh) surgery consisted of only a 3.0mm midline descent and a third group of controls (C) were unoperated. In the first experiment, rats from all groups were rapidly decapitated during 10:00-12:00 hr 1, 2 and 3 wk after surgery. Trunk blood was collected for measurement of B and End by RIA and thymus weights were measured. AHAL caused a significant increase in non-stress serum B concentrations (107±1.4 vs 6.7±1.1 ng/ml for C and Sh) one wk after surgery. Two wks after AHAL serum B had returned to C levels (10±1.5 ng/ml), but concentrations in Sh rats had increased to 34±2.0 ng/ml. At 3 wks after AHAL serum B levels increased to 31±7.9 ng/ml, whereas B concentrations in Sh blood were again decreased to C levels. Thymus weights in both AHAL and Sh groups showed a gradual 25% decline from starting weights during this experiment. Plasma End concentrations were very low 1 wk after AHAL and gradually increased (186±20 pg/ml, wk2; 200±49 pg/ml, wk3) approaching C levels (308±18 pg/ml, all intervals). Plasma End levels after SH (166±45 pg/ml, wk1) were similar at subsequent intervals. In the second experiment, LPS was administered 1 wk after AHAL and Sh surgery and the rats were killed 48hr later. LPS caused a 2-3x increase in serum B in Sh and C rats, but AHAL rats showed no further change in what were already elevated serum B levels at 48 hr (134±17.6 vs. 123±11.8 after LPS). The overall effect of LPS on thymic involution was highly significant with AHAL rats showing the greatest (70%) reduction in weight. Plasma End levels were considerably ($p<0.01$) increased after LPS in both AHAL and Sh rats, but not in controls. In summary, specific AHA damage produces dynamic changes in hormones known to modulate lymphoid function. Thymic involution was further intensified after AHA lesion plus exposure to endotoxin. These effects may reflect in part the chronology of post-AHA lesion adrenal steroid imbalances. Significant increases in plasma β-endorphin after brain damage plus endotoxin challenge also suggest opiod modulation of immune response in this preparation. Supported by BRSG SO7 RR05749.

380.7 DEVELOPMENTAL CONSEQUENCES OF PRENATAL EXPOSURE TO THYMOSIN: LONG TERM CHANGES IN IMMUNE AND ENDOCRINE PARAMETERS. M.P. O'Grady, N.R. Hall and A.L. Goldstein*. Biochemistry Dept., George Washington Univ. Medical Center, Washington, D.C. 20037

A growing body of evidence supports the concept of a bidirectional interaction between the neuroendocrine and immune systems. A thymic extract, thymosin fraction 5 (TF5), has been shown to modulate secretion of ACTH, beta-endorphin, GH, PRL, LHRH and TSH. However, almost all of the research on neuroendocrine actions of TF5 has been in adult animals or in vitro models. The neuroendocrine effects of TF5 in adults are temporary and depend on continuous presence of TF5. This experiment was designed to determine if an intrauterine critical period exists during which TF5 can cause long term changes in immune and/or neuroendocrine function.

Pregnant Swiss-Webster mice were injected (i.p.) with TF5 (0.5 mg in 0.05 cc saline) on days 16, 17 and 18 of gestation. The offspring were studied in adulthood. Immune measures included mitogen assays (PHA, Con A and PWM) and IL-2 production. Various organ weights were recorded: thymus, spleen, heart, kidneys, adrenals, ovaries, testes and preputial glands. Serum levels of thymosin alpha-1 (Ta1), a purified, biologically active component of TF5, were determined by radioimmunoassay.

The TF5-treated group had enlarged thymus glands compared to vehicle-injected controls ($p<0.02$) and TF5-treated females showed a decrease in ovary weight ($p<0.02$) compared to saline-treated females. TF5-treated males had larger adrenals than the saline males ($p<0.01$). Ta1 levels did not differ between the TF5 and vehicle groups. However, females had higher Ta1 levels than males ($p<0.001$) irrespective of treatment. Thymosin treatment lowered IL-2 production in females compared to saline females ($p<0.03$). The TF5-treated males also tended towards decreased IL-2 production but this was not statistically significant ($0.05<p<0.10$). The TF5-treated females displayed a reduced stimulation index to the mitogens, Con A and PWM ($p<0.005$).

These data suggest that intrauterine exposure to an immune system product, TF5, can alter neuroendocrine and immune function during adulthood. TF5 could affect endocrine development, which, in turn, alters immune responsiveness.

380.8 TYROSINE HYDROXYLASE POSITIVE NERVE TERMINALS CONTACT LYMPHOCYTES IN THE PERIARTERIOLAR LYMPHATIC SHEATH OF THE RAT SPLENIC WHITE PULP. S.Y. Felten, J. Olschowka and D.L. Felten. Dept. of Neurobiol. and Anat., Univ. of Rochester, Sch. of Med., Rochester, NY 14642.

The abundant evidence that sympathetic noradrenergic nerve fibers innervate smooth muscle associated with the splenic capsule and trabeculae (causing splenic contraction) and vascular smooth muscle (causing vasodilation and increased blood flow) has led to the assumption that NE containing nerve fibers in the spleen have no other functions. This bias persists even though several of the reports of innervation, including studies at the electron microscopic level mentioned the existence of nerve fibers that are surrounded by splenic cells with no relationship to smooth muscle, or nerve terminals in very close contact with lymphocytes, erythrocytes and reticular cells. Due to the bias that the autonomic nervous system innervates only cardiac muscle, smooth muscle and secretory gland, these findings were dismissed, usually with the statement that they were really platelets rather than nerve terminals. This study used immunocytochemistry of tyrosine hydroxylase to positively identify noradrenergic nerve terminals in the spleen at the electron microscopic level in order to determine whether there was any direct relationship between these terminals and cells other than smooth muscle.

Immunocytochemistry was used to demonstrate tyrosine hydroxylase (TH) positive profiles in the spleen of the adult Fischer 344 rat. At the light microscopic level, numerous varicose nerve profiles were seen in the white pulp, particularly surrounding the central arteries and their arteriole branches. At the electron microscopic level, varicosities were seen in close proximity to smooth muscle cells of the arteries, and directly abutting lymphocytes (presumable T-lymphocytes) of the nearby periarteriolar lymphatic sheath. There were no intervening cell processes between the TH-positive terminals and the lymphocyte. The opposing membranes were smooth and evenly spaced approximately 6 nm apart. Additional TH-positive nerve profiles were seen in the inner marginal zone, and within trabeculae. The correlation between this immunocytochemical staining and previously demonstrated histofluorescence for norepinephrine leads to the conclusion that lymphocytes in the splenic white pulp have direct associations with noradrenergic fibers of the sympathetic nervous system. This association provides a route by which the autonomic nervous system could directly influence specific immune system effector cells.

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380.9 DEVELOPMENTAL COMPARTMENTATION OF TYROSINE HYDROXYLASE (TH) - POSITIVE NERVE FIBERS IN THE SPLEEN OF FISCHER 344 RATS. K.D. Ackerman, D.L. Felten, and S.Y. Felten, Dept. of Neurobiol. and Anat., Univ. of Roch. Sch. of Med., Rochester, NY 14642.

In the adult rat spleen sympathetic noradrenergic (TH - positive) nerve fibers distribute principally within the white pulp, forming close associations with T lymphocytes in the periarteriolar lymphatic sheath and with ED-3 positive macrophages at the marginal sinus. This study was undertaken to explore the developmental sequence of this compartmentation using double-labelled immunocytochemistry for TH - positive nerve fibers and specific markers for lymphoid cells, including OX19 for T lymphocytes, ED3 for macrophages, and OX-4 for Ia positive cells.

TH - positive fibers were present in the hilar region at birth and first associated with the primitive PALS as a ring of fibers along the zone of seperation between a mixed lymphocyte population on the inside and macrophages and B lymphocytes on the outside. TH - positive fibers were not associated with the central artery at this stage. By day 7, a central arteriolar nerve plexus was present, extending between the central artery and the outer ring of nerve fibers. At 10-14 days, the central arteriolar nerve plexuses increased in density and there was a greater seperation between the central arteriolar plexus and the outer zone of fibers now present along the developing marginal sinus. From 14 days to adult, the central arteriolar nerve fibers decreased somewhat in density, but kept pace with the rapid increase in size of white pulp, while maintaining their compartmentation.

The early presence of TH - positive nerve fibers associated with developing compartments of the white pulp may provide an opportunity for bidirectional interactions affecting growth, differentiation, or compartmentation.

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380.10 NEONATAL THYMECTOMY ALTERS THE DEVELOPMENT OF NORADRENERGIC INNERVATION OF THE SPLEEN IN FISCHER 344 RATS. T.A. Romano*, K.D. Ackerman, S.L. Carlson, S.Y. Felten, and D.L. Felten (SPON: R.M. Herndon). Dept. of Neurobiol. and Anat., Univ. of Rochester Sch. of Med., Rochester, NY 14622.

Past studies from our laboratory have shown a parallel course of neonatal development for sympathetic postganglionic noradrenergic nerve fibers innervating the splenic white pulp, and migration of T lymphocytes into the periarteriolar lymphatic sheath (PALS) of the white pulp. Tyrosine hydroxylase (TH)-positive nerve fibers are visible along the central artery at postnatal days 1-3, extend outward from these arteries into the parenchyma where T lymphocytes form discernible PALS by 7-10 days, and further keep pace with the full extent of PALS development into adulthood. We hypothesized that if the arrival of T lymphocytes into the PALS plays a role in guiding the compartmentation of the nerve fibers or maintaining their presence, then neonatal thymectomy might alter this influence. Neonatal (3 day old) Fischer 344 rats were surgically thymectomized and were sacrificed at day 15 or 28. Sham-operated and unoperated rats were used as controls. Spleens were examined with fluorescence histochemistry (SPG method of de la Torre) for presence and appearance of catecholamine fibers. Spleens from sham-operated and unoperated rats showed robust innervation of both the vasculature and parenchyma at 15 and 28 days of age, as reported previously. Spleens of thymectomized rats showed apparently normal compartmentation, density of varicosities, and intensity of fluorescence at 15 days of age, compared with controls. However, by 28 days of age, the noradrenergic innervation of the spleen in thymectomized rats showed considerable variability. In rats where thymectomy appeared complete and the white pulp of the spleen was reduced significantly in size, the innervation of that white pulp was reduced compared with controls. In rats where thymectomy appeared incomplete, the splenic innervation did not appear reduced. These preliminary findings support a role for thymic development in the maintenance of noradrenergic innervation of the splenic PALS at 28 days of age. We hypothesize that this influence is due to arrival of T lymphocytes into the PALS, where they may exert either a direct or indirect effect on the nerve fibers.

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380.11 MAINTENANCE OF NORADRENERGIC SYMPATHETIC FIBERS IN AGED INVOLUTING THYMUS. D.L. Bellinger, D.L. Felten and S.Y. Felten. Department of Neurobiology & Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

Sympathetic postganglionic noradrenergic fibers innervate both primary and secondary lymphoid organs. Nerve fibers derived from neurons in the superior cervical ganglion enter the thymus with the vasculature and distribute in the cortex, particularly in the subcapsular region and around the vasculature at the cortico-medullary junction. Noradrenergic innervation of secondary lymphoid organs distributes into compartments where T lymphocytes reside; this innervation declines with age in both lymph nodes and the spleen. This study was undertaken to determine whether a similar decline in noradrenergic innervation occurs in the thymus. Thymuses were examined in Fischer 344 rats at 3, 8, 12, 17, 21, 24, and 27 months of age. The noradrenergic innervation remained stable in appearance at 3, 8, and 12 months of age. At 17 months of age and older, the density of innervation appeared to increase in the thymic cortex; however, the thymus at these ages was reduced significantly in weight compared with younger ages due to involution. Therefore, it appears that the total noradrenergic innervation is maintained in aged Fischer 344 rats even in the face of involution, resulting in an increased density of fibers. This increase in density of noradrenergic fibers may contribute a higher concentration of norepinephrine in the local microenvironment, where it can interact with adrenergic receptors on thymocytes.

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380.12 TIME COURSE OF DEPLETION OF NORADRENERGIC INNERVATION OF THE SPLENIC WHITE PULP IN AGED FISCHER 344 RATS AND ITS RELATIONSHIP TO DECLINING POPULATIONS OF SPECIFIC IMMUNE CELLS. D.L. Felten, K.D. Ackerman, D.L. Bellinger, and S.Y. Felten, Department Neurobiology & Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

The sympathetic postganglionic noradrenergic innervation of the splenic white pulp is reduced significantly in 27 month old Fischer 344 rats compared with 8 month old rats (S.Y. Felten et al., Neurobiol. of Aging 8: 159-165, 1987). The number and density of fluorescent varicosities, assessed by morphometry, are reduced in all compartments of the spleen, and the norepinephrine content, assessed by high performance liquid chromatography with electrochemical detection, also is reduced. In the present study, we examined the noradrenergic innervation of the spleen with fluorescence histochemistry of catecholamines and double-labelled immunohistochemistry for tyrosine hydroxylase (TH)-positive nerve fibers and specific markers for T lymphocytes (OX-19) and macrophages at the marginal sinus (ED-3), to assess intermediate ages between 8 and 27 months of age. Fischer 344 rats were sacrificed at 3, 8, 12, 17, 21, 24, and 27 months of age. Noradrenergic innervation of the splenic white pulp, assessed by both methods, remained constant through 12 months of age. By 17 months of age, a noticeable decline in the number and density of nerve fibers was evident. This decline continued through 27 months of age. Observations with double-labelled immunohistochemistry also revealed a decline in the number of OX-19-positive T lymphocytes, as well as a diminution of white pulp by 17 months of age. Similarly, the number of ED-3-positive macrophages at the marginal sinus decreased with age. This remarkable parallel in decline of noradrenergic fibers and specific populations of immune cells supports the notion that regulatory influences may be exerted in either direction, and that the decline of one may precipitate or influence the decline of the other. This is consistent with evidence from past observation in our laboratories that the presence of noradrenergic innervation of the spleen and lymph nodes in adults is necessary for immunocompetence (Livnat et al., J. Neuroimmunol. 10: 5-30, 1985).

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380.13 SPLENIC NOREPINEPHRINE TURNOVER IS INCREASED DURING AN IMMUNE RESPONSE IN MICE. S.L. Carlson, S.Y. Felten, S. Livnat* and D.L. Felten. Dept. of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642

The noradrenergic (NE) innervation of the spleen follows the vasculature and extends into the T cell zones of the splenic white pulp. NE released from the nerve terminals into the local splenic microenvironment may modulate or regulate lymphocyte function via interaction with adrenergic receptors on the target cells.

As part of a larger study of the role of NE innervation of the murine spleen, we have found that immunization induced by repeated injections of normal rabbit serum (NRS) is accompanied by alterations in NE content and turnover in the spleen. Adult male C3H/HeN mice were injected intraperitoneally four times over 8 days with 0.2ml of NRS (NRS-4x) or saline, and were sacrificed one day after the last injection. A second group of mice received one injection of NRS (NRS-1x) or saline, and were sacrificed two days later. The spleens were removed, weighed and frozen for subsequent measurement of catecholamines and their metabolites with HPLC, and for histofluorescence of the nerve profiles.

The spleens of the NRS-4x group were 2.8 times larger by weight than the saline controls, indicative of the lymphocyte proliferation occurring due to the immune response. Quantification of the total splenic NE (pmol/spleen) revealed that NE was decreased 43% in the NRS-4x and 37% in the NRS-1x groups. MHPG, a metabolite of NE, was significantly increased in the NRS-4x group, but was decreased in the NRS-1x group. The concentration of NE (pmol/mg protein) also was decreased in the NRS-4x and NRS-1x groups, while the MHPG concentration was decreased in the NRS-1x group.

Previous studies from our laboratory have shown that at least 95% of the NE in the spleen is contained in the nerve terminals, and therefore is not due to the presence of circulating catecholamines in the blood. We conclude that during an ongoing immune response, splenic NE is decreased due to a higher rate of turnover of the transmitter from the nerve terminals. The lack of an increase in MHPG at an early time point in the immune response (NRS-1x) may indicate that the NE turnover rate has not yet increased to the extent seen later during the immune response, or that different mechanisms may be involved during the early phase of a developing immune response. These results give additional support to the hypothesis that NE plays a role in the bidirectional communication between the peripheral autonomic nervous system and the immune system.

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380.14 **ß-ENDORPHIN INHIBITS PRIMARY HUMORAL IMMUNE RESPONSE IN RATS.** L.M. Hemmick and J.M. Bidlack. Center for Brain Research, Univ. of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

$ß_h$-endorphin 1-31 suppressed the rat primary humoral immune response to sheep red blood cells (SRBC). Sprague-Dawley male rats weighing 150-175g were injected i.p. with SRBC (10^8 cells/rat). Seven days after immunization, splenocytes sterilely prepared from immunized rats were cultured with $ß_h$-endorphin 1-31 at 10^{-7} cells/ml in RPMI 1640 medium containing 5% fetal bovine serum for 48 hr at 37°C in a 5% CO_2 incubator. Splenocytes were then tested for their ability to lyse SRBC targets in the presence of guinea pig complement. Plaques were counted under low magnification using an indirect light source. Treatment with 1 μM $ß_h$-endorphin 1-31 suppressed the primary humoral immune response by 31.4% ± 10.2%. Studies are underway to determine if this suppression is naloxone reversible and if other opioids are capable of suppressing the primary humoral immune response.

$ß_h$-endorphin 1-31 enhanced concanavalin A (Con A)-stimulated uptake of $^{45}Ca^{2+}$ by rat thymocytes (Soc. Neurosci. Abst., 12: 340, 1986). $ß_h$-endorphin 1-31 suppression of the humoral immune response to the T-cell dependent antigen SRBC may correlate to its enhancement of Con A-stimulated thymocyte $^{45}Ca^{2+}$ uptake, if the $^{45}Ca^{2+}$ uptake of the T-suppressor cell sub-population is selectively enhanced. In contrast to the thymocyte data, $ß_h$-endorphin 1-31 did not significantly affect either basal or Con A-stimulated $^{45}Ca^{2+}$ uptake by rat splenocytes, a more heterogeneous cell population than thymocytes.

In order to investigate possible long-term modulation of lymphocyte $^{45}Ca^{2+}$ uptake by opioids, 150-175g Sprague-Dawley male rats were made dependent on morphine by subcutaneous implantation of a pellet containing 75 mg morphine base. After 1 week, naloxone-precipitated withdrawal was used as the index of dependence in a representative morphine-treated rat. Thymocyte and splenocyte populations were prepared from morphine and placebo-treated rats. The basal and Con A-stimulated $^{45}Ca^{2+}$ uptake of addicted vs. placebo-treated thymocytes and splenocytes were then compared. Preliminary data showed that morphine dependence had no significant effect on thymocyte basal or Con A-stimulated $^{45}Ca^{2+}$ uptake. Dependence also did not significantly affect the ability of $ß_h$-endorphin 1-31 to enhance Con A-stimulated $^{45}Ca^{2+}$ uptake by rat thymocytes. In addition, morphine dependence did not significantly affect basal or Con A-stimulated $^{45}Ca^{2+}$ uptake by rat splenocytes.

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380.15 METHIONINE-ENKEPHALIN IS A MODULATOR/REGULATOR OF IMMUNE REACTIONS. B.D.Janković* and D.Marić* (SPON: S.K.Sobrian). Immunology Research Center, Vojvode Stepe 458, 11221 Belgrade, Yugoslavia.

During the past few years, several studies have provided evidence that opioid peptides can modulate immune function. A wide range of in vitro immunomodulatory effects have been described for neuropentapeptides. However, the in vivo manipulations with these opioids are still in the initial phase. We report here on variations in immune responsiveness in mice and rats treated with methionine-enkephalin (Met-Enk). Several schedules of intraperitoneal and intracerebroventricular injections, and various doses of Met-Enk (0.1 to 10 mg/kg) were employed in different immune paradigms. The following findings support the view that Met-Enk may be involved in mechanisms underlying the integrative function of the nervous, endocrine and immune systems. (a) Met-Enk exerted a dose-dependent, bidirectional effect on humoral and cell-mediated immune reactions. Thus, high doses (2-10 mg/kg) suppressed plaque-forming cell response, antibody production to particular and soluble antigens, local Arthus reactivity, delayed skin hypersensitivity, allograft rejection and inflammatory immune reactions. In contrast, low doses of Met-Enk (0.1-0.5 mg/kg) potentiated immune responsiveness. (b) Immunoregulatory activity of this peptide was much more pronounced after its administration through a cannula permanently inserted into the lateral ventricle of the rat brain. (c) Met-Enk treatment of rats sensitized for anaphylactic shock completely protected the animals from fatal shock. (d) Anti-inflammatory effect of Met-Enk was evident in rats sensitized with mycobacterial adjuvant for adjuvant arthritis. (e) High doses of Met-Enk suppressed both the incidence of neurological symptoms and intensity of histological lesions in the brain and spinal cord in rats sensitized for experimental allergic encephalomyelitis. In addition, high doses of Met-Enk affected the passive transfer of the disease and prevented further development of clinical signs. (f) The experiments with antagonists of mu- and delta-opioid receptors revealed that Met-Enk acts primarily via delta-receptors. These results suggest that Met-Enk is an important constituent of the signal repertoire operating in the neuroimmune system. (Supp. by the Republic of Serbia Research Fund, Belgrade, and Thymoorgan Pharmazie, Vienenburg, F.R.G.)

380.16 SUPPRESSION OF NATURAL KILLER (NK) CELL ACTIVITY IN RATS BY VARIOUS NARCOTIC AGENTS. B. Beilin*, F.C. Martin, Y. Shavit, S. Ben-Eliyahu*, S.H. Sohn*, J.C. Liebeskind. (SPON: S.C. Lee). Dept. of Anesthesiology, Brain Research Institute, and Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

Surgical stress and general anesthesia are known to suppress immune function. This effect may contribute to the problem of post-operative infections. Exposure to a form of stress known to activate endogenous opioids suppresses NK activity and accelerates tumor growth in rats. Systemic morphine mimics these effects. In the present study, we examined the effects of 3 opiates commonly used in general anesthesia on NK activity in rats.

Male Fischer 344 rats were injected (s.c.) with morphine (30 mg/kg), fentanyl (0.3 mg/kg), or sufentanil (0.06 mg/kg) at 3, 12 or 24 hours before splenectomy. Controls received saline. Animals were anesthetized with halothane, and spleens removed and dissociated into single cell suspensions. Spleen cells were co-cultured with chromium labeled YAC-1 target cells, and NK cell cytotoxicity was determined in a 4 hrs chromium release assay.

All 3 drugs significantly suppressed NK cytotoxicity by 25-50% of control values at 3 hrs after administration, and this effect was blocked by naltrexone. Fentanyl and sufentanil, and to a lesser degree morphine, also caused a significant suppression (15-40%) 12 hrs after drug administration. Sufentanil still caused a substantial but not significant suppression 24 hrs later.

These results show that the commonly used anesthetic opiates can suppress NK cell activity for up to 24 hrs. In light of the increasing clinical use of high-dose narcotic anesthesia, it will be important to investigate this phenomenon in human beings and to study the mechanisms by which opiates exert their immunological effects. (Supported by NIH grant NS07628 and the David H. Murdock Foundation for Advanced Brain Studies. Y.S. was supported by the MacArthur Foundation).

380.17 EFFECTS OF CORTICOSTERONE ON NATURAL KILLER (NK) CELL ACTIVITY *IN VITRO*. F.C. Martin, L. O'Farrell, S.E. Holley*, J.C. Liebeskind. Brain Research Institute and Dept. of Psychology, UCLA, Los Angeles, CA 90024.

The effects of acute stress on immune activity in rats have received considerable attention in recent years. Our group has reported NK suppression in rats 3 h after either footshock or 30 mg/kg morphine. The role of the pituitary-adrenal axis in these effects is important to understand, since corticosterone is immunosuppressive. We showed that adrenalectomy but not demedullation blocks the morphine-induced NK suppression, but we have not established whether adrenal cortical hormones are solely responsible for these effects. The goal of this study was to determine whether levels of corticosterone seen in morphine-treated animals suppress NK activity *in vitro* .

Corticosterone levels were measured in morphine and saline treated rats. From these results we chose 100 ng/ml as baseline and 500 ng/ml as the morphine-induced level.

Spleen lymphocytes from untreated rats were suspended in RPMI cell culture medium containing 0 (control), 10 (below physiological), 100 (baseline), 500 (morphine) or 1000 (upper end of physiological) ng/ml corticosterone, and incubated for 3 h at 37^{o} C. Cells were washed and tested in a standard chromium-release NK assay.

Even 10 ng/ml corticosterone caused suppression of NK activity (22%) compared to control, and 100 ng/ml suppressed NK activity by about 40%. However, 500 or even 1000 ng/ml caused only a slight further suppression over that produced by 100 ng/ml. The finding that there is little differential effect of corticosterone over the physiological dose range calls into question the hypothesized role of corticosterone in mediating the NK suppressive effects of stress and morphine. (Supported by NIH NS07628 and the David Murdock Foundation for Advanced Brain Studies)

380.18 MORPHINE SUPPRESSES THE CYTOTOXIC ACTIVITY OF NATURAL KILLER (NK) CELLS SIMULTANEOUSLY HARVESTED FROM SPLEEN, BONE MARROW, AND PERIPHERAL BLOOD. Y. Shavit, F.C. Martin, R. Yirmiya*, S. Ben-Eliyahu*, G.M.L.H. Angarita*, S.H. Wald*, R.P. Gale*, and J.C. Liebeskind. Departments of Psychology and Medicine, Brain Research Institute, UCLA, Los Angeles, CA 90024-1563.

Recent evidence suggests that opiates and endogenous opioids play a modulatory role in immune function. We have previously shown that systemically administered morphine suppresses splenic NK cell cytotoxicity in rats, an effect blocked by the opiate antagonist, naltrexone (Shavit, et al., Science, 223: 188-190, 1984). Moreover, intracerebroventricularly administered morphine suppresses splenic NK activity at a dose 1,000 times smaller than the systemically required dose; this effect also is blocked by naltrexone (Shavit, et al., PNAS, 83: 7114-7117, 1986). Some drugs are known to cause redistribution of lymphocytes in the different compartments of the immune system, and this process might account for the observed splenic NK suppression. The present study, therefore, examined the effect of systemically administered morphine on the cytotoxic activity of NK cells derived from several immune compartments: spleen, bone marrow, and peripheral blood.

Fischer 344 male rats were injected with morphine (30 mg/kg, s.c.) or saline. Three hours later, rats were anesthetized with halothane, peripheral blood was collected by heart puncture, bone marrow was collected from the femur, and spleens were removed. All cells were dissociated into single cell suspensions and incubated with carbonyl iron for 30 min at 37°C. Cells were then separated using separation media (Sepracell), washed twice in PBS, counted, and their final concentration adjusted to 1X10^{7} cells/ml. Cells were co-cultured with chromium labeled YAC-1 target cells, and NK cytotoxicity was determined in a 4-hr chromium release assay.

Morphine significantly suppressed the cytotoxic activity of NK cells derived from all three immune compartments to about 60-70% of control values. These data suggest that morphine-induced NK suppression is an overall suppression of a particular host-defense mechanism, rather than specific emigration of NK or other regulatory cells out of the spleen.

(Supported by NIH grant NS-07628 and a gift from the David H. Murdock Foundation for Advanced Brain Studies. Y.S. was supported by the MacArthur Foundation).

380.19 STRESS-INDUCED IMMUNOSUPPRESSION IN RATS: A NALTREXONE-INSENSITIVE PARADIGM. S. Ben-Eliyahu*, R. Yirmiya*, F.C. Martin, P.G. McKinley*, Y. Shavit, J.C. Liebeskind. (SPON: J.W. Lewis). Department of Psychology, UCLA, Los Angeles, CA 90024-1563.

Stress can adversely affect both cellular and humoral aspects of immune function. However, stress can also augment immune function. In our laboratory, we have shown that a form of footshock stress known to activate endogenous opioid systems suppresses the cytotoxic activity of NK cells in rats, an effect blocked by the opiate antagonist, naltrexone (Shavit et al., Science, 223:188, 1984). In contrast, a very similar form of footshock stress, which induces a nonopioid form of analgesia, had no effect on NK cell activity. We also found that several well established stressors, such as isolation, crowding, and restraint, had no effect on NK cytotoxicity. We now report that another stress paradigm, forced swimming, causes NK suppression not blocked by naltrexone.

Fischer 334 male rats, 11 weeks old, were assigned to 3 groups. Thirty minutes before the stress session, one group (n=12) was injected with saline, another (n=12) was injected with naltrexone (10 mg/kg, s.c.), and a third (n=14), which served as non-stressed control, was also injected with saline at this time. During the stress session, a weight of 45 g/kg b.w. was attached to the tails of rats of groups 1 and 2. Each rat was then placed in a tank of 37°C water for 3 minutes, followed by a 3-minute rest period. This procedure was repeated 5 times consecutively. One hour later spleens from all animals were removed and dissociated into a single-cell suspension. Cells were co-cultured with chromium-labeled YAC-1 target cells, and NK cytotoxicity was determined in a 4 hour chromium release assay.

Swimming stress significantly suppressed NK cell cytotoxicity in both saline- and naltrexone-treated rats compared with controls. The magnitude of the suppression (to about 75% of control levels) was similar in the two groups. These results suggest the existence of opioid and nonopioid mechanisms in stress-induced immunosuppression. More generally, these results reinforce the idea that stress is not uniform with respect to its effects on the immune system. (Supported by NIH grant NS07628 and the David H. Murdock Foundation for Advanced Brain Studies. Y.S. was supported by the MacArthur Foundation).

380.20 NATURAL KILLER (NK) CELL ACTIVITY IN VASOPRESSIN DEFICIENT RATS (BRATTLEBORO STRAIN). R. Yirmiya*, Y. Shavit, S. Ben-Eliyahu*, F.C. Martin, P.G. McKinley* and J.C. Liebeskind. Neuroscience Program, Brain Research Institute, and Department of Psychology, University of California, Los Angeles, CA 90024-1563.

Several lines of evidence suggest that the neurohypophysial hormone vasopressin is involved in regulation of the immune system. We explored this possibility in 3 experiments in which the cytotoxic activity of NK cells was compared between Brattleboro rats, which are homozygous for diabetes insipidus (DI) and lack vasopressin, and Long Evans (LE) rats (the strain from which the DI rats were derived). Additionally, we compared the effects of swimming stress and morphine administration on NK activity in these two strains.

In Exp. 1, spleens from 10 DI and 10 LE rats were removed and dissociated into single cell suspensions. Cells were co-cultured with chromium-labeled YAC-1 target cells, and NK cytotoxicity was determined in a 4 hr chromium release assay. In Exp. 2, 12 DI and 11 LE rats were subjected to swimming stress, while another 12 DI and 11 LE rats served as non-stressed controls. During the stress session, a weight of 45 g/kg b.w. was attached to the rats' tails. Each rat was then placed in a tank of 37° C water for 3 min, followed by a 3-min rest period. This procedure was repeated 5 times consecutively. One hr after the completion of the stress session, spleens of experimental and control animals were removed, processed and assayed for NK cell activity as described above. In Exp. 3, 14 DI and 18 LE rats were injected with morphine (30 mg/kg; s.c.), while another 13 DI and 17 LE control rats were injected with saline. Three hr after the injection, spleens of all rats were removed, processed and assayed for NK cell activity.

In all 3 experiments, NK cell activity was significantly higher in DI rats compared with LE rats. In Exp. 1, and in the control groups of Exp. 2 and 3, NK activity of DI rats ranged between 140-200% above that of LE rats. Both swimming stress and morphine significantly suppressed NK activity in DI and LE rats (to about 80% of their respective non-stressed and saline-injected controls). These results suggest that vasopressin is involved in tonic regulation of NK cell activity, but not in mediating the immune-suppressive effects of stress and morphine. (Supported by NIH grant NS-07628 and a gift from the David H. Murdock Foundation for Advanced Brain Studies. Y.S. was supported by the MacArthur Foundation).

381.1 ULTRASTRUCTURAL LOCALIZATION OF AN OCTADECANEUROPEPTIDE (ODN) DERIVED FROM DBI IN THE RAT BRAIN. H. Alho, D. Jenkins, P. Bovolin, A. Guidotti and E. Costa. FIDIA-Georgetown Institute for the Neurosciences, Georgetown University Medical School, Washington, D.C. 20007

A 10-kDa endogenous neuropeptide that modulates gamma-aminobutyric acid (GABA) receptor function has been termed diazepam binding inhibitor (DBI) because of its capability to displace diazepam from its specific brain recognition sites. DBI has been shown to be localized in neurons and specific populations of glial cells in the rat brain (Alho et al., Science, 229, 1985). Tryptic digestion of DBI generates an octadecaneuropeptide (ODN) that shares with DBI the ability to displace specifically bound beta-carbolines and produce proconflict action when injected intraventricularly in rodents. ODN-like immunoreactivity (LI) is highly concentrated in neurons but is virtually absent in glial cells. In primary cultures of cortical cells ODN-LI is localized in GABAergic neurons and can be released by depolarization with high potassium and veratridine. However, a direct evidence for subcellular and ultrastructural localization of ODN in neurons is lacking. An antiserum against synthetic ODN was raised in rabbits and electron microscopical immunohistochemical studies were performed employing the peroxidase-anti-peroxidase method and biotin-avidin-colloidal gold methods. Normal and colchicine pretreated rat brains were perfused with formaldehyde/glutaraldehyde fixative and vibratome sections were used to study the ODN-LI in different brain areas at electron microscopical level. The ODN-LI was compared to the glutamic acid decarboxylase (GAD)-LI. The peroxidase ODN-LI was found in neurons throughout the cytoplasm of numerous axon terminals and few pericarya and dendrites. In the cerebellum, ODN-LI was identified in Golgi cells and axon terminals. The immunoreactive material was concentrated in synapses around the Purkinje cells and in the molecular layer of cerebellum. In the glial stuctures no specific staining was detectable. The GAD-LI was localized in identical neuronal stuctures. The present results demonstrate the localization of ODN-LI in synapses and establish the presence of ODN-LI in neuronal sructures that are important to synaptic transmission. The findings support the suggestion of the neuromodulatory role of ODN in GABAergic transmission.

381.2 ANATOMICAL LOCALIZATION OF DIAZEPAM BINDING INHIBITOR (DBI) AND OCTANEUROPEPTIDE (ODN)-LIKE IMMUNOREACTIVITY IN RAT BRAIN. P. Bovolin, H. Alho, A. Guidotti and E. Costa (SPON: M.R. Santi). Fidia-Georgetown Institute for the Neurosciences, Georgetown University Medical School, Washington, D.C. 20007.

An endogenous neuropeptide (DBI) that displaces benzodiazepines and beta-carbolines from their specific modulatory site on $GABA_A$ receptors, has been recently isolated and purified from rat, bovine and human brains. DBI injected intraventricularly into rodents has a proconflit action and antagonizes the anti-conflict action of diazepam in the Vogel behavioral test. It also decreases the duration of Cl^- channel opening bursts elicited by GABA in primary cultures of spinal cord neurons. In rat brain, DBI is the precursor of a new family of peptides including an octadecaneuropeptide (ODN) that shares with DBI the ability to displace specifically bound beta-carbolines from their binding sites. DBI- and ODN-like immunoreactivity (LI) has been localized in GABAergic neurons in primary cultures of cortical cells and DBI-LI has been localized in neurons and specific population of glial cells in rat brain at the light microscopical level. Immunohistochemical studies were performed using antisera raised in rabbit against DBI purified from rat brain and synthetic ODN. For these experiments, normal and colchicine pretreated rat brains were perfused with 4% paraformaldehyde and consecutive vibratome (25 um) and cryostat (20 um) sections were stained with the peroxidase-anti-peroxidase method. The preabsorption of anti-DBI antiserum with 10 ug/ml of DBI peptide and anti-ODN with 20 ug/ml of ODN peptide completely abolished the staining, while the preabsorption of ODN antiserum with 20 ug/ml DBI peptide did not alter significantly the staining intensity in neurons. DBI- and ODN-LI was detected in several brain regions, including cortex, hippocampus, amygdaloid area, habenular area and cerebellum. An intense DBI-LI but not ODN-LI was observed in Bergman glial cells in cerebellum and in the tanycytes around the third ventricle. After colchicine pretreatment ODN and DBI positive neurons were detected in few pyramidal cells of hippocampus, small neurons in subiculum, various size neurons in cortex, large neurons in reticulothalamic nucleus, small neurons in subgeniculate nucleus and in Golgi, Stellate and Purkinje cells of cerebellum. Studies conducted on consecutive sections detected positive reactions to ODN-LI and DBI-LI in neurons of the same brain areas and nuclei, but DBI-LI was constantly weaker and no ODN-LI was found in glial cells. The present results support the suggestion that DBI may act as an ODN precursor and that DBI might be processed differently in glial cells and neurons.

381.3 CGRP IN HUMAN NERVOUS SYSTEM AMD CEREBROSPINAL FLUID: HIGH LEVELS IN THE LOCUS CERULEUS. J. K. Tiller-Borcich M.D., H. Capili, G. S. Gordan M.D. (SPON: E.W. Yund) Departments of Pathology and Internal Medicine, UC Davis, VAMC, Martinez, CA 94553

A quantitative survey of calcitonin gene-related peptide(CGRP) in human brain, peripheral nerve and cerebrospinal fluid(CSF) was performed using radioimmunoassay(RIA) with antisera against synthetic hGRPP. High levels(approximately 2000-15000 fmol/mg protein) were found in the dorsal spinal cord, dorsal nerve and trigeminal nerve. Relatively large amounts(500-2000) were found in parts of the hypothalamic-pituitary axis, peripheral nerve and for the first time, in the locus ceruleus. Low levels of CGRP (less than 500) were detected in the cerebrum, subcortical nuclei and cerebellum. CGRP, not previously reported in the CSF, was detectable in all of 27 CSF specimens with mean values of 39 + 4.5 pmol/L(SE). Simultaneous plasma CGRP levels were higher and, when elevated by antihypertensive treatment, were not increased in the CSF, just as astronomical plasma levels of calcitonin in medullary carcinoma of the thyroid are not reflected in CSF. Our data confirm and extend the results of previous human and animal studies with species variation: humans have low CGRP levels in subcortical nuclei whereas high levels have been found in rat caudate-putamen and amygdala. The high level of CGRP in the locus ceruleus(LC), the major source of noradrenergic transmisssion in the CNS, is in harmony with the presumed functions of the LC and the very potent hemodynamic activity of CGRP. This work was supported, in part, by the Veterans Administration Medical Research Service.

381.4 Atrial Natriuretic Factor (ANF) Concentrations In Discrete Brain Regions Of Female Rats. N.W. Hoffman,* A.A. MacPhee*, and A.A. Gerall (SPON: G. Dohanich). Alton Ochsner Medical Foundation and Dep. of Psychol., Tulane University, New Orleans, LA 70118

ANF is a set of polypeptides secreted from cardiac atria that promote natriuresis, diuresis, and vasodilation. Recently, immunocytochemical, radioimmunoassay (RIA), and receptor binding studies have revealed ANF-immunoreactivity and binding sites in the rat's brain. RIA of central ANF levels has either been performed on samples from relatively large brain areas (Morii, N., Biochem. Biophys. Res. Commun., 127:413, 1985) or regionally discrete brain tissue that had not been extracted for ANF (Samson, W.K., Endocrinol., 117:1279, 1985). In the present study, ANF-immunoreactivity was compared in ANF-extracted samples of microdissected brain regions and pituitaries of female rats.

Forty eight Sprague Dawley female rats approximately 180 days of age maintained on a 14 hr light: 10 hr dark illumination cycle were studied. Whole brains and pituitaries were rapidly removed after decapitation and frozen in dry ice. The following brain regions were microdissected (Palkovits, M., Brain Res., 59: 449, 1973): organum vasculosum of the lamina terminalis (OVLT), medial preoptic area (MPOA), median eminence (ME), suprachiasmatic nucleus (SCN), supraoptic nucleus (SON), and the fourth ventricular grey region (FVG) containing locus coeruleus. Samples were boiled for 10 min and homogenized in 0.1 N acetic acid. Supernatants were applied to methanol-activated Sep-Pak C18 cartridges and eluted with 80% MeCN in .1% TFA, then taken to dryness in a Speed-Vac. Extracted ANF levels were then measured by RIA. The percent ANF recovery by this procedure is 90%, and the intra- and inter-RIA coefficients of variation are 6.4 and 9.7, respectively. Regional ANF concentrations (ng/mg protein ± SEM) are presented below.

MPOA	ME	OVLT	SCN
1.67 ± 0.06	1.13 ± 0.04	0.70 ± 0.05	0.70 ± 0.03
SON	SCN	Pituitary	
0.40 ± 0.04	0.24 ± 0.02	0.01 ± 0.00	

ANF concentrations at the seven regions significantly differed from one another ($p<.01$) with the exception of the SCN vs OVLT ($p>.05$). The finding that concentrations vary across discrete regions supports a neurosecretory role for brain ANF. Highest concentrations were present in the MPOA, a region associated with the control of vasomotor and electrolyte balance, drinking behavior, temperature, and phasic release of gonadotropins. Accordingly, ANF may participate in regulating these processes. Additionally, high ME ANF concentrations indicate a role in mediating pituitary hormonal output.

381.5 DISTRIBUTION OF NEUROPEPTIDE Y, NEUROTENSIN, SEROTONIN AND VASOACTIVE INTESTINAL POLYPEPTIDE IN RELATION TO LUTEINIZING HORMONE RELEASING HORMONE IN THE COMMON MARMOSET.
Clive W. Coen and Kevin T. O'Byrne*. Dept. Anatomy & Human Biology, King's College London & *MRC Reproductive Biology Unit, Edinburgh, United Kingdom.

Luteinizing hormone (LH) release in the common marmoset (Callithrix jacchus) is stimulated by treatment with the hypothalamic neuropeptide LH releasing hormone (LHRH). Few other details are available concerning hypothalamic involvement in reproductive processes in this species. In order to initiate various anatomical and physiological studies we have examined the distribution of hypothalamic LHRH; we have also assessed the correspondence between the distribution of processes with immunoreactivity (IR) for certain neurotransmitter-related antigens and those regions containing the LHRH perikarya.

Using peroxidase-antiperoxidase immunohistochemistry with LHRH antisera kindly donated by B. Flerko and L.H. Jennes we have detected LHRH cell bodies around the organum vasculosum of the lamina terminalis (OVLT), within the periventricular preoptic area and in the paraventricular and supraoptic nuclei. The correspondence between LHRH perikarya and other neurochemically identified processes was assessed in both adjacent and single sections, the latter being achieved with two-colour detection methods involving various combinations of the conventional brown diaminobenzidine reaction product, benzidine blue induced by ammonium nickel sulphate and the purple staining obtained with α-naphthol and pyronin.

The regions containing LHRH perikarya were characterized by a marked intensity of neuropeptide Y-IR, the supraoptic area displaying a slightly less dense distribution than the other sites and the caudal paraventricular region receiving processes predominantly medial to the LHRH cell bodies. The distribution of neurotensin-IR was strikingly similar to that of neuropeptide Y. Although serotonin-IR was predominantly lateral and medial to the LHRH perikarya at the level of the OVLT, the medial LHRH cell bodies immediately caudal to that point lay within a dense concentration of immunoreactive processes; the supraoptic region showed only a moderate density and at the level of the caudal paraventricular nuclei the intensely immunoreactive processes were predominantly situated medial to the LHRH cell bodies. Vasoactive intestinal polypeptide-IR was also located lateral to the LHRH cell bodies around the OVLT but the other sites containing LHRH demonstrated an apparently uniform medium density of immunoreactive processes.

These observations offer only indirect evidence for possible neural interactions; they do, however, indicate some of the possible transmitter candidates for the neuromodulation of LHRH release and consequently for the hypothalamic control of ovulation.

381.6 DISTRIBUTION OF NOVEL NEUROPEPTIDES, NEUROMEDIN U AND PANCREASTATIN IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM (CNS).
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Neuromedin U (Minamino et al., Biochem. Biophys. Res Comm., 130:1078, 1985) and pancreastatin (Tatemoto et al., Nature 324:476, 1986) are two recently discovered peptides. To determine their distribution and hence further elucidate their possible biological rôles their cellular localisation was studied by immunocytochemistry and tissue levels evaluated by radioimmunoassay throughout rodent and porcine CNS.

Neuromedin U and pancreastatin immunoreactivities were found in rat and pig tissues respectively. Neuromedin U-immunoreactive (IR) fibres were distributed throughout the brain. IR cell bodies were mostly restricted to the arcuate nucleus although a few were found in the central amygdaloid nucleus. The relative densities of neuromedin U-IR neuronal systems in the brain were reflected in the extractable tissue levels. Significant concentrations were found in the hypothalamus, thalamus, amygdala, nucleus accumbens, tegmental ganglia and medulla oblongata. In the spinal cord a few fibres were localised to the dorsal horn and the area around the central canal.

Pancreastatin-IR fibres were also found in many brain regions including hypothalamus, cortex, hippocampus and brainstem. The posterior pituitary contained a small number of fibres. IR cell bodies were present in the arcuate nucleus and large numbers of neurones were apparent in paraventricular and supraoptic hypothalamic nuclei. In the brain, the highest extractable pancreastatin IR levels were recorded in the hypothalamus, cortex, thalamus and hippocampus. In contrast to neuromedin U, pancreastatin IR was abundant in the spinal cord. IR fibres were concentrated in laminae I-II of the dorsal horn (a proportion of which presumably derive from pancreastatin-IR cells observed in the dorsal root ganglia), intermediolateral cells columns and the ventral horn. The localisation of neuromedin U and pancreastatin to extra- and hypothalamic regions suggest multiple rôles and thus both peptides are potentially important in neural regulation of the CNS.

381.7 THE VASOPRESSIN AND OXYTOCIN IMMUNOREACTIVE SYSTEMS IN THE BRAIN AND UPPER SPINAL CORD OF THE PRIMATE MACACA FASCICULARIS.
A.R. Caffé*, F.W. van Leeuwen, P.C. van Ryen*[1] and T. van der Woude*. Netherlands Institute for Brain Research, Amsterdam, and [1]Department of Neurosurgery, University Hospital, Utrecht, The Netherlands.

The various extrahypothalamic vasopressin (VP) cell groups in the rat brain are largely responsible for the extensive central VP pathways, part of which is also sexually dimorphic (De Vries et al., J. Comp. Neurol., 233: 236, 1985). Studies conducted on the human brain, demonstrated VP cells in the bed nucleus of the stria terminalis (BST) and the presence of extensive central VP and oxytocin (OXT) pathways, which are different from the rat (Fliers et al., Brain Res., 375: 363, 1986). However, due to suboptimal tissue treatment, no complete picture of the distribution of these neuropeptide systems in the human brain might have emerged.

Therefore adult monkeys of both sexes were either untreated or intraventricularly treated with 1 mg colchicine. After a survival time of 48 hours, during which Temgesic was administered to colchicine monkeys, the animals were perfused with 6% paraformaldehyde. 100 um Vibratome sections of the brain and cervical spinal cord were stained for the presence of VP (using antisera directed against: VP, rat neurophysin and guinea pig glycopeptide) and purified OXT.

Apart from the known hypothalamic paraventricular, supraoptic and suprachiasmatic nuclei, VP cell groups were found in the diagonal band of Broca (DBB), BST, medial amygdaloid nucleus, dorsomedial hypothalamic nucleus, locus coeruleus (LC), nucleus tractus solitarius (NTS) and the substantia gelatinosa of the dorsal horn. In addition, dense VP innervation was observed in the medial septum, DBB, BST, anterior and cortical amygdaloid nuclei, molecular layer of the subiculum and CA1 region of the hippocampus, paraventricular nucleus of the thalamus, nucleus reuniens, periaquaductal gray, area of Forel, ventral tegmental area, LC, parabrachial nuclei and the NTS. In contrast to the rat, areas such as the lateral septum and the lateral habenula were not innervated and no sexually dimorphic extrahypothalamic VP system was found in this monkey species.

No OXT cells were observed outside the monkey hypothalamus. Dense OXT innervation occurred in the cortical amygdaloid nucleus, NTS and the marginal zone of the spinal cord.

The results demonstrate that the number of extrahypothalamic VP cell groups in the monkey is much larger and their location more widespread than in the rat. In addition, the VP and OXT fiber systems in the macaca fascicularis brain is quite different from that of the rat both in density and in brain regions innervated. However, they resemble that of the human brain.

381.8 AN IMMUNOHISTOCHEMICAL STUDY OF CORTICOTROPIN-RELEASING FACTOR-LIKE IMMUNOREACTIVITY (CRFLI) IN MONKEY BRAIN.
S. L. Foote, C. I. Cha*, D. A. Lewis, and J. H. Morrison. Div. Neurosci., Res. Inst. Scripps Clinic, La Jolla, CA 92037.

Immunohistochemistry was used to examine the distribution of CRFLI in brains of two monkey species (Saimiri sciureus, Macaca fascicularis). The results indicate that both similarities and differences exist between rodent and primate in CRFLI distribution. In monkey, like rat, dense immunoreactivity was evident in parvocellular neurons of the paraventricular nucleus and in fibers extending into the median eminence. However, CRFLI perikarya previously observed in other hypothalamic nuclei in rats were not evident in monkey. Also in contrast to previous rat studies, CRFLI cells were evident in several thalamic nuclei, especially the intralaminar complex. There were also two large groups of CRFLI neurons in the brainstem which have not been previously described: a group just lateral to the mesencephalic tegmentum throughout the rostral-caudal extent of the midbrain, and labeled perikarya throughout the inferior olive. The distributions of CRFLI fibers also exhibited similarities and differences between monkey and rat. The most striking terminal fields not previously described are within and adjacent to the substantia nigra pars compacta, in certain subdivisions of the interpeduncular nucleus, and in cerebellar cortex.

Most labeled cortical neurons were small, bipolar cells with radially-oriented dendrites, although some, especially in layers II and IV, were multipolar. Regional differences were evident in the density and laminar distribution of labeled cells: anterior cingulate cortex contained the greatest density (primarily in layers II-V); prefrontal, parietal and occipital regions had an intermediate density (cells most numerous in layers II-superficial III and IV); some temporal regions exhibited a distinctive band of labeled cells restricted to layer IV; the lowest density of labeled cells was in motor cortex (confined to layers II and III). Most labeled processes were radially-oriented, with regional and laminar distributions closely paralleling those of labeled neurons. Some tangential fibers were present in layer I. These findings indicate that CRF is contained in a subpopulation of intrinsic cortical neurons that have a heterogenous distribution in primate neocortex.

Thus, there appear to be substantial differences between rodent and primate in the cellular distribution of CRFLI in brain. However, technical differences between experiments (antiserum sensitivity/specificity, lack of colchicine pretreatment in the present study) may account for some of these contrasting distributions. Antisera generously provided by W. Vale and J. Rivier, The Salk Institute.

381.9 GABA PEPTIDES IN RAT BRAIN: DISTRIBUTION AND REGIONAL RESPONSES TO GAMMA-VINYL-GABA. C.M. Mangione*, T.N. Ferraro, D.S. Garant*, G.T. Golden, T.A. Hare, R.G. Fariello. Thomas Jefferson Univ., Philadelphia, PA and VAMC, Coatesville, PA

GABA in the central nervous system exists both in free and conjugated forms. Administration of gamma-vinyl-GABA (GVG), a relatively specific inhibitor of the enzyme GABA-transaminase, has been documented to increase levels of both free and conjugated GABA. To date, the only endogenous conjugated forms of GABA identified have been dipeptides; however, their distribution is not well characterized and their physiological function is unknown. The regional profile of GABA peptides in rat brain was determined and compared to the regional profile of free GABA by measurement of GABA levels in acid extracts of discrete brain areas before (free GABA) and after (total GABA) acid hydrolysis. The difference between levels of total and free GABA is taken as a measure of conjugated (peptide) GABA. In normal rats, the level of peptide GABA was highest in substantia nigra (SN), hippocampus (HP) and superior colliculus (SC) whereas striatum (ST), cerebellum (CB) and frontal cortex (FC) contained lower levels. This order roughly corresponds to the sequence determined for free GABA. The production of GABA peptides was stimulated by treating rats with a single dose of GVG (2000 mg/kg, i.p.). Animals were sacrificed at 6 or 24 hr after treatment and regional levels of free and peptide GABA were determined. In all regions except CB, free and peptide GABA levels were greater in the 24 hr as compared to the 6 hr group. The greatest percentage increases for peptide GABA were found in SC, HP, and ST. The greatest percentage increases for free GABA were found in FC, HP and CB. In order to distinguish between metabolic and synaptic pools of GABA peptides, nigral levels were measured 24 hr after a single injection of GVG in rats having undergone unilateral transection of the striatonigral pathway. Hemitransection resulted in significant depletion of both free and peptide GABA levels in the ipsilateral nigra compared to the contralateral, non-transected nigra. This result was seen in both saline- and GVG-treated animals and suggests a relationship between GABA peptides and nerve terminal activity. (Supported by U.S. Veterans Administration and The Epilepsy Foundation of America).

381.10 LOCALIZATION OF NEUROMEDIN U-LIKE IMMUNOREACTIVITY IN RAT AND GUINEA-PIG SPINAL CORD, BRAIN AND INTESTINE. S.J. Augood*, J.R. Keast* and P.C. Emson. (SPON: B. Dixit) MRC Unit, Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, England.

Neuromedin U-8 (NMU-8) is a peptide recently isolated from porcine brain by Minamino and colleagues (Biochem. Biophys. Res. Commun. 130:1078, 1985), which stimulates smooth muscle contraction in blood vessels and the uterus. Antisera were raised in two New Zealand white rabbits, against NMU-8 conjugated to bovine thyroglobulin. Animals were boosted 8 weeks later and usable antisera obtained 4 weeks after the first boost. Both antisera had similar properties and only one (Rb 59) was used for further studies. It was used at a dilution of 1:60,000 in a radioimmunoassay (RIA) to determine levels of NMU-like immunoreactivity (NMU-LI) in 0.5 M acetic acid or 0.1 N HCl extracts of rat spinal cord, brain or gut. NMU-LI in these and guinea-pig tissues was characterized by HPLC separation of extracts (on a linear gradient of 16-44% acetonitrile/water/0.1% TFA) and detection of NMU-LI by RIA. Additional samples of duodenum, ileum and distal colon were taken from both species, fixed overnight by immersion in a picric acid/formaldehyde mixture (Zamboni's fixative) and processed for detection of NMU-LI in frozen sections of whole wall thickness or whole mounts of myenteric or submucous plexuses, by fluorescence immunohistochemistry. Final antiserum dilution was 1:200.

In RIA the antiserum had < 0.001% cross-reactivity with the C-terminal hexapeptide of pancreatic polypeptide and no detectable cross-reactivity with neuropeptide Y or vasoactive intestinal peptide. Pre-incubation of antiserum with any of these peptides (up to 10^{-5}M) had no effect on the NMU-LI seen with immunohistochemistry; staining was abolished by pre-incubation with 10^{-6}M NMU-8. In rats the highest level of NMU-LI was in the ileum (7.96 ± 0.95 pmol/g, n=4) and the lowest in the cortex and striatum (0.51 ± 0.15 pmol/g, n=4); HPLC studies showed that, with either extraction method, at least two molecular forms of NMU-LI were present, in rats and guinea-pigs. None corresponded exactly with the peak position of porcine NMU-8. In rat and guinea-pig small intestine, subpopulations of submucous and myenteric neurons were stained. The reaction in guinea-pig tissues was much less intense. No endocrine cells were stained. NMU-LI was also in nerve terminals in both ganglionated plexuses, forming dense baskets around a small population of unstained cells, and in nerve fibres close to the mucosal epithelium. Innervation of the mucosa and submucosa was very sparse in the colon. The high concentrations of NMU-LI in the intestine and its location in enteric neurons suggests a possible role in regulation of gastrointestinal function.

381.11 DISTRIBUTION OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP)-LIKE IMMUNOREACTIVITY IN THE BAT BRAIN. Lois K. Laemle and John R. Cotter. Depts. of Anatomy, UMDNJ-New Jersey Med. Sch., Newark, NJ 07103 and SUNYAB, Buffalo, NY 14214.

Vasoactive intestinal polypeptide (VIP) has been shown to be involved in regulation of central energy metabolism, pituitary hormone release, body temperature, and cerebral blood circulation, however, the mechanisms governing the regulation of these functions are poorly understood. One approach is to compare the distribution of this peptide in species with differing life-styles and, if possible, under different physiological conditions. Therefore, we have mapped the distribution of VIP in the brain of the little brown bat, Myotis lucifugus, a species in which these functions undergo broad cyclical variation. Myotis hibernates for up to 8 months of the year, during which time body temperature falls from 24°C to 5°C, and heart rate drops from 64 beats/sec. to 11 beats/sec.

VIP was localized immunocytochemically in euthermic, hypothermic, and hibernating animals using Bouin's fixed, 10um paraffin sections, and the unlabeled antibody enzyme method of Sternberger ('79). Immunoreactivity was abundant in olfactory portions of the brain, in all regions of the neocortex, in certain midline hypothalamic and midbrain nuclei including the suprachiasmatic nucleus, anterior hypothalamic area (AHA), paraventricular and periventricular nuclei, dorsomedial and ventromedial nuclei, dorsal and linear raphe nuclei, central gray, and interfasicular nucleus, and in several medullary nuclei, and at least one major subcortical pathway, the stria terminalis.

Our observations in the hypothalamus are of particular interest. VIP-like immunoreactivity in the AHA varied in a manner which corresponded to changes in the physiological state of the animal. Densitometry readings and computer-assisted plots of immunoreactive varicosities showed that the density of VIP- immunoreactivity in the AHA was greatest in euthermic animals, and decreased dramatically in hypothermic and hibernating animals. The density of VIP-like immunoreactivity in other brain centers remained constant. These observations in conjunction with previous studies which indicated a role for the AHA in regulation of body temperature and cardiovascular rate suggest a causal relationship between the physiological changes associated with hibernation in Myotis lucifugus and the decreased VIP innervation of the AHA. The presence of VIP-immunoreactive neurons in the periventricular and paraventricular nuclei may suggest a mechanism for VIP mediation of pituitary hormone release.

382.1 EFFECTS OF SPINAL CORD STIMULATION ON SEGMENTAL REFLEX PATHWAYS IN MAN. J.P. Hunter, P. Ashby, and R.G. Vanderlinden. Dept. of Anatomy and Playfair Neuroscience Unit, University of Toronto. Toronto, Ontario, Canada. M5T 2S8.

Epidural spinal cord stimulators are implanted in patients suffering from chronic pain and in some instances produce symptomatic relief. The actions of these stimulators in man are unknown. We examined 16 patients with implanted stimulators. In most, the cathode was in the epidural space at vertebral level T9/10 and the anode was a metal disc implanted in the paraspinal muscles. Nerve action potentials were recorded from the common peroneal and sural nerves using fine needle electrodes insulated except at the tip. Postsynaptic potentials in single spinal motoneurons were derived from changes in firing probability of voluntarily activated motor units. Spinal cord evoked potentials were recorded using surface electrodes or an epidural electrode at the T12 vertebral level.

When activated at 25-50 Hz spinal cord stimulation (SCS) produced paresthesiae in the trunk and lower limbs. At 3 Hz muscle twitches could be observed in muscles innervated at the segmental level of the cathode and, with stronger stimuli, in muscles of the lower limbs (particularly during voluntary contraction). During SCS nerve action potentials could be recorded from both mixed and cutaneous nerves in the lower limbs. The refractory period was less than 5 msec implying that SCS produces antidromic activation of these afferents. SCS causes short latency periods of increased firing probability in the motoneurons of all lower limb muscles examined. The facilitation occurred in flexor and extensor motoneurons bilaterally. The rise times and the variations in amplitude during paired stimulation are similar to those of the composite Ia EPSP. The facilitation has been obtained in a patient with presumed degeneration of the pyramidal tract. It is likely that this facilitation most likely results from antidromic activation of Ia afferents. There are later periods of decreased or increased firing probability which have not yet been examined in detail. The effects of continuous 25-50 Hz SCS on the facilitation of motoneurons from muscle and cutaneous afferents and on spinal evoked potentials was also examined.

It is concluded that SCS of the type described generates antidromic volleys in primary afferents which could reduce orthodromic volleys by collision. SCS also produces PSP in spinal neurons at segmental levels well below the cathode and could potentially modify transmission in spinal reflex pathways.

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382.2 EXCITATORY TRANSMITTERS AND VENTRAL ROOT POTENTIALS PRODUCED BY CUTANEOUS STIMULI IN AN ISOLATED AMPHIBIAN LEG-SPINAL CORD PREPARATION. J.C. Hackman, A.M. Holohean*, J.L. Vega*, D.X. Zhang* and R.A. Davidoff. Neurophysiology Lab, VAMC and Dept. of Neurology, Univ. of Miami Sch. of Med., Miami, FL 33101.

The morphology and physiology of the large diameter primary afferents which mediate touch/pressure sensations and the unmyelinated fibers which respond to noxious stimuli have been well studied, but the transmitters involved in transmission to secondary spinal neurons is still unclear. The present experiments made use of the isolated frog leg-spinal cord preparation (Syková & Vyklický, Physiol. bohemoslov. 28:227,1979) to investigate the role of excitatory amino acids (EAAs) and substance P in the generation of ventral root (VR) potentials (VRPs) produced by non-noxious and noxious stimuli.

Frogs were anesthetized and after laminectomy spinal cords, with leg attached via sciatic nerve, were placed in a plexiglass chamber. Severed VRs were placed across a sucrose gap to record VRPs. The leg still attached to the spinal cord via the sciatic nerve and dorsal roots was placed in an adjacent chamber and covered with Ringer's solution. The spinal cord was superfused with HCO_3^--Ringer's solution bubbled with 95%O_2:5%CO_2 and maintained at 15°C. Von Frey hairs were used to stimulate touch/pressure receptors and 1.0 M acetic acid was used to activate nociceptors. This concentration of acetic acid was sufficient to elicit vigorous wiping and escape behavior when applied to the leg of an intact frog.

Control VR depolarizations evoked by 10 sec applications of a 4.93 gram von Frey hair and 10 sec applications of 1.0 M acetic acid were 2.1±0.4 mV (n = 7) and 4.5±0.6 mV (n = 10), respectively. The addition of 1.0 mM Mg^{++} to the superfusate reduced the VR responses evoked by the von Frey hair and acetic acid by 40-80%. Similar results were observed when the N-methyl-D-aspartate (NMDA) antagonist 2-amino-5-phosphonovaleric acid (10 uM) was applied to the cord. The broad-spectrum EAA antagonist kyurenate (1.0 mM) completely blocked VRPs produced by both touch and noxious stimuli. In contrast, the substance P antagonist spantide (20 uM) had little effect on the touch responses, but significantly reduced the acetic acid-evoked elicited responses.

In summary, in an in vitro frog preparation EAA receptors appear to participate in both noxious and non-noxious stimuli, while substance P appears to participate in noxious responses. (Supported by VAMC MRIS #s 1769 and 3369 and USPHS #17577).

382.3 **TIME DEPENDENT CHANGES IN THE RESPONSE PROPERTIES OF DORSAL HORN NEURONS AFTER DORSOLATERAL FUNICULUS LESIONS.** L. M. Pubols, H. Hirata, and P. B. Brown, Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97209.

The spinal cord dorsolateral funiculus (DLF) contains the axons of descending projections to the dorsal horn, some of which are thought to be inhibitory, particularly with regard to nociceptive inputs. In pentobarbital-anesthetized cats, the effects of unilateral DLF lesions were evaluated at < 1 (acute) to 56 days postoperatively (d.p.o.). Microelectrodes were used to record the responses of dorsal horn neurons to natural mechanical stimulation of the skin and to electrical stimulation of A fibers in the sural nerve.

As indicated by the number of cells per electrode penetration that could be driven by natural stimuli, the responsiveness of the dorsal horn was below normal both ipsilateral and contralateral to acute DLF lesions. By 28 d.p.o. responsiveness was above normal ipsilateral to the lesion, but remained depressed contralaterally. The percentage of cells that could be driven by sural nerve stimulation (% SN+) and the percentage that had a receptive field in the skin region supplied by this nerve (% RFin) were below normal at 3 d.p.o, but increased linearly over the next 4 weeks to above normal levels. Results from the two animals studied at survival times longer than 30 days (43 and 56 d.p.o.) suggested that these percentages may return to normal with prolonged survival. As shown in the following table, the differences in these percentages between 15 normal animals and 8 lesion animals studied at 14-30 d.p.o. were highly significant.

	$\bar{x}$(normal)	$\bar{x}$(14-30 d.p.o.)	t	d.f.	p
SN+	31.6%	54.8%	4.155	21	<.001
RFin	35.7%	55.2%	4.037	21	<.001

The percentage of cells exhibiting spontaneous activity as a function of survival time showed a trend which was opposite to that for %SN+ and %RFin, declining from a higher than normal level at 3 days, to below normal at 30 days. Surprisingly, the proportions of cells that responded only to low intensity mechanical stimulation, only to high intensity stimuli, or differentially to both, was very similar to normal in lesion animals at all survival times.

The spontaneous activity data of the present study support the concept of immediate release of descending inhibition by DLF lesions. Measures of responsiveness to peripheral inputs, however, indicated a more gradual increase in excitability, and may reflect a different process. Failure to see an increase in the proportion of cells responding to high intensity stimuli after DLF lesions may indicate that differential inhibition of nociceptive inputs by DLF projection is true for some, but not all, dorsal horn cells and/or descending pathways (Support: NIH, NS19523).

382.4 EFFECTS OF SODIUM PENTOBARBITAL ON THE ACTIVITY OF DIFFERENT CLASSES OF SPINAL NEURONS. S.C. Nam*, K.S. Paik* and J.M. Chung (SPON: J. Calverley). Marine Biomedical Institute and Departments of Anatomy & Neurosciences and Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Sodium pentobarbital is used commonly as an anesthetic drug for humans and experimental animals. Its mechanism of action, however, is not yet clear. Furthermore, there are contradictory reports of its effect on the activity of spinal neurons. The purpose of this experiment was to test the effects of graded doses of sodium pentobarbital on the peripherally evoked activity of both dorsal horn cells and motoneurons in the cat spinal cord.

Twenty-five decerebrate-spinal cats were used in this study. After surgical decerebration, a tracheostomy was performed and the animal was paralyzed with gallamine triethiodide while ventilating with a respirator. The spinal cord was sectioned at the T11 level after exposing the cord by laminectomies at segments T10-L1 and L3-S1. In some animals, the activity of dorsal horn cells was recorded with a carbon filament filled glass microelectrode in the L7-S1 spinal cord. In other animals, the activity of motor axons was recorded as single unit activity from a filament of L7 ventral root. The recorded activity was fed into a computer to compile peristimulus time histograms. The activity of the spinal neurons was evoked by stimulation of the common peroneal nerve and the evoked activity was compared before and after intravenous injection of sodium pentobarbital in small increment doses. Only one cell was studied in each animal.

The activity of 10 motor axons and 15 dorsal horn cells was studied. In general, both A and C fiber evoked activity gradually declined with graded doses of sodium pentobarbital. Approximate average doses required for the reduction of activity to 50% of control values were: 5 mg/kg for the A responses of motor axons; 2 mg/kg for the C responses of motor axons; 20 mg/kg for the A responses of dorsal horn cells; and 15 mg/kg for the C responses of dorsal horn cells. The evoked activity by peripheral C fibers was more sensitive to the drug than that by A fibers. The evoked activity in motor axons was much more sensitive to sodium pentobarbital than the dorsal horn cells so that 10 mg/kg of the drug practically abolished the evoked activity in motor axons by peripheral C fibers. The effect of pentobarbital varied widely between activities of different dorsal horn cells, and the activity of many dorsal horn cells was enhanced with a smaller dose (2-5 mg/kg) of the drug followed by reduction with a higher dose.

From the above results, we conclude that different classes of spinal neurons have differential sensitivity to sodium pentobarbital. (Support by NIH grants NS21266 and NS11255 and by NIH RCDA NS00995).

382.5 SYNAPTIC INPUTS TO LUMBAR SPINAL NEURONS PROJECTING TO THE PONTO-MEDULLARY RETICULAR FORMATION IN THE CAT. Y. Sahara, Y. Xie* and G.J. Bennett. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Extracellular recordings have shown that spinoreticular tract (SRT) neurons receive highly convergent inputs and suggested that excitatory and inhibitory processes interact complexly. In order to define the synaptic inputs to SRT neurons, intracellular recordings were made from antidromically identified SRT neurons in the lumbar spinal cord (L4-S1) of pentobarbital-anesthetized cats. Stable intracellular recordings were obtained from 34 neurons. The neurons were activated from the ipsilateral (n = 8) or contralateral (n = 26) medial pontomedullary reticular formation. Fifteen neurons were also activated from the contralateral spinothalamic tract, indicating that SRT neurons have branching axons. These neurons were divided into 4 groups based on their locations and responses to electrical stimulation of the ipsilateral sciatic nerve. Type 1: In 20 neurons, IPSPs were evoked by stimulation of cutaneous nerves (SP: superficial peroneal nerve and Sur: sural nerve; less than twice threshold: <2T). The central latency of the IPSP ranged from 3.5 to 5.7 ms. Stimulation of a muscle nerve (GS: gastrocnemius-soleus nerve, >2T) also evoked an IPSP. These neurons were located medially and in the deeper laminae (2.2-3.95 mm from the surface). Although some of these neurons had spontaneous discharges, receptive fields were never found. Type 2: In 5 neurons, EPSP-IPSPs were evoked by stimulation of SP, Sur (<2T) and GS (>2T). The central delay of the EPSP ranged from 1.0 to 1.7 ms. Four of these neurons were characterized as wide-dynamic-range (WDR) neurons, which had low-threshold and high-threshold mechanoreceptive inputs and large receptive fields. These neurons were located laterally and 1.0-1.7 mm below the surface. Type 3: In 6 neurons, located 1.95-3.3 mm below the surface, IPSP-EPSPs were evoked by stimulation of SP, Sur (<2T) and GS (>2T). The central latency of the IPSP ranged from 1.75 to 3.2 ms. Two of the 6 neurons were WDR neurons. Type 4: In 3 neurons, located 2.85-3.7 mm below the surface, EPSP (single spike)-IPSPs were evoked by stimulation of SP, Sur (<2T) and GS (>2T). The central latency of the EPSP ranged from 1.3 to 4.4 ms. The present results show that (1) stimulation of low-threshold cutaneous and muscle afferents produces only IPSPs in many SRT neurons, and (2) SRT neurons receive several different patterns of peripheral inputs via multiple synaptic pathways, suggesting that the SRT might be functionally heterogeneous and relay highly integrated information.

382.6 NON-UNIFORM RELEASE PROBABILITIES UNDERLIE QUANTAL TRANSMISSION AT AN EXCITATORY SYNAPTIC CONNECTION IN THE CAT SPINAL CORD. B. Walmsley, F.R. Edwards*, and D.J. Tracey. Neural Research Laboratory, School of Anatomy, University of New South Wales, P.O. Box 1, Kensington NSW 2033, Australia.

Primary afferents from muscles of the hindlimb ascend in the dorsal columns and terminate on dorsal spinocerebellar tract (DSCT) neurons in Clarke's column. Previous studies using deconvolution procedures have demonstrated that excitatory postsynaptic potentials (EPSPs), evoked in DSCT neurons by impulses in single group I muscle afferents, fluctuate in amplitude between discrete levels separated by approximately equal, or quantal, increments (Tracey & Walmsley, J. Physiol., 350, 599-614; Walmsley et al., J. Neurosci., in press, 1987). However, further interpretation of these results requires the application of some kind of probabilistic model.

In a previous study, we demonstrated that simple binomial statistics do not describe the fluctuations in amplitude of single fiber EPSPs recorded in DSCT neurons (Walmsley et al., J. Neurosci., in press, 1987). In the present study we have developed and applied two procedures to find a more appropriate model, using the deconvolution results as a guide.

In a first stage we have demonstrated that the amplitude fluctuations of single group I fiber EPSPs can be well described by a strictly quantal process, unconstrained by any general statistical model such as Poisson or binomial. The unconstrained quantal model indicates that that each EPSP is composed of the sum of a number (1-30) of underlying quantal events, and that furthermore, there is extremely little variability in the amplitude of the single quantal event (<3% C.V.).

In a second stage we have proceeded from the quantal description to examine a probabilistic model in which each underlying quantal event is associated with a particular, but independent, release probability (i.e. the most general case of a compound binomial model). The results of this analysis indicate that a compound binomial model provides a good description of the fluctuations in single group I fiber EPSPs evoked in DSCT neurons. The probability of transmitter release may be at, or close to unity for many release sites and may vary considerably between the remaining sites. In addition to these 'active' release sites, a number of 'silent', or zero-probability, release sites may also exist.

382.7 SOMATOTOPIC ORGANIZATION OF HINDLIMB CUTANEOUS AFFERENTS IN THE DORSAL HORN OF THE CHICK. C. J. Woodbury* (SPON: W. T. Newsome, III), Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY, 11794.

Cutaneous afferents project somatotopically to discrete laminae of the dorsal horn (DH) in mammals. Little is known about how this map develops. As a first approach I am studying the central projections of hindlimb cutaneous afferents in hatchling chicks, in which much work has been done on the development of peripheral projection patterns.

Peripheral receptive fields (RF) and central terminations of selected cutaneous nerves have been mapped. RFs were recorded while lightly stimulating the skin. The central projections were then labeled by applying HRP or HRP-ligand conjugates to the proximal cut end of a nerve or, in the case of afferents from toe skin, by injecting markers subcutaneously. Frozen sections of spinal cord and DRGs were processed with TMB and the locations of label in the DH reconstructed. The hindlimb nerves studied include the cutaneous femoris lateralis (CFL), medialis (CFM), caudalis (CFC), and the cutaneous surae lateralis (CSL), which combined innervate most of the thigh and proximal shank. As in mammals, these nerves project heavily into ipsilateral superficial DH; surprisingly, in the chick each nerve projects not to one but to two discrete regions. These dual projections remain separated from one another throughout their rostrocaudal (RC) extent by a region devoid of label. Both projections from each nerve appear to map somatotopically. For example, nerves that innervate skin on the proximal thigh (CFL and CFC) project laterally in the DH relative to the projections from nerves with more distal RFs (CSL and CFM). Afferents from toe skin project even more medially. Further, any mediolateral shift along the RC extent of one projection is mirrored by a similar shift in the other. To test whether these dual projections represent different fiber types or modalities, cholera toxin-HRP (CT-HRP), which preferentially labels large diameter afferents (Robertson and Grant, Neurosci.Abs. 12:1568, 1986), was applied to cut nerves. In each case the medial projection of a nerve was more robustly labeled than its lateral projection, indicating that the two projections are indeed distinct.

These results suggest that two somatotopic maps representing different modalities exist across the mediolateral axis of the DH of the chick. Further, they support the assertion of Brinkman and Martin (Br.Res.56:43,1973) that in the chick, DH laminae 1 and 2 lie lateral rather than dorsal to lamina 3. (Supported by NSF grant BNS 85-18927 to S. A. Scott.)

382.8 Differences In Membrane Properties of DRG Neurons Supplying HTMRs, D-hairs and Other Low Threshold Mechanoreceptors. L. M. Mendell, H. R. Koerber and R. E. Druzinsky*. SUNY-Stony Brook, NY 11794.

Individual somata (n=176) were impaled in the L7 dorsal root ganglion of chloralose anesthetized cats. In agreement with previous results from this laboratory (Neurosci. Lett. 63: 1986) baseline duration of somal action potentials (AP) correlated with both peripheral fiber conduction velocity (PFCV) and receptor type innervated. Most notably A-delta neurons supplying HTMRs had wider spikes than those supplying D-Hairs mainly as a result of a shoulder on the descending limb of the spike. Examination of the after hyperpolarization potentials (AHP) of A-beta and A-delta neurons revealed an inverse correlation between AHP half width and conduction velocity. Within the A-delta group HTMR-AHPs exhibited substantially longer half widths than those of D-Hairs. Hyperpolarizing currents (2nA and/or 5nA; 50ms) were delivered through the microelectrode and the magnitude of inward rectification was calculated as the ratio of the membrane potential 30 ms after pulse onset to the peak membrane hyperpolarization. Over all cells (A-beta and A-delta) we noted that rectification was most prominent in cells with high values of axonal conduction velocity and low values of resistance and AHP. Accordingly, we also found an inverse relationship between cell resistance and axonal conduction velocity. Within the A-delta group D-Hair somata exhibited considerably more rectification than HTMR somata which displayed little or none. The strength of the rectifier in both HTMR and D-Hair somata diminished as axonal conduction velocity decreased but there was no correlation with somal resistance. Differences in rectification between HTMRs and D-Hairs were independent of conduction velocity. No consistent difference in input resistance or RMP was noted between HTMR and D-Hair somata, and so the magnitude and absolute level of peak hyperpolarization produced by these currents were similar. Thus the greater inward rectification associated with D-Hair somata reflects a difference in an intrinsic membrane property. D-Hair somata resemble cells with A-beta axons in having a well developed inward rectifier although A-beta cells displayed even stronger rectification. HTMRs resemble axotomized A-beta sensory neurons which lose rectification (J. Physiol. 270: 1977). Our findings confirm that somal membrane of cells supplying D-Hairs differs from that in cells supplying HTMRs. This difference is independent of parameters related to cell size such as input resistance. Furthermore, cells with A-delta axons differ on the average in AHP, resistance and rectification from those with A-beta axons. Supported by NS 23725 (HRK) and NS 16996 and NS 14899 (LMM).

382.9 Correlation of Rostrocaudal Extent of Monosynaptic Field Potentials Evoked by Single Action Potentials in Identified Single Cutaneous Sensory Afferents and Their Bouton Distributions. P. B. Brown, H. R. Koerber and L. M. Mendell. West Virginia University, Morgantown, WV 26506, and SUNY-Stony Brook, NY 11794.

Single identified fibers penetrated in the dorsal columns of alpha-chloralose anesthetized cats with microelectrodes containing HRP (12-15%; 35-50 Mohms) were stimulated (18/sec) intracellularly while averaging (n=1024) the monosynaptically evoked cord dorsum potentials (CDPs) simultaneously at four rostrocaudal locations centered over the dorsal horn. HRP was then iontophoresed into the axon (450 msec pulses; 1.67/sec; total charge 80-126 nA-min). Sections (100 um) were cut in the para-sagittal plane from a single block of tissue encompassing the full extent of the active zone and processed for HRP. Boutons were counted in 230 um rostrocaudal bins and summed mediolaterally across all sections. CDP amplitude profiles and bouton distributions were compared for fibers innervating slowly adapting type 1 (SA1), and hair follicle and field mechanoreceptors (RAs). Rostrocaudal distribution of bouton densities (4.92mm-11.55mm; 5000-8000 boutons) varied in agreement with the extent of the CDP amplitude profile for individual fibers. Extent of bouton distributions and CDPs varied according to type of peripheral receptor innervated with SAIs routinely having the greatest rostrocaudal extents for both, often being twice the rostrocaudal extent of RA afferents innervating the same area of hindlimb skin. The rostrocaudal range of boutons for an afferent never exceeded the range of active recording sites, but the extent of active recording sites activated by a single SA1 was occasionally slightly larger than the bouton distribution. Therefore, either the full rostrocaudal extent of boutons was not always labeled or volume conduction causes a potential distribution which is longer than the extent of the current sinks (EPSPs). There was no apparent correlation between fiber conduction velocity and the rostrocaudal extent of an individual fibers' bouton or CDP distribution. The relative amplitude of the CDP recorded at an electrode could be approximated from the bouton distribution and electrode location by assuming that synaptic transmission at each bouton results in an equal current sink and that the recorded potential decreases with distance from the bouton. These results show that SAIs have a larger spatial distribution of boutons in the rostrocaudal axis which produce a larger spatial distribution of amplitude profiles than do fibers innervating RA receptors. Furthermore, within the limits of resolution used, the full rostrocaudal ensemble of boutons of a primary cutaneous sensory axon is capable of generating EPSPs in dorsal horn cells, and the amplitude of the CDP appears to be proportional to the density of boutons. Supported by NS 23725 (HRK), NS 16696 and NS 14899 (LMM) and NS 12061 (PBB).

382.10 Spinal Processing of Temporal Information in Sensory Fibers Depends on Receptor Type. H. R. Koerber and L. M. Mendell, SUNY-Stony Brook, NY 11794.

The peripheral receptor of cells impaled in the L7 dorsal root ganglion of cats anesthetized with alpha-chloralose was identified as rapidly adapting (RA) - hair follicle or field - or slowly adapting (SA) using hand held probes. The cell was then stimulated intracellularly and field potentials were averaged simultaneously from 4 positions on the dorsal cord surface straddling the L7 entry zone. The field potentials at the position exhibiting the maximum response were examined following: a) 2 action potentials (APs), conditioning (C) and test (T) separated by 50ms and evoked every 1500ms (n=128) and b) a single AP evoked every 56ms (n=1024). For RA fibers the conditioning effect was consistently a reduction in the peak amplitude of the potential produced by T, with hair follicle responses exhibiting more depression than those from field receptors. The field potentials produced by SA fibers reliably displayed less depression or even facilitated in response to the same C-T sequence. Field potentials evoked at 18/sec were depressed compared to those obtained at 0.67/sec stimulation, and to a greater extent for RA afferents. Thus for these cells (all A-beta fibers- 38-85 m/s) the central amplitude modulation "matched" the frequency modulation of the cutaneous receptor: fibers supplying RA receptors exhibited frequency depression of their central responses whereas those supplying SA fibers either facilitated or were subject to modest depression. Examination of field potential waveforms revealed a longer rise time on the average for those associated with RA receptors and within this group those with the lowest peripheral threshold had the most prominent polysynaptic component. Thus frequency depression associated with RA afferents may result from the lability of their polysynaptic pathways. Therefore, one difference between the central action of SA and RA fibers may reside in the networks activated by such fibers. However, the same differences were also noted between SA afferents and a subset of RA afferents producing field potentials with correspondingly brief rise times. Further examination of RA afferent responses with long rise times but with a distinguishable early component revealed marked depression of this (monosynaptic?) component. This may imply intrinsic differences in terminals of RA and SA afferents. In addition to differences associated with receptor types we noted less depression and more facilitation as afferent CV decreased. A-delta fibers innervating D-Hairs and high threshold mechanoreceptors (HTMRs) evoked more facilitation than A-beta fibers with HTMRs consistently yielding the greatest amount of facilitation. We conclude that central processing of temporal information in the dorsal horn differs systematically for afferents supplying different receptor types. Supported by NS 23725 (HRK) and NS 16996 and NS 14899 (LMM).

382.11 RELEASE OF ENDOGENOUS AMINO ACIDS FROM THE SPINAL CORD OF FREELY MOVING RATS MONITORED BY MICRODIALYSIS. D. H. Smullin, S. R. Skilling* and A. A. Larson. Department of Veterinary Biology, University of Minnesota, St. Paul, MN 55108.

The amino acids glutamate, aspartate, GABA, glycine and taurine have been proposed as neurotransmitters in the mammalian spinal cord. We have developed a method for monitoring the extracellular fluid levels of these amino acids in the freely moving rat using microdialysis.

Male, Sprague-Dawley rats were implanted with 0.2 mm diameter, 50,000 MW cut-off dialysis tubing transversely through the dorsal half of the spinal cord at the region of T-12 or through the CSF at the region of the cauda equina. The animals were perfused with Ringer's solution at 15 µl/min and samples were collected in 10 min aliquots. The aliquots were analyzed for amino acids using HPLC with OPA derivitization. A concentration of 180 µM veratridine perfused through the spinal cord dialysis tubing for 10 min produced a marked increase in extracellular levels of glutamate and aspartate in the spinal cord. A 1mM lidocaine-HCl perfusion through the dialysis tubing for 50 min produced a decrease in the extracellular levels of glutamate, aspartate and glycine, but not taurine. A dose of 40 mg/kg pentobarbital (ip) had no detectable effect on the extracellular levels of glutamate, aspartate, glycine or taurine in either the spinal cord tissue or the CSF as compared to control injections of the vehicle.

The use of *in vivo* microdialysis in the spinal cord for studying the release of neuroactive substances promises to be a powerful tool for the investigation of spinal mechanisms. (Supported by USPHS Grants DA04090 and DA04190, and NIDA Training Grant DA07234)

382.12 EFFECT OF INTRATHECAL PRETREATMENT WITH NEUROTOXINS ON ADENOSINE RELEASE FROM RAT SPINAL CORD SYNAPTOSOMES. M.I. Sweeney*, T.D. White, and J. Sawynok. Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

Morphine increases endogenous adenosine release from dorsal spinal cord synaptosomes in a manner which is dose-dependent, Ca^{2+}-dependent and opiate receptor-mediated. Noradrenaline (NA) produces a similar increase in the release of adenosine from dorsal spinal cord synaptosomes which is due to release of a phosphorylated nucleotide. Adenosine release is implicated in spinal analgesia by morphine but not NA (Soc. Neurosci. Abstr. 1986, 12:1018). However, the neuronal source of adenosine and the phosphorylated nucleotide is unknown. In the present study, this source of adenosine released from dorsal spinal cord was investigated. Discrete subpopulations of spinal neurons were destroyed by intrathecal (i.t.) administration of the following neurotoxins: 60 µg capsaicin which destroys primary afferent terminals in the substantia gelatinosa; 100 µg 6-hydroxydopamine (6-OHDA) which destroys descending noradrenergic neurons; 100 µg 5,7-dihydroxytryptamine (5,7-DHT) which destroys descending serotonergic neurons. In all cases, appropriate vehicle solutions were administered i.t. to a control group of animals and with 5,7-DHT, animals were pretreated i.p. with desipramine to protect NA-terminals. Approximately 7 days after pretreatment, animals were sacrificed, synaptosomes prepared from the dorsal half of the spinal cord, and adenosine release evoked by K^+ (24 mm), morphine (10 µM) and NA (50 µM) assessed. Adenosine was quantitated by HPLC with fluorescence detection of the etheno-derivative. In the capsaicin pretreated group, significant increases in tail flick, hot plate and pressure test latencies were observed. In these rats, K^+- and morphine-evoked adenosine release from synaptosomes were reduced to 38 ± 11% ($p < .05$) and 47 ± 9% ($p < .05$) of control values respectively, while NA-evoked release remained unchanged. Pretreatment with 6-OHDA reduced NA levels in the ventral spinal cord to 3% of control values but had no significant effect on K^+- or morphine-evoked adenosine release. However, NA-evoked release was increased to 225 ± 20% ($p < .05$) of control values, probably due to the development of receptor supersensitivity. 5-7-DHT reduced 5-HT levels in the ventral spinal cord to 11% of control values but had no significant effect on adenosine release by any agent. These results suggest that in the spinal cord, morphine releases adenosine from primary afferent terminals, but not descending noradrenergic or serotonergic terminals. K^+-evoked release of adenosine appears to originate from the same source. The origin of purines released by NA is still not clear. (Supported by MRC Canada)

382.13 MEMBRANE PROPERTIES AND AFFERENT-EVOKED SYNAPTIC RESPONSES OF SUBSTANTIA GELATINOSA NEURONS IN RAT SPINAL CORD SLICES. M. Yoshimura* and T.M. Jessell. Center for Neurobiology and Howard Hughes Medical Institute, Columbia University, New York, N.Y. 10032

The terminals of most primary sensory neurons that convey nociceptive information synapse with neurons in the substantia gelatinosa (s.g.) of the spinal cord. The analysis of synaptic transmission between nociceptive afferents and s.g. neurons has proved difficult in vivo. We have therefore developed an in vitro slice preparation that permits intracellular recording from s.g. neurons.

Adult rats (100-150 g) were anesthetized, a lumbosacral laminectomy performed and a 1.5 cm length of spinal cord with attached dorsal roots was transferred to oxygenated Krebs solution at 4-6^{o} C. Transverse slices (500 μm) with attached dorsal roots were cut on a vibratome and maintained in a recording chamber at 36±1^{o} C. Intracellular recordings of up to 5 h in duration were obtained from 130 s.g. neurons (mean resting membrane potential -67 mV, mean input resistance 245 MΩ). Action potentials (mean amplitude: 76 mV; mean duration: 0.9 ms) were evoked by depolarizing current. Neurons could be subdivided into 3 distinct classes on the basis of their membrane properties. 20 % of neurons exhibited a time-dependent inward rectification at membrane potentials more negative than -75 mV. Rectification was blocked by extracellular Cs^{++} but not by Ba^{++}, suggesting the presence of an Ih current. In 30 % of neurons, membrane depolarization from potentials more negative than -70 mV, to -50 mV produced an early transient outward rectification that was blocked by 4-aminopyridine (2 mM), possibly reflecting the presence of IA current. The remaining 50 % of s.g. neurons exhibited both inward and outward rectification.

Stimulation of dorsal roots at low intensity (1.5-2 V, 0.2 ms) evoked monophasic epsp's (50-100 ms; to 20 mV) in over 80 % of s.g. neurons. The epsp latency was constant and followed repetitive stimulation at high frequency (5-10 Hz) suggesting that sensory-evoked monophasic epsp's are monosynaptic. The mean conduction velocity of afferent fibers that initiated short latency epsp's was 2.9 m/s (range 0.9-5.5 m/s). In some s.g. neurons, increasing the intensity of dorsal root stimulation (8 V, 0.2 ms) evoked a second class of longer but still constant-latency epsp's. The mean conduction velocity of dorsal root afferents that initiated the long latency epsp's was 0.5 m/s (range 0.4-0.6 m/s) possibly reflecting C fiber input.

Perfusion of slices with l-glutamate (0.5-3 mM) evoked membrane depolarizations that were associated with an increased frequency of spontaneous epsp's in 80 % of s.g. neurons that exhibited afferent-evoked monophasic epsp's. However, in the presence of TTX only 40-50 % of these s.g. neurons retained l-glutamate sensitivity. The amino acid antagonists kynurenate (0.9-1.5 mM) and 2-APV (0.2-0.25 mM) decreased the amplitude of afferent-evoked epsp's in some but not all s.g. neurons. These results indicate that transmission at some afferent synapses with s.g. neurons is mediated by activation of excitatory amino acid receptors.

382.14 RECIPROCAL VARIATIONS IN PLASMINOGEN ACTIVATORS (PAS) IN MEMBRANES FROM DEVELOPING MOUSE SPINAL CORDS.: B.W. Festoff, C.B. Kahler*, C.S. Maben* and J.S. Rao*. Dept. of Neurology, UKMC, Kansas City, KS. and Neurobiology Research Laboratory at VAMC, Kansas City, MO 64128.

We studied the activities of PAs in cytosol (S_2) and membrane-bound (P_2) fractions of mouse spinal cord homogenates from embryonic day 12 (ED12) to post-natal day 60 (PD60). We used a sensitive chromogenic assay based on a synthetic tripeptide specific for plasmin (HD-leu-val-lys-PNA; S-2251) and samples were assayed in the presence/absence of purified human plasminogen (plgn) with/without fibrin monomer (fm) and absorbance was read at 405 nm. Fm can discriminate tissue (tPA) from urokinase-type (uPA) PAs, since tPA must bind to fibrin before it can activate plgn. At constant protein concentrations P_2 uPA showed two sharp peaks of specific activity, at birth ED21 (OD 0.83) and at PD15 (OD 0.86). Just after birth, at PD3 P_2 uPA was at its lowest level (OD 0.22). A broader peak, from PD20-PD30 continued until two months of age (OD 0.5-0.65). P_2 tPA showed much lower activity and its troughs were exactly opposite to uPA peaks at birth and at PD15. In contrast, soluble S_2 activities for both PAs remained quite low (OD 0.05-0.21) throughout spinal cord development.

These results suggest that significant fluctuations in membrane-bound uPA activity coincide with major developmental remodelling situations in murine spinal cord. These are at birth and during the major period of synapse elimination in muscle (ED9-PD20) Other data suggest that this uPA is bound to a specific receptor on spinal cord cells.

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REGENERATION: OPTIC NERVE

383.1 NEURITE OUTGROWTH FROM RETINAL EXPLANTS FROM THE ADULT MOUSE. R.L. Meyer and J. Miotke* Developmental & Cell Biology, Developmental Biology Center, Univ. California, Irvine, CA 92717.

Retinal explants from adult mammals offer an attractive system to study the effects of nerve injury and to explore methods of promoting CNS nerve regeneration with in vitro methods. We here describe our efforts at developing such a system and report some initial characterization of the neurite outgrowth.

One optic nerve of adult Swiss Webster or CJ57J mice was crushed in the orbit and 8 days later both retinas removed. Each retina was chopped into 500 μm squares and placed on glass coverslips coated with polylysine and laminin. They were then maintained in DMEM-HEPES with 10% fetal calf serum and 0.4% methylcellulose under 5%CO_2 and air.

About 90% of explants became attached. As early as 24h, neurites were observed growing out of the retinal explants with the crushed nerve and by 48h 2/3 of these explants had obvious neurites. These neurites grew directly on the substrate and extended for several hundred μm. Virtually no neurites were observed from explants from the normal retina at this time. The number of neurites increased with time and by 1 week some outgrowth from the normal retina was observed.

To further determine that these neurites originated from retinal ganglion cells, they were further characterized with immunohistochemistry using monoclonal antibodies. They were found to be strongly positive for heavy neurofilament which preferentially stains ganglion cells and horizontal cells in the intact retina. They were also positive for light neurofilament, negative for GFAP and stained with the Bodian silver method. Finally, in CJ57J mice, the neurites were positive for Thy-1.2, a ganglion cell specific marker. We suggest these neurites may represent regenerating optic axons in vitro.

(This work was supported by funds from the Monsanto Corporation.)

383.2 IMMUNOREACTIVITY TO GAP-43 IN AXOTOMIZED AND REGENERATING RETINAL GANGLION CELLS OF ADULT RATS. A.M. Lozano, S.K. Doster, A.J. Aguayo and M.B. Willard. Neurosciences Unit, Montreal General Hospital, McGill Univ., Montreal, QUE, H3G 1A4 and Dept of Anatomy and Neurobiology, Washington Univ. Med. School, St Louis, MO, 63110.

GAP-43, a protein which is expressed at higher levels in certain neurons during developmental and regenerative axonal growth has not been observed to increase after injury to CNS neurons of adult mammals. Because axotomized retinal ganglion cells (RGCs), which do not normally regenerate in adult rats, regrow axons into peripheral nerve grafts (PNG) used as optic nerve (ON) substitutes (Soc. Neurosci. Abst. (1986) 12:700), we have investigated whether the growth of these CNS axons into the PNG is accompanied by an increase in GAP-43 immunoreactivity.

Whole-mounts of adult rat retina were stained with an affinity purified polyclonal anti GAP-43 antibody at various times after the ON was cut near the optic disc (n=19 rats) or after the ON was replaced by a PNG (n=35). In 15 unaxotomized retinas, no reactivity to anti GAP-43 was observed nor was there detectable immunoreactivity in frozen sections of the ON. However, between 6 and 25 days after axotomy, the axons and some somata of RGCs reacted with the antibody; no reactivity was observed after 25 days. The time course of the reactivity was the same regardless of whether the ON was axotomized or axotomized and replaced with a PNG to allow regeneration of RGC axons. GAP-43 immunoreactivity was also detected in axons in frozen sections of the graft, 1 and 2 months after grafting, but it was not determined if such axons originated from RGCs or from peripheral neurons that also send fibers into these grafts. The induction of RGC immunoreactivity appeared to be influenced by the location of the injury along the RGC axon. No induction was observed when the optic nerve was cut more than 6 mm from the eye (n=13), nor was immunoreactivity observed after axons which had regenerated into a graft were induced to resume growth by crushing the graft 7 to 10 mm from the optic disc (n=4). The reinduction of growth in the latter case was confirmed by retrograde labelling.

These observations indicate that some RGCs in adult rats respond to axotomy with an increased immunoreactivity to GAP-43 antibody, regardless of whether or not they regenerate into the nerve grafts. This response appears only when axons are severed near the retina. We do not know whether this immunoreactivity is a consequence of increased synthesis of GAP-43, increased accumulation of GAP-43 synthesized at a basal rate, or some other change induced by axotomy.

383.3 LONG-TERM PRESERVATION OF INTRINSIC RETINAL NEURONS AFTER AXOTOMY INDUCED DEATH OF RETINAL GANGLION CELLS. D. A. Carter*, M. Vidal-Sanz* and A. J. Aguayo. Neurosciences Unit, The Montreal General Hospital and McGill University, Montreal, Quebec, H3G 1A4, CANADA.

In adult rats, the transection of the optic nerve (ON) within the orbit is followed by the death of nearly 90% of the axotomized retinal ganglion cells (RGCs). Although RGC survival and axonal regrowth can be enhanced in these animals by peripheral nerve segments grafted to the ON stump, the RGC population that survives axotomy and grafting only approximates 25 % of normal (Vidal-Sanz et al., 1987, J. Neurosci, in press). In the present study, the long-term effects of such extensive retrograde degeneration of RGCs on intrinsic retinal neurons (IRNs)- amacrine, bipolar, horizontal and receptor cells- have been investigated using standard morphometric techniques as well as antibodies that recognize molecules expressed characteristically by certain classes and subclasses of IRNs. Because many of the IRNs synapse with RGCs in the intact retina, it was considered important to investigate protracted effects of RGC loss on the entire population of retinal neurons.

In female Sprague-Dawley rats weighing approximately 200 g, the ON was cut within the orbit (Axotomy group) or totally replaced by a 3 cm autologous segment of peripheral nerve (Axotomy and Graft group). Retinas from animals of both groups were examined up to 15 months after axotomy and compared to intact retinas of rats of the same age. Immunoreactivity to several antibodies (Ab) was used in retinal radial sections and flat mounts: Ab-MAP 1A and RT 97 for the ganglion cell and nerve fibre layer; HPC-1, an antibody that recognizes amacrine cells preferentially; Ab-tyrosine hydroxylase, Ab-somatostatin, Ab-substance P and Ab-glutamic acid decarboxylase, which distinguishes subclasses of amacrine cells; asv48 , an antibody that immunoreacts with synaptic vesicle components present in higher concentration in the external and internal plexiform layers; RET-B1 antibody for bipolar cells, VC1.1 antibody for horizontal cells and Ab-opsin for photoreceptors.

Although the loss of RGCs in both experimental groups of animals caused a 20% reduction in the thickness of the retina, the density and appearance of the different classes and subclasses of IRNs, detected by the immunocytochemical techniques used in this study, were indistinguishable from normal. These findings suggest that local interactions within the retina may suffice for the prolonged survival of local circuit neurons. It is not known if their apparent survival is accompanied by changes in their synaptic connectivity within the retina.

383.4 **GALVANOTROPIC REGENERATION IN THE MAMMALIAN VISUAL SYSTEM.** **M.F. Zanakis, M.J. Politis and B.J. Albala American BioInterface Corp., New York, NY and Anatomy Dept., Univ. of Saskatchewan, Canada**

Recent *in vivo* work has demonstrated a regenerative response in the lesioned mammalian spinal cord following the application of static DC electric fields. Unfortunately, this is not the ideal system to study central nervous system (CNS)regeneration due to the inherent problems of tissue heterogeneity. These studies utilized the lesioned rat optic nerve model in order to determine whether the mammalian CNS is indeed capable of significant regeneration following the application of electric fields. Rat optic nerves were crushed behind the orbit, and a *galvanotropic neural guide* device (TRAXON™) was placed over the lesion, with the anode just proximal to the lesion, and the cathode 3mm distal to the lesion (delivering 1µA to the tiussue). After 3 weeks, the animals were sacrificed, and the optic nerve 3mm distal to the lesion was cross-sectioned for histological analysis using fluorescent antibodies to neurofilament protein. Control animals received A) an inverted current electrode (anode distal), or B) an inactive electrode, or C) an active electrode but the retinae were avulsed 1 weeks prior to sacrifice (which destroys the retinal contribution of axons to the optic nerve, but not the possible contribution of regenerated peripheral nerves). The results demonstrated that in every animal with actively implanted (cathode distal) electrodes with intact retinae, a large number of axons had regenerated through the scar and 3mm into the distal portion of the nerve, as indicated by the presence of neurofilament-positive profiles. Additional toluidine blue staining and electron microscopy showed that the axons had grown in fascicles, and were either myelinated or unmyelinated. In contrast, none of the control nerves showed signs of axons in the distal segment. The avulsed retina animals also showed no signs of regenerated axons in the nerve, suggesting that peripheral nerves did not contribute to the regenerated axonal population observed in the cathode distal-intact retina group. Thus, these results suggest that damaged optic axons had regenerated through the lesion and into the optic nerve. In a limited number of animals in each of these groups, the optic tract (close to the optic radiations) was also sectioned for neurofilament-positive profile determination. Preliminary results indicate that the optic tract contralateral to the treated nerve contained a greater number of axonal profiles than the contralateral tracts of control animals, suggesting that regenerated axons may be capable of entering the optic tract. In summary, these studies add additional support to the claims that the CNS can regenerate following the application of electric fields.

383.5 PHOSPHORYLATION OF PROTEINS IN REGENERATING GOLDFISH OPTIC NERVE IS INDEPENDENT OF THEIR SYNTHESIS AND EXPORT INTO RETINAL GANGLION CELL AXONS. Denis C. Larrivee and Bernice Grafstein. Dept. of Physiology, Cornell Unversity Medical College, New York, NY 10021.

When ^{32}P-labelled phosphate was injected into goldfish eyes, approximately 20 optic nerve proteins became strongly labelled, as shown by two-dimensional electrophoresis and autoradiography. Most of these proteins were present in the retinal ganglion cell axons and many changed their phosphorylation during regeneration: 5 proteins (including a 45 kD protein closely related to the growth-associated protein GAP-43) showed an increase in incorporation of phosphate whereas 4 others showed a decrease. However, the changes in protein phosphorylation were not correlated with changes in incorporation of ^{3}H-proline. These results suggest that either 1) the phosphorylation of many optic axon proteins is not closely coupled to their synthesis, or 2) phosphorylation accompanies synthesis, but phosphate groups on newly synthesized proteins are actively removed by protein phosphatases. To distinguish between these possibilities we blocked protein synthesis in the retinal ganglion cells by intraocular injection of cycloheximide. The experiments were carried out on animals in which the optic tracts had been severed 3 weeks earlier, in order to maximize protein synthesis and export into the axons. One hour after injection of the inhibitor a combination of ^{32}P and ^{3}H-proline was injected into the eye. Seventeen hours later the animals were sacrificed and the nerve proteins subjected to two-dimensional electrophoresis and autoradiography. The inhibitor reduced the incorporation of ^{3}H-proline into total nerve protein by approximately 85% but reduced the incorporation of ^{32}P by only 50%. In most of the individual phosphoproteins the incorporation of proline was reduced by more than 85% whereas the incorporation of ^{32}P was reduced by less than 50% in 18 of the 20 proteins examined and by less than 30% in 11 of these. The fact that the phosphorylation of all proteins was significantly less affected than their synthesis indicates that phosphorylation of these proteins is unlikely to be closely coupled to their synthesis.

To determine whether phosphorylation occurred in the ganglion cell bodies or in the axons of the optic nerve, axonal transport was blocked by intraocular injection of vincristine in experiments that duplicated the protocol used with cycloheximide. Vincristine reduced ^{3}H-proline incorporation into total nerve protein by 90% but reduced ^{32}P incorporation by only 55%. In 17 individual proteins examined, incorporation of ^{3}H-proline was reduced by more than 90%. However, 15 of these proteins showed a reduction in phosphorylation of less than 55% and 12 of them less than 30%. Thus, most of the phosphoproteins appear to be phosphorylated in the optic nerve.

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383.6 OPTIC NERVE REGENERATION IN CARP IS ASSOCIATED WITH ACCUMULATION OF APOLIPOPROTEIN-A-I (PARTICULARLY ONE ISOFORM) AND INCREASED EXPRESSION OF APOLIPOPROTEIN-A-I TRANSCRIPTS. M. Schwartz[1], A. Harel[1]*, C. Stein-Izsak[1], M. Fainaru[2]*, Z. Schafer[2]*, and T. Vogel[3]* Dept. of Neurobiology, The Weizmann Institute of Science[1], Kaplan Hospital[2] and Biotechnology General (LTD)[3], Israel.

Injury to the fish optic nerve initiates pronounced changes in the composition of proteins derived from the surrounding non-neuronal cells. One of the injury-induced alterations is in the appearance of a pulse-labeled 28kDa polypeptide in conditioned media (CM) of these nerves (Rachailovich, I. and Schwartz, M., Brain Res., 306; 149, 1984). In this report, we provide evidence that this polypeptide is immunologically cross reactive with apolipoprotein-A-I (apo-A-I) from fish plasma. Apo-A-I was found in both intact and injured fish optic nerves. Injury to the optic nerve was associated with accumulation of apo-A-I, particularly of one isoform.

Apo-A-I was purified from fish plasma by density gradient centrifugation, followed by gel filtration of the high density lipoprotein fraction on Sephadex G-200. The purified protein was identified as apo-A-I based on molecular weight (28kDa) as determined by gel electrophoresis and amino acid composition. No immunological cross-reactivity was observed between fish plasma apo-A-I and antibodies directed against mammalian apo-A-I. Therefore, antibodies were prepared against fish plasma apo-A-I. Using these antibodies, by Western blot analysis we were able to show that the 28kDA polypeptide cross-reacts immunologically with the apo-A-I from fish plasma. Immunoblotting of two-dimensional gels of substances derived from CM of non-injured fish optic nerves revealed two polypeptides (pI 6.49, 6.64) that were stained with antibodies raised against the purified plasma apo-A-I. In regenerating preparations, and additional polypeptide (pI 6.73) was stained.

Messenger RNA derived from non-neuronal cells of fish optic nerves hybridized in slot blots with nick-translated cDNA probes of human apo-A-I. The hybridization signal was higher in injured (regenerating) than in non-injured nerves. In the former, higher levels of apo-A-I transcripts were found in the distal stump than in the segment close to the optic disk.

Apolipoprotein-E (apo-E) is known to accumulate in peripheral nerve of mammals after injury. No appreciable levels of apolipoprotein corresponding to mammalian apo-E could be detected among the fish apolipoproteins by polyacrilamide gel electrophoresis. It is, therefore, suggested that apo-A-I in regenerating nerves of fish CNS plays a role similar to that of apo-E in regenerating nerves of mammalian PNS. Both may be involved in elimination of myelin-degradation products.

(This work was carried out with support from the US Army Medical Research and Development Command to MS.)

384.1 PLASTICITY OF RETINAL AND NONRETINAL AFFERENTS IN THE CHICK VENTRAL LATERAL GENICULATE NUCLEUS FOLLOWING TECTAL REMOVAL AT HATCHING. G.R.Ten Eyck,* R.M. Kraemer,* L.M. Sokolowski,* and W.J. Crossland, (SPON: J.A. Rafols) Dept. of Anatomy and Cell Biology, Wayne State Univ. Schl. Med., Detroit, MI 48201.

The chick ventral lateral geniculate nucleus receives projections from the retina, optic tectum, and visual wulst. We previously showed plasticity of the retinal projections to the chick ventral lateral geniculate nucleus (GLv) following optic tectum lesions. In this study, we examined further the effects of optic tectum removal on transneuronal atrophy and plasticity in the GLv.

Newly hatched chicks were anesthetized with ether and subjected to unilateral tectal removal. After a postoperative survival of 78 days one group of animals was perfused with formalin, embedded in paraffin, and sectioned in the frontal plane for light microscopy; a second group was perfused with an aldehyde mixture, sectioned on a vibratome, and samples of the center of the GLv embedded in epon for electron microscopy.

The volume of the GLv (both ipsilateral and contralateral to the tectal removal) was reconstructed from tissue sections which were measured using a digitizing morphometry system (Bioquant II, R & M Biometrics) in ten chicks. The GLv volume ipsilateral to the lesion was 13% larger than that of the contralateral GLv.

Eight animals prepared for electron microscopy were analyzed using point-counting stereology in a single-blind study of 1) the number of synaptic active zones per unit area in the GLv neuropil lamina, 2) the volume fraction of the tissue occupied by retinal and nonretinal synaptic terminals (containing active zones), and 3) retinal and nonretinal vesicle containing profiles (not containing active zones).

The areal density of retinal and nonretinal active zones did not differ significantly between the GLv contralateral and ipsilateral to the removed tectum, however, the volume fraction was significantly increased for both synaptic terminals and vesicle containing profiles ipsilateral to the tectal removal.

The results indicate sprouting of axonal terminals in the GLv without an increase in synaptic membrane specializations, and without any overall atrophy of the GLv volume. Furthermore, the bulk of the sprouting occurs in nonretinal terminals which may include intrinsic cells of the GLv.

This study was supported in part by NIH grants EY01796 (W.J.C.), and EY-04068 (Core Equipment Grant for Vision Research), and a Grant from the Michigan Eye Bank (W.J.C.). L.M.S. was supported by a summer fellowship from the Neuroscience Program at Wayne State University.

384.2 GUINEA PIG VAGAL MOTONEURONS EXHIBIT EXTENSIVE PERIKARYAL AND DENDRITIC SPROUTING DURING RETROGRADE REACTION

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During retrograde reaction, vagal motoneurons of the guinea pig show complex changes of their perikaryal and dendritic surface in addition to chromatolysis. Within a few days, flap-like processes appear on the neuronal surface. They contain varying numbers of smooth-surfaced vesicles or cisternae, a feltwork of filamentous material and, occasionally, microtubules and dense-core vesicles. These upfoldings cover large parts of the perikarya or interdigitate with processes of adjacent neurons. Moreover, the shape of the dendrites becomes highly irregular, and growth--cone-like profiles appear in the neuropil. These changes reach their maximum two to three weeks after axotomy, but seem to persist for several months. Both perikaryal and dendritic processes are frequently seen in contact with presynaptic terminals.

Local astrocytes react with hypertrophy of their processes, an increase of glial filaments and intense lamellae formation. Within a few days, neurons seem to be completely wrapped by glial lamellae stacks.

Acetylcholinesterase (ACHE) activity was used as a cytochemical marker to distinguish neuronal from glial processes. Interestingly, the guinea pig vagal neurons do not show the rapid loss of ACHE activity that has been described in other species. Rather, a sub-cellular redistribution of activity seems to occur after nerve transection. At the light microscopical level, staining of the neuronal perikarya slightly increases. After axotomy, electron microscopic cytochemistry demonstrates strong activity in peri-nuclear cisternae, endoplasmic reticulum and Golgi complexes. Staining is also especially prominent on the neuronal plasmalemma. The newly formed processes were consistently found to be ACHE--positive.

It is concluded that the morphological changes observed correspond to a sprouting process in an unusual location, i.e. adult CNS environment. This could indicate a mechanism that possibly is involved in the reorganization of synaptic afferent inputs. It might also serve protective functions, since preliminary data suggest that guinea pig vagal motoneurons are less susceptible to cell death than those of other species.

384.3 MONOAMINERGIC ADAPTATION TO NEONATAL NOREPINEPHRINE DEPLETIONS IN RAT BRAIN. M.H. Teicher, N.I. Barber*, R.J. Baldessarini, S.P. Finklestein, E. Marsh*, J. Zorc*. Harvard Medical School; Mailman Research Center, McLean Hospital, Belmont, MA 02178.

We reported that the combination of GBR-12909, a selective inhibitor of neuronal uptake of dopamine (DA), and 6-hydroxydopamine (6OHDA) administered to 3-day-old rat pups resulted in a 95-99% depletion of cortical and hippocampal norepinephrine (NE), without acute effects on DA or serotonin (5HT) levels (Teicher et al., Devel. Brain Res., 1986, 30:124-128). In the present study we evaluated possible long-term compensatory changes in monoaminergic systems following profound neonatal NE depletion by such treatment. Stakowiak et al (Brain Res. 1984, 291:164-167) and Breese et al. (J. Pharm. Exp. Ther. 1984, 231:343-354) reported that neonatal depletion of DA was followed by increased striatal concentrations of 5HT, presumably by a compensatory process of axonal sprouting and increased innervation by 5HT-containing axonal terminals arising from raphe nuclei (Berger et al. Brain Res. 1985, 336:354-358; Snyder et al. J. Comp. Neurol. 1985, 245:247-281).

3-d-old rat pups were given GBR-12909 (40 mg/kg ip), followed in 1 h by intracisternal 6OHDA (125 µg in 20 µl saline-ascorbic acid solution), and were sacrificed at 14, 17, 22, 40 and 60 d of age. Regional HPLC/EC assays of NE, DA, and 5HT were performed in hippocampus (HIPP), hypothalamus (HYPO), striatum (STR), and prefrontal cortex (PFCTX). Data were compared to vehicle injected littermate controls sacrificed at the same time.

At 60 d of age, HIPP NE was decreased by 93%, and 5HT was increased by 71% ($p<.02$); the increase in HIPP 5HT content emerged slowly, and was not discernable at 40 d of age. At 60 d HYPO NE was 77% depleted and 5HT tended to increase (43%). Despite a 97% reduction in PFCTX NE, the 5HT content of this region was not significantly increased at this age.

Levels of DA were virtually identical to control levels in the STR, HYPO and PFCTX of 60-d-old rats. It was surprising that there were no significant effects of NE depletion on DA concentration. Perhaps a transient change in DA levels preceded slower and more sustained alterations in other neurochemical processes. In HYPO, levels of DA were transiently increased at 14 and 17 d (by 63% and 95% respectively) but had returned to control levels at 22 d. The PFCTX displayed an even greater transient change, with DA levels exceeding control concentrations by 2-fold at 17 d and 3-fold at 22 d of age, but were not significantly greater than controls at 40 d.

These results suggest that neonatal depletion of NE is followed by regionally-specific time-dependent compensatory alterations in other monoaminergic systems that include sustained slow increases of 5HT, particularly in the HIPP. Such developmental processes may enable these neonates to survive and adapt to massive damage to this presumably important neurotransmitter system.

384.4 COMBINED SEPTAL AND ENTORHINAL LESIONS PRODUCE MORE EXTENSIVE SUPRAGRANULAR MOSSY FIBER SPROUTING THAN DO ENTORHINAL LESIONS ALONE. H.H. Tjossem and J.R. West. Alcohol and Brain Research Lab, Dept. of Anatomy, Univ. of Iowa, Iowa City, IA 52242.

In the hippocampal formation of rats, the removal of CA4-derived or entorhinal afferents to the dentate gyrus causes sprouting of granule cell axons (mossy fibers) onto proximal segments of the granule cell dendrites (Laurberg, S. and Zimmer, J., J. Comp. Neurol. 200:433, 1981). We find that the removal of septal afferents in conjunction with entorhinal lesions causes additional supragranular mossy fiber sprouting. Four groups of young adult albino rats were used, three of which received electrolytic lesions while the fourth received no treatment. One group received bilateral septal lesions, one group received unilateral entorhinal cortex lesions and one group received combined septal and entorhinal lesions. Thirty days following the lesions, animals were perfused for the Timm-stain of heavy metal cations. Lesion sections were examined to document the extent of tissue destruction. Two series of coronal sections were taken from the rostral hippocampal formation and stained with either the Timm procedure for mossy fiber analysis, or for acetylcholinesterase histochemistry to document the success of the septal lesions. Qualitative analysis of the normal group showed a few bundles of black-staining mossy fibers radiating through the granule cell layer with some coursing around the free end of the infrapyramidal granule cell layer to reach the supragranular region where limited punctate staining is often seen. The mossy fiber distribution of the septal lesion group did not appear different from normal rats. Animals which received entorhinal lesions had marked, punctate mossy fiber staining in patches scattered throughout the supragranular zone of the dentate gyrus. Combined septal and entorhinal lesions enhanced this mossy fiber sprouting. The combined lesions produced a continuous, heavily stained band of mossy fiber puncta throughout the supragranular zone of the dentate gyrus. Thus, in the rat dentate gyrus, the loss of septal afferents, when combined with removal of entorhinal afferents, will augment supragranular mossy fiber sprouting even though bilateral septal lesions have no striking effect of their own. These findings have important implications for recovery of function following multiple injuries to the central nervous system and to disease processes such as those associated with Alzheimer's disease where multiple foci of pathology occur. (Supported by NIAAA grant AA06192 to J.R.W.).

384.5 SEX DIFFERENCES IN HIPPOCAMPAL REACTIVE FIBER GROWTH: STEROID INTERACTIONS J. K. Morse, S. W. Scheff, and S. T. DeKosky. Depts. Anatomy and Neurology, Lexington VA and Sanders-Brown Research Center on Aging, Univ. of Kentucky, Lexington, KY 40536.

We have previously reported that steroids play an important role in the sexually dimorphic lesion-induced fiber outgrowth observed in the hippocampus. Normal male and female rats demonstrate equivalent reactive growth. Adrenalectomy (ADX) results in a significant increase in fiber outgrowth in females but fails to alter the male's response. Gonadectomized (GDX) females significantly decrease their reactive response, while GDX males maintain control levels. The growth response in GDX females is normalized by estrogen (E) or testosterone (T) replacement. A dimorphic response is also seen in GDX/ADX animals. Females respond to control levels while males display decreased fiber outgrowth. T is apparently essential to the male mechanism of growth, while E is sufficient but not necessary for the female response. The present study further examined steroid interactions with these sexually dimorphic mechanisms of reactive outgrowth.

Ninety day Sprague-Dawley rats of both sexes were randomly assigned to one of five treatment groups: 1) Controls 2) GDX/ADX (asteroidal) 3) GDX/ADX + T 4) GDX/ADX + E and 5) GDX/ADX + glucocorticoids (hydrocortisone). Ten days after GDX/ADX surgery, all rats underwent a unilateral entorhinal cortex ablation. Fifteen days later changes in axon sprouting were determined by assessing the reactive outgrowth of the hippocampal commissural-associational afferents.

The interpretation of sexually dimorphic aspects of fiber outgrowth change depending upon the baseline used for comparison. Compared to normal controls, T enhanced outgrowth in GDX/ADX females but had no effect in GDX/ADX males. When compared to asteroidal (GDX/ADX) baseline rats, T facilitates fiber growth in both males and females. This surgical procedure (GDX/ADX) decreases outgrowth in the males resulting in a lower baseline. The baseline for the females is unchanged with GDX/ADX. Estrogen enhanced growth in both GDX/ADX females and males when compared to normal or asteroidal controls. Glucocorticoids are inhibitory in both sexes when compared to normal controls, but do not play the same role when asteroidal baselines are used. In females they appear to regulate lesion-induced outgrowth while in the male under the GDX/ADX condition they appear to have little effect making their role uncertain. (Supported by NIH grant NS21541 and the VA Medical Research Service)

384.6 THE ROLE OF CONTRACTILE ACTIVITY AND SYNAPTIC FUNCTION IN MOTOR NEURON SPROUTING. M.M. Wines, D.G. Garrett,* and M.S. Letinsky. UCLA School of Medicine, Ahmanson Laboratory of Neurobiology, Los Angeles, California 90024.

In a previous publication we demonstrated that application of the compound formamide to amphibian striated muscle selectively eliminated contractile activity without significant alterations to either presynaptic release of acetylcholine or muscle fiber action potentials. In addition, motor neurons within these paralyzed preparations were seen to sprout unmyelinated outgrowths from their terminal arborizations (Soc. Neurosci. Abstr. 11:916, 1985). More recently we have produced chronic contractile and electrical inactivity in amphibian striated muscle (cutaneous pectoris; Rana pipiens) by repetitive exposure of the tissue to the postsynaptic blocking agent alpha-bungarotoxin (α-BTX). Analogous to the response seen with formamide incubation, motor neurons in these toxin treated preparations are also seen to sprout. However, the onset of this response as well as the morphology and frequency of sprouting is notably different than that seen to occur after selectively eliminating only contractile activity. To date, postsynaptic blockade with α-BTX has been maintained without interruption for up to 35 days. Relatively small amounts of sprouting (3-8% of the observed terminals) occur up until approximately two weeks of inactivity, afterwhich the number of terminals bearing sprouts increases dramatically (up to 54% at five weeks of inactivity). The onset for the initiation of sprouting in amphibian muscle is markedly slower than the response seen to occur after α-BTX application to mammalian muscles (Holland and Brown, Science 207:649, 1980). In addition, formamide induced inactivity produces lengthy, ornate terminal outgrowths in contrast to the short, rudimentary outgrowths seen to follow α-BTX incubation. The observed results suggest that the method used to promote contractile inactivity (i.e., presence or absence of synaptic function and muscle fiber action potentials) may modify the sprouting mechanism. Periods of α-BTX induced inactivity beyond the five week maximum are currently being maintained. Supported by USPHS grant NS13470.

384.7 INTRASPINAL SPROUTING OF RAT PRIMARY AFFERENTS AFTER DEAFFERENTATION. D.L. McNeill* and C.E. Hulsebosch (SPON: K. Chung). Marine Biomed. Inst. and Depts. Anat. & Neurosci., Univ. Tex. Med. Br., Galveston, TX 77550.

In 1958, Liu and Chambers first reported increased silver deposition in the dorsal horn on the chronically denervated side in the cat spinal cord after unilateral dorsal rhizotomies above and below a spared root. The increase in silver reaction product was interpreted as terminal sprouting of the spared primary afferents in response to spinal cord deafferentation. The issue of primary afferent sprouting after spinal cord deafferentation remains controversial. Recently, Hulsebosch and Coggeshall (1981) observed a significant increase in the number of unmyelinated primary afferent fibers in the spared root of juvenile rats when compared to the contralateral control root, which was interpreted to be sprouting (or branching) elicited by spinal cord denervation. In the present study, we used fluoride-resistant acid phosphatase (FRAP), an enzyme present in a subpopulation of small diameter primary afferent perikarya and their terminals in laminae I and II of the dorsal horn, to test presynaptic sprouting of primary afferent fibers in the dorsal horn of juvenile rats using a modification of the spared root paradigm. One month old Sprague-Dawley rats were anesthetized and a unilateral dorsal rhizotomy was performed three segments above and below T9 or T10 which was the chronic "spared" root. As a control, 26 days following the initial surgery, rats were reanesthetized and the deafferentation procedure was repeated on the contralateral side three segments above and below the spared segment, thus eliminating all but the primary afferent fibers from the chronic and control spared roots. Four days later, the animals were perfused with 4% paraformaldehyde, the T8-T11 spinal cord was removed, frozen, serially sectioned at 20 µm, mounted on glass slides and reacted for FRAP enzyme activity by a modified Gomori procedure. In all rats (n=5), the density of FRAP reaction product was greater on the chronically denervated side than on the contralateral acutely denervated side. In addition, FRAP reaction product extended medially in the spared segment and cranially beyond the spared segment. To rule out normally occuring asymmetrical projections of primary afferents, the spared root surgery was performed bilaterally on 56 day old littermates which were sacrificed 5 days later. No asymmetrical projection patterns were found. We hypothesize that the increased and extended FRAP reaction product medially in the spared segment and into adjacent denervated segments on the chronic side is due to sprouting of presynaptic terminals of primary afferent fibers in the dorsal horn. Ultrastructural verification of this hypothesis is now underway.

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384.8 IMMUNOLABELING OF SYNAPTIC REMODELING IN NEONATALLY DEAFFERENTED OLFACTORY CORTEX. R. E. Westenbroek*[1], L. E. Westrum[2], A. E. Hendrickson[3], J-Y. Wu[4], and N. A. Nougek-Goebl*[3]. Depts. of Neurosurg.[1,2,5], Biol. Struct.[1,2,3], and Ophthalmol.[3], Univ. of Wash., Seattle, WA 98195 and Dept. of Physiol.[4], Penn. State Univ., Hershey, PA 17033.

Plasticity and reorganization of afferents within the CNS following deafferentation is a topic of much current interest. The three-layered olfactory cortex (OC), with its well-established afferents and efferents, serves as an excellent model for such studies. Olfactory bulb (OB) removal in neonatal rats results in dramatic spread or sprouting of association fibers into the deafferented superficial apical dendritic fields (layer Ia) of the OC. We are studying the developmental plasticity in OC using immunocytochemical localization of neurotransmitters including antisera to cholecystokinin (CCK; graciously provided by Dr. M. C. Beinfeld) and glutamic acid decarboxylase (GAD). Sprague-Dawley rats are being used. Newborns under deep ether anesthesia have one OB removed. Following about 3 months survival the animals are sacrificed, perfused, and Vibratome sections from OC are processed by the PAP method of Sternberger modified for electron microscopy (EM). In normal adults, and in contralateral, unoperated control sections of OC, GAD-positive terminals mostly contain flat to pleomorphic vesicles and form symmetric type 2 contacts onto dendritic shafts and branches throughout layer I, superficial (Ia) and deep (Ib). CCK-positive terminals normally occur in deeper layer I (Ib) and cell layer II but are rare superficially (Ia). They form mainly type 2 and occasionally type 1 (asymmetric) contacts. In normals primary afferents from the OB form type 1 asymmetric contacts only. In the deafferented material there appear to be greater numbers of labeled GAD terminals throughout Ia and Ib. Here not only do these GAD-positive terminals form type 2 contacts, but several of them form clearly asymmetric type 1 synapses onto dendrites of all sizes, including spines. CCK-labeled axons and terminals now occur more frequently in superficial Ia where they were usually rare or absent in controls. These numerous CCK-positive terminals usually contain pleomorphic or round vesicles and sometimes form contacts of either type, but more often lack a distinct contact. The findings clearly show modifications in synaptic patterns of immunocytochemical-labeled terminals which might be compatible with the process of atypical reinnervation of deafferented postsynaptic sites and possible ingrowth of new axons.

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384.9 NERVE GROWTH FACTOR RECEPTOR AND CHOLINE ACETYLTRANSFERASE COLOCALIZATION WITHIN THE RAT FOREBRAIN: RESPONSE TO FIMBRIA-FORNIX TRANSECTION. P.E. Batchelor*, S.N. Blaker*, D.M.Armstrong, and F.H. Gage , (Spon: A. Miller). Dept. of Neurosciences, UCSD, La Jolla, CA 92093.

The distribution of nerve growth factor receptor (NGF-R) and choline acetyltransferase (ChAT) within the rat brain were simultaneously mapped by a double immunocytochemical procedure. A specific polyclonal antibody to ChAT (gift of L.B. Hersh and G. Bruce) was used in combination with peroxidase to identify cholinergic neurons while the monoclonal antibody 192-IgG (C.E. Chandler, et al., J. Biol. Chem. 259(11) 6882-6889, 1984: gift of E.M. Johnson) in combination with alkaline phosphatase was used to visualize NGF-R distribution. It was found that a distinct subpopulation of cholinergic neurons expressed NGF-R. This population included the large long-projecting magnocellular forebrain neurons located in the septum and vertical limb of the diagonal band, the large midline cells located within the columns of the fornix rostrally and dorsal hippocampal commissure caudally, and cells of the nucleus basalis magnocellularis. By contrast, no double immunoreactivity was observed in the smaller shorter projecting intrinsic ChAT positive neurons located in the caudate-putamen, nucleus accumbens, cortex and caudal and temporal hippocampal formation.

In order to more fully determine the functional significance of the presence of NGF-R on populations of cholinergic neurons, complete aspirative lesions of the fimbria-fornix and supracallosal striae were made. Eight weeks following FF transection the large midline ChAT-positive neurons which exhibited neuronal sprouting (S. N. Blaker, et al. Soc. Neurosci. Abstr. 12:1379, 1986) also expressed NGF-R. In contrast, the small intrinsic ChAT-positive cells of the hippocampus did not express NGF-R. In addition, a second NGF-R-positive sprouting response was observed in the dentate gyrus and CA3 regions of the hippocampus. These large AChE-negative, NGF-R-positive, fibers have the distribution and morphological appearance of anomolous fimbria-fornix transection-induced sympathetic nonadrenegic fibers of the superior cervical ganglion and stain for tyrosine hydroxylase immunoreactivity (R. Loy and R.Y. Moore, Exp. Neurol. 57:645-650, 1977).

In view of the elevated levels of NGF in the hippocampus following fimbria-fornix transection (S. Korsching, et al., Neurosci. Lett. 66:175-180, 1986) and the presence of NGF-R only in sprouting neurons, we conclude that the sprouting response observed in these different cell populations may be due to their responsiveness to the increase in NGF within the hippocampus following fimbria-fornix transection.

384.10 **TRANSECTION OF THE INFRAORBITAL NERVE PROMOTES THALAMIC FIBER INGROWTH INTO EMBRYONIC NEOCORTICAL TRANSPLANTS IMPLANTED INTO THE BARRELFIELD OF ADULT MICE.** R.S. Erzurumlu and F.F. Ebner, Center for Neural Science, Brown University, Providence, R.I. 02912.

Embryonic neocortical transplants survive and differentiate within the cortex of an adult host, but the damaged host fibers from specific thalamic nuclei fail to innervate these implants. The present series of experiments was carried out to determine if blocking of sensory activity in the periphery would promote the regeneration of mature thalamic fibers from the ventrobasal nucleus (VB) into grafts of immature neocortex implanted into the PMBSF region of SI cortex. Anesthetized adult mice underwent unilateral infraorbital (IO) nerve cautery or transection at the IO foramen 2 days prior to transplantation into the contralateral cortex. The ingrowth of VB axons in this experimental group was compared to that seen after grafting into normal adult animals.

Procedure: strips of embryonic (E14) parietal cortex were dissected into Eagle's medium and suctioned into a square glass capillary filled with the same medium. The capillary was inserted into the host SI cortex parallel to the pial surface and the graft floated into place after expelling the host tissue that had been isolated within the capillary. Thirty days later both experimental and control animals received HRP injections into the VB nucleus ipsilateral to the transplant (0.02 ul, Sigma Type VI). HRP injections were made using a horizontal approach to avoid traversing the hemisphere containing the graft. HRP was visualized by $CoCl_2$ enhancement of the DAB-GOD reaction.

The control cases confirmed previous observations that very few or no labeled fibers cross the interface between normal host cortex and the grafts, even when dense anterograde and retrograde labeling is present in the host cortex both medial and lateral to the transplant. In contrast, all cases with prior IO nerve cut contained numerous large caliber thalamic fibers that traverse the border, enter the transplant and then form elaborate terminal arbors within the graft. The distribution of thalamic fibers was not uniform throughout the graft. Instead, they clustered in discrete zones that were often surrounded by aggregates of granule cells reminiscent of layer IV barrels in mouse SI cortex. Adjacent AChE-stained sections showed a completely different distribution of cells and fibers from the HRP labeling. Gross anatomical dissection of the IO nerve after perfusion showed that many fibers had regenerated into the whisker pad. We are investigating the possibility that whisker stimulation will activate neurons in the grafts that show elaborate VB fiber innervation. (supported by NS-13031 + the Mathers Foundation)

384.11 NONREGENERATIVE AXONAL GROWTH WITHIN THE MATURE MAMMALIAN BRAIN. K.A. Crutcher and C.F. Marfurt. Dept. of Anatomy, Univ. of Utah School of Med., Salt Lake City, UT 84132 and Dept. of Anatomy, NW Center for Med. Ed., Indiana Univ. School of Med., Gary, IN 46408.

Extensive axonal elongation occurs during CNS development, but is limited to the PNS in adult mammals. The favorable environment for axonal growth provided by the PNS also permits regeneration of central neurons, as demonstrated by the peripheral nerve grafting experiments of Aguayo and coworkers. The reverse situation, i.e., elongation of peripheral axons within the mature CNS, is rare but may be occuring when sympathetic axons appear in the rat hippocampal formation following septohippocampal denervation. Preliminary results presented last year suggested that sympathohippocampal fibers may be restricted to the perivascular environment. In order to extend these observations we undertook more rigorous ultrastructural analysis of HRP-labeled sympathetic fibers within the dentate gyrus.

The superior cervical ganglia were injected with WGA-conjugated HRP and the dentate was sectioned and reacted at pH 6.0 in order to preserve ultrastructural morphology. Stabilization of the TMB reaction product with ammonium molybdate resulted in significant preservation of the granules which were easily identified at the EM level. In order to obtain some index of the extent to which labeled fibers were associated with blood vessels, quantification was undertaken both from 1 micron-thick plastic sections and from random EMs.

As reported previously, HRP-labeled profiles were present in association with hippocampal blood vessels, particularly above and below the granule cell layer within the dentate gyrus. In addition, HRP-labeled axons and vesicle-filled profiles (many of the vesicles were of the small dense-cored variety) were encountered within the neuropil, without any vascular association. In one plastic section, and in a sample of electron micrographs (n=98), two-thirds of the labeled fibers were in a perivascular location and the majority of these were directly apposed to the basal lamina. The other third, however, were present within the neuropil. In some cases, labeled axons within the neuropil were present in fascicles but individual vesicle-filled varicosities were more common. In no case were membrane specializations observed indicative of synaptic contacts.

These results establish the presence of axons of peripheral origin within the neuropil of the mature mammalian brain following specific denervation. The specific changes in the target tissue that permit axonal elongation in this instance are not known but NGF has been implicated as one factor that may permit such ingrowth. Furthermore, the presence of sympathetic fibers in the hippocampal neuropil would presumably result in peripheral autonomic influences on hippocampal function. If a similar sprouting response occurs in the human hippocampal formation e.g., in Alzheimer's disease, then such an innervation could be clinically relevant.
(Supported by NIH grant #NS-17131 and NSF grant #BNS-8501269.)

384.12 RECEPTIVE FIELD PLASTICITY AND SOMATOTOPIC ORGANIZATION OF PLEURAL MECHANOSENSORY/NOCICEPTIVE NEURONS OF APLYSIA. A.J. Billy* and E.T. Walters (SPON: D. Johnston). Dept. Physiol. & Cell Biol., Univ. Texas Med. Sch. Houston, TX 77225.

Sensory neurons in the LE and VC clusters of Aplysia express several intensively studied forms of plasticity in their central (synaptic and soma) regions. Much of this plasticity is readily produced by noxious stimuli that injure the peripheral receptive fields of the sensory neurons (Walters, J.Neurosci. 7:400, 1987). These central forms of plasticity may normally be coordinated with (and may share cellular mechanisms with) plastic mechanisms in the peripheral regions of these cells which act to restore sensory function to injured areas of the body. To test this hypothesis we have begun to examine the effects of peripheral injury on the receptive fields of the VC sensory neurons. Because the somatotopic organization of these clusters was only partially known (Walters et al., J. Neurophysiol. 50:1522, 1983) we first examined the relationship between soma position in the VC clusters and receptive field position in normal animals. Nerve stimulation indicated that axons of VC neurons occur in all nerves of the pedal and pleural ganglia, with cells innervating the anterior part of the foot and body wall in the anterolateral part of the cluster, and cells innervating progressively more posterior parts of the body distributed in a rough continuum to the posteromedial borders. Although there is considerable variation in the size and shape of fields, a remarkably precise boundary exists along the midline, so that receptive fields of sensory cells in each cluster are restricted to the ipsilateral side of the body. At least 3 classes of peripheral plasticity are indicated: (1) Increases or decreases in the size and sensitivity of a receptive field often accompany repeated application of the test stimuli (von Frey hairs). We have not yet systematically investigated this short-term plasticity. (2) Functional denervation of a region produced by a cut across part of the tail was partially reversed in 8 of 27 preparations after 1-3 weeks by apparent reinnervation from ipsilateral VC neurons. (3) In 7 of 27 cut tail preparations there was clear extension across the midline by contralateral receptive fields near the cut. No comparable extension across the midline was seen in 9 uncut preparations. As a first step in testing whether long-term peripheral plasticity is activity-dependent we are examining the effects of explicitly stimulating the contralateral side of the tail at the time of the cut. Thus far, 40% (6 of 15) of the medial contralateral fields extend across the midline in stimulated animals while only 12% (9 of 75) show extension in animals not stimulated contralaterally.

385.1 CELL CYCLE MODULATION OF HISTOCOMPATABILITY ANTIGEN EXPRESSION ON HUMAN ASTROCYTOMA (U373MG) CELLS. Michael E. Barish and Mary E. Thornton*, Departments of Developmental and Cell Biology & Physiology and Biophysics, University of California, Irvine, California 92717.

Recent years have seen an increased interest in expression of histocompatability antigens on brain cells. In particular, cells expressing class II major histocompatability complex (MHC) gene products have been found on glial cells surrounding various types of brain lesions, and it is thought that antigen presentation by astrocytes may play a role in the genesis of autoimmune reactions in the brain such as those associated with multiple sclerosis.

Many laboratories including ours have observed that the level of expression of class II MHC antigens on cultured primary astrocytes stimulated by gamma-interferon or other agents can be variable -- within a given dish only some cells show high levels of expression. Because we felt that an understanding of this variability would give some clues as to the normal control of class II MHC expression in the brain, we have studied a human astrocytoma cell line (U373MG, ATCC HTB 17) that is normally class II MHC positive using FITC-conjugated monoclonal antibody against HLA-DR (clone L243, Becton-Dickinson).

We observed using fluorescence microscopy and flow cytometry that normal expression of HLA-DR varied greatly between cells. Within an average culture approximately 60% of cells showed fluorescence levels above background. We hypothesized that HLA-DR expression might be linked to the cell cycle. We have thus used two color flow cytometry to correlate DNA content (measured using propidium iodide) with HLA-DR expression, and have observed that cells expressing higher levels of HLA-DR are more likely to be in S, G2 or M phases than low expressors. This correlation is being investigated further. It suggests that at least in astrocytoma the proliferative state may be permissive for HLA-DR expression.

385.2 MONOCLONAL ANTIBODY 8A2 RECOGNIZES A DEVELOPMENTALLY REGULATED GLYCOLIPID IN THE CHICK RETINA. J. Drazba* and V.P. Lemmon (SPON: C.F. Lagenaur). Dept. of Neurobiology, Anatomy and Cell Science and Ctr. for Neuroscience, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

A monoclonal antibody, 8A2, has been obtained that binds to axons in the chick nervous system. Immunohistochemical experiments indicate that this antibody binds to an antigen that is developmentally regulated in the chick visual system. Strong binding of 8A2 to the developing retinal ganglion cell axons is seen throughout the period when they are forming topographically organized projections within the central nervous system. In vitro experiments on dissociated E-6 chicken retina indicate that the antigen is not present on all the cells, but very strongly labels the cell surface of large, multipolar cells which generate long neurites. At later developmental stages the 8A2 antigen appears on other cell types in the retina. For example, at E-15, in addition to strong staining of the optic fiber layer and optic nerve, the inner plexiform, inner nuclear and outer plexiform layers also express the antigen. In the adult retina the 8A2 antigen has largely disappeared from the optic fiber layer and optic nerve, but persists in the inner plexiform, inner nuclear and outer plexiform layers. Initial biochemical characterization of the antigen reveals that it is a lipid present in the aqueous phase of chloroform/methanol/water extraction of embryonic chick retina and is sensitive to neuraminidase but not protease digestion. The relationship between the 8A2 antigen and other glycolipids such as the JONES antigen (Constantine-Paton, M. et al., Nature, 324:459, 1986) will be discussed.

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385.3 FREEZE-FRACTURE STUDIES ON UNMYELINATED AXOLEMMA OF RAT CERVICAL SYMPATHETIC TRUNK: CORRELATION WITH SAXITOXIN BINDING. J.A. Black and S.G. Waxman. Dept. of Neurology, Yale University School of Medicine and V.A. Medical Center, New Haven, CT 06510

In freeze-fracture replicas, intramembranous proteins are visualized as "bumps", or particles, within the generally smooth lipid layer background. The functional identity of these particles remains obscure, with considerable speculation regarding a population of large diameter (>10 nm) particles present in high concentration at the node of Ranvier. It has been suggested that some of these large diameter particles may represent voltage-sensitive sodium channels, which are known to be present in high density at nodes. In order to further examine the possible correspondence between large diameter particles and sodium channels, the density and size distribution of particles within unmyelinated axolemma of the rat cervical sympathetic trunk was examined. This fiber tract is composed nearly entirely of unmyelinated axons, and has been recently utilized in saxitoxin-binding studies to obtain an estimate of the density of sodium channels in unmyelinated axolemma (Pellegrino et al., 1984)

Rat cervical sympathetic trunks were excised and immersion-fixed in a solution containing 2% glutaraldehyde and 2% para-formaldehyde in 0.14 M phosphate buffer with 0.1 M sucrose. The tissue was cryoprotected, frozen in a slush of Freon 22 and fractured and replicated in a Balzers 301 device.

Unmyelinated axolemma of rat cervical sympathetic trunk exhibits a highly asymmetrical partitioning of the particles. On each fracture face, the particles appeared to be randomly distributed. Quantification of the density and size distribution of particles within the unmyelinated axolemma are summarized below and are expressed as mean ± SD:

	Density	% > 9.6 nm	Diameter
PF	1234±355.2	16.3±5.0	8.0±0.4
EF	110±52.4	27.7±13.7	8.2±0.9

From these data, the calculated density of large diameter particles within unmyelinated axolemma from rat cervical sympathetic trunk is 231/μm^2. Saxitoxin binding studies on rat cervical sympathetic trunk suggest a sodium channel density of ~200/μm^2 for unmyelinated axolemma. These observations are consistent with a correspondence between large diameter particles and voltage-sensitive sodium channels.

[Supported by NIH and V.A. Medical Service]

385.4 MACROMOLECULAR STRUCTURE OF UNMYELINATED AND MYELINATED AXOLEMMA FROM RAT CORPUS CALLOSUM. S.G. Waxman and J.A. Black. Dept. of Neurology, Yale University School of Medicine and V.A. Medical Center, New Haven, CT 06510.

The corpus callosum of the rat contains unmyelinated and myelinated fibers that exhibit a distinct overlap in their axonal diameter spectra. Thus, the largest unmyelinated fiber has an axonal diameter of ~0.6 um, while the smallest myelinated fiber has a diameter of ~0.4 um. Previously, it has been demonstrated that the axolemma of unmyelinated fibers displays a markedly different ultrastructure than that of axonal membrane ensheathed by compact myelin. However, a correlation between axolemmal ultrastructure and axonal diameter of unmyelinated and myelinated fibers has not been reported.

Adult rats were fixed by perfusion with a solution of 2% glutaraldehyde and 2% paraformaldehyde in 0.14 M phosphate buffer. Corpus callosum were excised, rinsed in buffer and cryoprotected in graded glycerol solutions. The tissue was rapidly frozen and fractured and replicated in a Balzers 301 device. Replicas were examined with a JEOL 100CX electron microscope.

Both unmyelinated and myelinated axons displayed a highly asymmetrical partitioning of intramembranous particles (IMPs). The particles appeared to be randomly distributed along the fracture faces of unmyelinated and myelinated internodal axonal membrane. Quantification of IMP densities (mean ± SD) are given:

Condition	PF	EF
Internode (all dia.)	1502±203.6	190±40.8
intern. <.5 μm dia.	1507±66.1	
intern. ≥.5 μm dia.	1498±265.2	
Unmyelinated (all dia.)	773±276.4	123±59.9
unmyel. <.5 μm dia.	711±201.8	
unmyel. ≥.5 μm dia.	1162±415.7	

The data demonstrate that the P-face ultrastructure of internodal axolemma is not dependent upon axonal diameter. However, the structure of unmyelinated axolemma appears to be dependent upon axonal diameter. Moreover, the data suggest that unmyelinated axolemma is ultrastructurally different than myelinated internodal membrane for axons of the same diameter.

[Supported by NIH and V.A. Medical Service]

385.5 **AXONAL TRANSPORT AND NEURONOTOXICITY OF A HYBRID CYTOTOXIN COMPOSED OF ANTI-THY 1.1 MONOCLONAL ANTIBODY (OX7) DISULFIDE COUPLED TO THE RIBOSOME INACTIVATING PROTEIN SAPORIN.** R.G. Wiley & F. Stirpe*. Neurology Dept., Vanderbilt University and VAMC, Nashville, TN 37212, and Istituto di Patologia Generale, Universita di Bologna, Italy.

Antibodies to neuronal surface determinants offer the possibility of targeting drugs and other agents to neurons via selective endocytosis. In the present study, we sought to determine if a monoclonal antibody (OX7) with high affinity for rat neuronal Thy 1.1 could be used as a carrier to deliver the ribosome inactivating cytotoxin, **saporin**, to rat neurons. **Saporin** was disulfide coupled to **OX7** to yield a conjugate with 0.68 moles **saporin**/mole of **OX7**. **OX7-SS-Sap** (0.025-8.9 ug) was dissolved in saline and unilaterally pressure microinjected into the cervical vagus nerve, tongue or caudate nucleus of 45 anesthetized, adult male Sprague-Dawley rats. After 1-12 days, rats were reanesthetized and perfused transcardially with aldehyde fixative. Cresyl violet stained sections of the brain and vagal ganglia were analyzed for neuronotoxicity. Doses of **OX7-SS-Sap** >0.35 ug reliably destroyed neurons of the dorsal motor nucleus and nodose ganglion of the vagus after vagal injection. At similar doses, caudate injection of **OX7-SS-Sap** destroyed ipsilateral substantia nigra neurons after caudate injection, a finding that was also evident in sections prepared for catecholamine histofluorescence (FAGLU). 8.9 ug of **OX7-SS-Sap** produced incomplete destruction of hypoglossal neurons after tongue injection. Indirect peroxidase immunohistochemistry demonstrated the presence of antibody in the nucleus tractus solitarius, dorsal motor nucleus of the vagus and nucleus ambiguus after vagal injection of **OX7-SS-Sap** or **OX7** alone. Pretreatment of **OX7-SS-Sap** with dithiothreitol to reduce the disulfide bond dramatically reduced the neuronotoxicity observed after vagal or caudate injections. Injections of **OX7** alone or **saporin** alone did not reproduce the neuronotoxicity of intact **OX7-SS-Sap**. We interpret these results to indicate that **OX7** was effective as a carrier to direct saporin into neurons resulting in suicide transport. Hopefully, this same strategy can be applied in other ways, both experimentally and therapeutically. (This work supported by the Veterans Administration.)

385.6 CONTROL OF GANGLIOSIDE GM_2 EXPRESSION IN TWO NEURONAL CELL LINES. K.M. Walton and R.L. Schnaar, Departments of Pharmacology and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

The NG108-15 neuroblastoma x glioma hybrid cell line expresses four major ganglioside species: GM_3, GM_2, GM_1, and GD_{1a}. When the cells are induced to differentiate in culture (in the presence of 1 mM sodium butyrate), ganglioside GM_3 expression decreases to <20% of control levels, while GM_2 expression increases 10-fold. The expression of gangliosides further along the same biosynthetic pathway (GM_1 and GD_{1a}) does not change (Dahms, N.M. and Schnaar, R.L., J. Neurosci., 3:806, 1983). We previously reported that the activity of the enzyme which synthesizes GM_2, UDP-GalNAc:GM_3 N-acetylgalactosaminyltransferase, increased 4 to 8-fold during differentiation (Walton, K.M. and Schnaar, R.L., Soc. Neurosci. Abstr., 11:1066, 1985). We now report that the activity of the enzyme which uses GM_2 as a substrate to synthesize GM_1 (UDP-Gal:GM_2 galactosyltransferase) **decreases** to 10% of control levels, ensuring a selective increase in GM_2 expression. Thus, ganglioside glycosyltransferases are coordinately regulated to generate a specific change in ganglioside GM_2 expression.

In the PC12 rat pheochromocytoma cell line, GM_2 expression undergoes an unusual form of regulation. A ganglioside with relatively high chromatographic mobility (on TLC) was found to be transiently expressed during PC12 cell growth in culture. Although this ganglioside was undetectable in either sparse or dense PC12 cell cultures during a four day period at intermediate cell density it became the major ganglioside species expressed, then disappeared. This unusual mode of transient ganglioside expression may be important in normal developing neuronal tissue. The ganglioside was purified, and its chromatographic mobility compared to that of standard gangliosides. In two different solvent systems, the ganglioside co-migrated with purified human GM_2. Determination of the neutral sugar composition of this ganglioside revealed only three sugars (galactose, glucose, and N-acetylgalactosamine) at a ratio of 1.0:1.3:0.8, values characteristic of GM_2. Thus PC12 cells transiently express GM_2 as their major ganglioside during growth in culture.

The ability of these neuronal cell lines to tightly control the expression of ganglioside GM_2 during growth and differentiation may implicate it in an as yet unknown function during neuronal maturation. Supported by NIH grants HD14010 & GM07626.

385.7 ALLELIC VARIANTS OF ACETYLCHOLINESTERASE: EVIDENCE THAT A SINGLE GENE ENCODES THE FAMILY OF OLIGOMERIC FORMS IN AVIAN NERVES AND MUSCLE. R.L. Rotundo A.M. Gomez*, C. Fernandez-Valle*, and W.R. Randall*, Dept. of Anatomy and Cell Biology, University of Miami School of Medicine, Miami, FL 33101.

Acetylcholinesterase (AChE) in electrically excitable cells exists as a complex family of forms distinguished by their subunit composition, hydrophobicity, and association with non-catalytic subunits. Muscle cells synthesize and assemble dimeric and tetrameric forms of the enzyme which are destined for secretion or accumulation on the cell surface. In addition, a subset of tetramers are further assembled in the Golgi apparatus into asymmetric forms consisting of three tetramers covalently linked to a collagen-like tail. In neurons, a similar array of forms are assembled including an amphipathic tetramer which is destined for accumulation on the axonal plasma membrane (Rotundo and Carboneto, PNAS 89, 1987). The molecular basis for this diversity is unknown.

We have now found two allelic forms of the AChE catalytic subunit expressed in quail tissues, α and β, with apparent molecular weights of 110 Kd and 100 Kd respectively determined by SDS gel electrophoresis. The expression of each allele in our quail sample population is approximately α=0.7 and β=0.3 and the distribution of heterozygous and homozygous α and β individuals indicates that the two alleles segregate as a single autosomal locus. We have studied the synthesis and assembly of these allelic AChE polypeptides by metabolically labeling tissue-cultured cells with 35S-methionine, immunoprecipitation of the labeled AChE polypeptides using a monoclonal antibody and analysis by SDS gel electrophoresis under reducing and non-reducing conditions. The expression of the two allelic polypeptides is co-dominant and their assembly into disulfide-linked dimers appears to be random and proportional to the relative abundance of each allele in the culture. When tissue-cultured neurons or myotubes are prepared from single quail embryos all molecular forms of AChE isolated from a given embryo exhibit the same allelic subunit composition. Furthermore, both the cell associated and secretory forms of AChE from neurons and muscle show the same allelic composition. Together, these studies indicate that the two allelic forms of the AChE catalytic subunit are equivalent with regards to assembly into multimeric forms and subcellular distribution. The observation that all AChE oligomeric forms from neurons or muscle cells, whether membrane-bound, secreted, or intracellular, isolated from individual embryos share the same subunit composition strongly suggests that they all arise from a single gene. More definitive evidence based upon ongoing mating studies will be presented. This research was supported by grants from the NIH, Muscular Dystrophy Association, and the Sloan Foundation to RLR.

385.8 THE TWO ALTERNATIVELY SPLICED FORMS OF THE 1B236/MAG GENE TRANSCRIPTS ARE DEVELOPMENTALLY REGULATED C. Lai*, K.-A. Nave, L.H. Farber, M. Brow*, A.B. Noronha*, R.H. Quarles*, F.E. Bloom, J.G. Sutcliffe, and R.J. Milner. Research Institute of Scripps Clinic, La Jolla, CA 92037 and NINCDS, Bethesda, MD 20014.

The rat brain protein, 1B236, which was isolated and characterized as a brain-specific gene product, has been recently shown to be closely related to and possibly identical to the myelin-associated glycoprotein (MAG). We have chosen the provisional name 1B236/MAG to describe these molecules. We have characterized two full-length cDNA clones of 1B236/MAG specific mRNA that encode distinct 1B236/MAG proteins. One protein is 626 amino acids long, with a single membrane-spanning domain separating a heavily glycosylated N-terminus from a 92 residue C-terminus. The other is identical to the first except that 54 amino acids at the C-terminus are replaced by a different sequence of 10 amino acids. Both forms are encoded by the single 1B236/MAG gene which spans 16kb and contains 13 exons. A comparison of the nucleotide sequences of the two cDNAs with the genomic sequence indicates that the two mRNA forms arise by the alternative inclusion or omission of the 45 base exon 12. Exon 2 is also alternatively spliced but this event is not coordinate with the splicing of exon 12 and has no effect on the amino acid sequence. We present evidence demonstrating that these two mRNAs are developmentally regulated in the brain with one form peaking in expression at the time of most active myelination while the other form increases in expression into early adulthood. Similar measurements on 1B236/MAG expression indicate that the form encoding the shorter protein is predominant in the peripheral nervous system. We also present data showing the aberrant expression of these 1B236/MAG mRNAs in the mutant mouse *quaking*.

The common N-terminus of these proteins is composed of five domains related in sequence to each other and to immunoglobulin-like molecules. This domain organization is reflected in the structure of the gene which reveals that each Ig-like domain is encoded by a separate exon. The 1B236/MAG primary sequence is most homologous to N-CAM and is also related to the PDGF receptor and the poly IG receptor. We propose a model for the structure of these proteins and suggest that they may be involved in cell-cell or other recognition processes through interactions with the N-terminal domains. Supported in part by grants from NIH (NS 20728, GM 32355), NIAAA (AA 06420) and McNeil Pharmaceuticals.

385.9 GANGLIOSIDE "RECEPTORS" ON RAT BRAIN MEMBRANES: DETECTION USING GANGLIOSIDE-DERIVATIZED PROTEIN LIGANDS. M. Tiemeyer*, Y. Yasuda*, and R.L. Schnaar, Departments of Pharmacology and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Gangliosides, which consist of a sialic acid-containing oligosaccharide chain linked to a ceramide lipid moiety, are major glycoconjugates of the neuronal cell surface. Although gangliosides have been proposed as recognition molecules in cell-cell adhesion and transmembrane signalling, clear evidence for complementary ganglioside binding proteins on brain membranes has been lacking. Analysis of direct ganglioside binding to membranes is complicated by their amphipathic nature, leading to non-specific binding and insertion. Therefore, we have undertaken the direct demonstration of ganglioside receptors in brain using synthetic ligands consisting of gangliosides covalently linked to a hydrophilic protein carrier. We refer to these ligands as neogangliproteins (NGP).

Gangliosides GM_1 and GT_{1b} were purified from bovine brain and derivatized to contain a primary amine in their ceramide moiety. The resulting glycolipid products, which contained the proper sialic acid/amine ratio, were covalently linked to bovine serum albumin (BSA) using a disuccinimidyl crosslinking reagent. The resulting conjugates contained 3-10 gangliosides per protein molecule. Upon SDS-PAGE analysis the derivatized protein migrated differently than underivatized BSA or ganglioside, and a ganglioside-specific toxin probe demonstrated the association of ganglioside with protein. The resulting GM_1 and GT_{1b} neoganglioproteins were readily radioiodinated, generating the desired high-specific activity ganglioside ligands.

Binding of ^{125}I-NGP's to rat brain membranes was determined utilizing a detergent-washed P2 fraction. High affinity saturable binding of both GM_1- and GT_{1b}-NGP was readily apparent. Binding was inhibited >90% in the presence of excess unlabeled ligand. The apparent K_D was 30 nM for GM_1-NGP and 1 nM for GT_{1b}-NGP. Mild trypsin treatment of the membranes eliminated NGP binding (tested with GM_1-NGP). Binding of GT_{1b}-NGP to membranes was inhibited half-maximally by 230 nM native GT_{1b}. Other gangliosides (GM_1 and GM_2) were 9- to 20-fold less potent inhibitors than GT_{1b}. No other lipid tested (phospholipids, neutral glycosphingolipid, or sulfatide) was as potent as GT_{1b} in inhibiting GT_{1b}-NGP binding to brain membranes. Oligosaccharide derived from GT_{1b} (10 µM) inhibited binding of both GT_{1b}- and GM_1-NGP to membranes more potently than did oligosaccharide derived from GM_1. These data suggest that the new ganglioside derivatives described may be fruitful tools for detecting and defining brain ganglioside receptors. Supported by NIH grants HD14010 and MH18030.

385.10 A CELL SURFACE EPITOPE WHICH IS PRESENT ON A SOME BUT NOT ALL MOTOR NEURONS. D.T. Stephenson*, P.A. St. John, J.L. Barker, and P.D. Kushner. (SPON: L.C. Fritz) ALS Research Center, Pacific Presbyterian Medical Center, San Francisco, CA 94115 and Lab. of Neurophys., NINCDS-NIH, Bethesda, MD 20892.

Motor neurons fall into different categories based on location, morphology, electrical properties, and synaptic connectivity. The issue this study addresses is whether there are categories of motor neurons based on cell surface determinants. The monoclonal antibody, Tor 23, was made to Torpedo cholinergic synaptosomes (JNC 43 775) and, in Torpedo, binds the external synaptosome membrane (PNAS 80 7342), where it recognizes two polypeptide antigens, one of which is a presynaptic form of AChE (JNC, in press). Tor 23 has a broad species cross-reactivity, binding motor neuronal elements in rodents and humans (Muscle & Nerve, in press). In the rat, Tor 23 does not appear to recognize AChE but does cytolocalize to the perimeter of the motorneurons of layers VIII and IX of the spinal cord. In addition, in each 10 µm section, one to twelve neurons of the intermediate grey, laminae III through VI, are stained. These rare neurons are typically small in size (averaging 10µm x 18µm) and fusiform in morphology. A FACS analysis provided verification that Tor 23 labels the surface of somata. Cells were dissociated from the spinal cord of embryonic rats, E15, incubated with Tor 23 and subsequently with a fluorescent second antibody. The FACS analysis of the spinal cord cell suspension indicated that 10-15% of the total viable cells gave a positive immunofluorescent signal for Tor 23, although whether the labeled cells are motor neurons has yet to be directly addressed.

Does this antibody bind all motor neurons? First, does Tor 23 bind only motor neurons? In mapping Tor 23 in the rat brain, we found that Tor 23 binding is not restricted to motor neurons but is present on rare and select neurons in several other brain areas (Neurosci. Abs XII 247.9). An examination of the brainstem has revealed that certain of the motor nuclei are positive, others are negative. Neurons of the cranial nerve nuclei, V, VII, VIII, and ambiguus, are positive. The other cranial nuclei are negative.

In summary, in the spinal cord, the major cell type identified by Tor 23 is the ventral horn motor neuron. By FACS analysis, antibody binding is to the neuronal cell body surface. And, although motor neurons represent a single class of CNS neurons, we have identified a subclass on the basis of the cell surface epitope defined by Tor 23. Because Tor 23 was made to a structure derived from the branchial arches (the electric organ), the particular property we are identifying may be related either functionally or in phylogeny and ontogeny to the branchial arch system. By this analysis, Tor 23 may be helpful 1) in defining the cellular lineages of motor neurons and their different functional categories and 2) in preparing pure motor neuron populations.

385.11 LOCALIZATION OF N-CAM mRNA AND PROTEIN IN THE DEVELOPING MOUSE CNS. D. Goldowitz, D. Barthels and W. Wille. Dept. of Anatomy, Thomas Jefferson Univ., Phila., PA 19107, and Inst. of Genetics, Univ. Koln, Koln, FRG.

Cellular recognition and adhesion must be important phenomena in forming a structure as complex as the mammalian CNS. The well characterized neural cell adhesion molecule (N-CAM) has been proposed to be involved in developmental events such as neural induction, neural crest migration, and axon fasciculation. The present study uses N-CAM in situ hybridization and immunocytochemistry of the developing normal and genetically mutant murine cerebellum and dentate gyrus to further investigate the possible role of N-CAM expression during CNS formation.

N-CAM mRNA and gene product were detected by in situ hybridization (a 600bp probe, Barthels et al., EMBO. J., in press, labeled with biotin or tritium) and immunocytochemistry (the antibody a gift of C. Goridis), respectively. These probes do not distinguish among the various forms of N-CAM. Mice of varying ages were deeply anesthetized and transcardially perfused with a solution of 3pt 95% ethanol:1pt acetic acid. Brains were removed, dehydrated, embedded in paraffin, cut and stained.

Immunocytochemical staining for N-CAM activity in the developing cerebellum was, as reported by others, densest in the developing molecular area and tracts of white matter. The most prominent N-CAM immunoreactivity in the developing dentate gyrus coincided with the appearance of the granule cells' axonal extensions, the mossy fibers. The trilaminar synaptic region of the granule cell molecular layer was highlighted by N-CAM immmunoreactivity by 10 days postnatal.

In situ hybridization allowed us to examine the cellular site of N-CAM production. As suggested by the immunocytochemistry, N-CAM mRNA is detected at high levels in almost all neuronal populations. However, two exceptions relevant to this study are: 1) migrating cerebellar granule cells, which are also extending neurites, have low-to-nil expression of hybridizable N-CAM mRNA, 2) recently generated dentate granule cells, which are presumably starting to elaborate axons, have low-to-nil hybridizable N-CAM mRNA. Examination of N-CAM expression in the cerebellum of mutant mice provides correlative information: a migrational defect, as in the reeler mouse, has no obvious effect on N-CAM expression while disruption of the granule cell-to-Purkinje cell contact, as in the lurcher mouse, is accompanied by marked decrease in N-CAM hybridization product in the granule cell but not in the Purkinje cell. These findings are suggestive that higher levels of N-CAM expression seem to be correlated with the later stages of axonal growth such as fasciculation and/or synaptogenesis rather than initial axon outgrowth.

385.12 NERVOUS SYSTEM SPECIFIC ANTIBODIES IN FROG. T. Nagy and T.A. Reh. Dept. Med. Physiol., Univ. Calgary, Calgary, Alta. T2N 1N4.

Monoclonal antibodies to neural and glial cell surface molecules have proven to be invaluable in the characterization and study of nervous system tissue cultures. However, most of these probes have been developed for use in chicks and mammals. Since the frog has long been used as an experimental animal for the study of neural development, we have been raising monoclonal antibodies directed against similar cell surface antigens in this species.

Midlarval staged **Rana pipiens** or **Rana catesbienna** were anaesthetized and the retinas and brains homogenized in 0.32M sucrose. A membrane fraction was prepared by differential centrifugation and used to immunize Balb/c mice (1 mg I.P.) at 2 to 3 week intervals (4 injections total). Five days after the final boost, their spleen cells were fused with SP2 myeloma cells by a standard protocol and the resulting hybridomas were screened by ELISA and indirect immunofluorescence on cryostat sections of tadpole retina. Positive wells were cloned by limiting dilution.

Of the nervous system specific antibodies that were produced, three were characterized more fully and are the subject of this report. One neuron specific antibody, 2D3, binds to the entire differentiated retina, with the exception of the photoreceptor outer segments, as well as to the zone of germinal neuroepithelial cells located at the peripheral margin of the larval retina. In dissociated retinal cell culture, the antigen reognized by 2D3 is present only on neurons, and on all neurons and their processes. The antigen is present throughout the larval CNS and peripheral nervous system, but is not present on any other tissue at all stages examined. This antibody also binds to all germinal neuroepithelial cells in **Rana** embryos and tadpoles, but is not present in **Xenopus laevis** or **Bombina orientalis**. Immunoblot analysis indicates that the 2D3 antibody recognizes a 180 - 200 kd sialated glycoprotein, similar to N-CAM, that is specific to **Ranids**.

The second neuron-specific antibody we characterized (2C6) does not bind to germinal neuroepithelial cells; this antigen is only present on cell surfaces of differentiated neurons. In dissociated cell cultures, all neurons, and only neurons express the antigen. While the 2C6 antigen is present throughout the CNS, it is expressed in very high levels in peripheral nerves. Initial characterization of this antigen indicates that it is a cell surface glycolipid and probably a frog specific ganglioside. We also characterized a glial-specific antibody (4D5). This cell surface antigen is present on Mueller cells in the retina and the radial glial of the CNS. In addition, the antibody binds to all germinal neuroepithelial cells in the larval CNS and in dissociated retinal cell cultures it only binds to a population of flat, phase dark cells. Immunoblot analysis indicates the antigen is a protein with two subunits (200, 66 kd). These antibodies have already proven useful in identifying various nervous system cell types in dissociated cell cultures and may also be good markers for the study of neural induction in early embryos.
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386.1 ROLE OF CALCIUM AND SODIUM IONS ON PHOTOTRANSDUCTION IN CRAYFISH. B. Fuentes-Pardo and J. Hernández-Falcón*. Depto. Fisiología, Fac. Medicina, UNAM. Apdo. Postal 70250, México, D.F., MEXICO.

Light stimulation of the retinular cells in the crayfish induces a depolarization, i.e., the receptor potential (RP), which consists of an initial fast and transient phase dependent upon the stimulus intensity, and of a slow, stable phase associated to the stimulus duration. It has been stablished that in some animal species the transient phase is a function of Na^+ entrance to the cell, whereas the stable phase depends on the free Ca^{++} in the cytosol. However, in the crayfish it has not been yet possible to establish clearly the role played by these ions in the phototransduction process. The aim of this paper is to establish the role of Ca^{++} and Na^+ in the RP generation of the retinular cells in the crayfish. We made intracellular recordings of retinular cells from eyestalks placed first in darkness, then under dim light and then again under darkness immersed in Van Harreveld's saline solution normal or modified in its Ca^{++} or Na^+ concentration or in both simultaneously. The RP was evoked by light flashes of 1800 lux intensity and of 15 us duration, applied every 2 min during 70 min. In each response, the amplitude of the fast phase and of the slow phase, the depolarization and repolarization velocities, as well as the response duration were measured. In the normal solution , we found two types of behaviors in the photoreceptor response; in the first type, the RP maintained a tendency to increase in all the variables studied, and little decay during illumination accompained by a fast recovery during the dark adaptation. In the second type we found a tendency to decrease of all the variables, a great decay during illumination and a low recovery level during the last darkness stage. With the modified saline solutions, the general characteristics of both types of responses remained practically unchanged, altough slight variations were observed. Thus, in the low Ca^{++} solution, there was an increment in the stable phase amplitude and in the response duration, but a diminution in the depolarization and repolarization velocities. In the solution with low Ca^{++} and low Na^+ content, all the parameters decreased; we even found disappeareance of the stable phase. These changes were reversed when the preparation was returned to the normal saline solution. These results suggest that the two RP phases depend inversely on the extracellular Ca^{++} levels; a reduction in Ca^{++} induces both a reduction in the amplitude of the transient phase and in the depolarization velocity and an increment in the amplitude of the stable phase, which in turn, lenght the response duration. The increase of extracellular Ca^{++} seems to determine the Na^+ entrance to the cell, which produces an increment in the transient phase and a halt in the stable phase generating mechanism. This latter effect, in turn, causes a reduction in the response duration.

386.2 VOLTAGE-DEPENDENCE OF LIGHT-INDUCED CURRENTS IN TYPE B PHOTORECEPTOR SOMATA OF HERMISSENDA. C. Chen* & D.L. Alkon. Laboratory of Cellular and Molecular Neurobiology, NIH-NINCDS, Rockville, MD 20852

Previous voltage-clamp studies of the nudibranch mollusc Hermissenda Type B photoreceptor indicated that light elicits an inward Na^+ current, an outward K^+ current, and a slow reduction of a steady-state K^+ current (Alkon & Sakakibara, Biophys. J., 48: 983-995, 1985). Here we use brief (50ms) light flashes of various intensities (max. 1.8×10^3 Joules/M^2.sec) to more thoroughly characterize the current-voltage relation of these currents with a minimum of light adaption. Two-microelectrode voltage clamp was performed on the Type B photoreceptor somata. Membrane potential was changed gradually from -90mV to +50mV to minimize activation of voltage-dependent conductances. The I-V plot of the peaks of the overall light-induced current, which was clearly voltage-dependent, showed a maximum inward current at -50mV, a negative slope below -50mV and a positive slope above -50mV. Surprisingly, the current-voltage relation of the light-induced current in Hermissenda was similar to the transient Na^+ current of squid axon (Cole & Moore, J. Gen. Physiol., 44: 123-167, 1960) and the early inward dark current of Limulus ventral photoreceptor (Lisman et al., J. Gen. Physiol. 79:187-209, 1982), while it was quite different from the light-induced current-voltage relation of Limulus (Millecchia & Mauro, J. Gen. Physiol. 54:331-351, 1969). Three components underlying the light-induced current were obtained by isochronal measurements and characterized according to their kinetics and ionic selectivity. A fast inward Na^+ component, mainly responsible for the light-induced current amplitude and voltage-dependence, reversed at +20mV and reached its peak about 500ms after the onset of the light flash. Another fast component of the Hermissenda light-induced current reached its peak at 1 second, as an outward current from -60mV to -30mV. Below -60mV or above -30mV either the inward Na^+ current or a late inward component obscured the fast outward component. The fast outward component was completely blocked by 10mM Ba^{2+}-ASW and in previous studies was unaffected by removal of external Na^+ but varied with external K^+. A delayed voltage-dependent inward component reached its peak 6 seconds after the onset of the light stimulus and was reduced by external Ba^{2+}. This current is caused by the Ca^{2+}-dependent inactivation of steady-state K^+ conductances (Alkon & Sakakibara 1985). The delayed component began to appear at -70mV, nonlinearly increased from -70mV to +20mV until it reached its maximum value and then gradually decreased above 20mV. At fixed membrane potentials, the overall light-induced current increased exponentially with light intensity (log units).

386.3 REGIONAL DISTRIBUTION OF cGMP-ACTIVATED CHANNELS IN THE PLASMA MEMBRANE OF THE ROD PHOTORECEPTOR. Shu-Ichi Watanabe* and Gary Matthews, Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

Ion channels are not distributed equally throughout the rod photoreceptor of the vertebrate retina: the light-receptive outer segment seems to contain only the cGMP-activated, light-sensitive conductance (1, 2, 3), while the inner segment contains a variety of other conductances (4). Thus, there appears to be a mechanism that excludes inner segment ion channels from the outer segment. To determine if this sorting process also excludes light-sensitive, cGMP-activated channels (g_{cG}) from the inner segment, we looked for cGMP-activated channels in inside-out patches obtained from inner segments of rods mechanically dissociated from toad retina.

Of 49 inner-segment patches, 39% had no detectable channel activity of any sort. Such silent patches may have been vesicles, and thus only patches showing some sort of channel activity (N=30) were examined. Of the active inner-segment patches, 57% showed a conductance increase when cGMP was applied to the intracellular membrane face. In parallel experiments on outer-segment patches from the same preparations, 100% of inside-out patches responded to cGMP. The maximal g_{cG} elicited by a saturating dose of cGMP was smaller in inner- than in outer-segment patches. In 30 inner-segment patches, maximal g_{cG} averaged 51 ± 32 pS (mean ± s.e.m.), while in 66 outer-segment patches the average was 676 ± 112 pS. The single-channel conductance was similar in inner- and outer-segment patches. Thus, although the cGMP-activated conductance was present in the plasma membrane of both inner and outer segments, channel density was considerably lower in the inner segment. It is unclear whether g_{cG} in the inner segment has a function or simply represents imperfect operation of the channel segregation mechanism.

The amplitude of maximal g_{cG} varied widely across patches in both outer- and inner-segment recordings (0 to 959 pS for inner segments and 15 to 4300 pS for outer segments), suggesting local variation in channel density in both inner and outer segments. Within the inner segment, there was no indication that channel density was higher nearer to the cilium connecting the inner and outer segments.

Inner-segment patches often contained channels other than g_{cG}. We have carefully examined only calcium-activated K-channels, which were present in 47% of inner-segment patches. These K-channels were also found in 2/23 outer-segment patches, which was surprising given the results of refs. 1-3. It seems likely that these K-channels in the outer segment do not open under physiological conditions in the intact cell. (Supported by NIH grant EY03821.)

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386.4 LIGHT-STIMULATED PHOSPHORYLATION OF PROTEINS IN THE LIMULUS VENTRAL AND LATERAL EYES. S. C. Edwards, A. C. Wishart* and B-A. Battelle. C. V. Whitney Laboratory and Dept. of Neuroscience, University of Florida, St. Augustine, FL 32086.

We have been investigating light-stimulated protein phosphorylation in the lateral and ventral eyes of the horseshoe crab, Limulus polyphemus. We have demonstrated that light stimulates the phosphorylation of a 46 kD protein in the lateral eye and a photoreceptor cell body-rich fraction of the ventral eye (P-fraction) and a 122 kD protein in the ventral eye P-fraction. Now we describe the dynamics of the phosphorylation and dephosphorylation of these proteins.

Light-stimulated phosphorylation of the 46 kD protein is rapid. Intact ventral eye P-fractions and slices of the lateral eye were dark adapted overnight and incubated in the dark with $^{32}PO_4$ for an additional 1.5 hrs. When these tissues were exposed to a 1 msec burst of bright, white light, the 46 kD protein was phosphorylated within 15 sec of the flash. The 46 kD also became phosphorylated in lateral and ventral eye preparations maintained in constant light. In those experiments, the tissues were maintained in room light for at least 20 min prior to the addition of labelled phosphate. These latter results may indicate that in constant light the 46 kD protein is in a steady state between phosphorylated and nonphosphorylated forms.

The rate of dephosphorylation of the 46 kD protein differs depending on the conditions of its phosphorylation. When the 46 kD protein was phosphorylated by exposing dark-adapted tissues to a bright flash of light, the protein remained phosphorylated for at least 30 min after the flash. However, when light-adapted cells were transferred to the dark, the protein became dephosphorylated within 5-10 min. The reasons for these differences in the rate of dephosphorylation are not yet clear.

The identity of the 46 kD protein is presently unknown. It corresponds to a minor silver-stained band on SDS-PAGE in both the lateral eye and ventral eye P-fraction. It appears to be membrane bound since it is enriched in the 130,000 x g pellet.

The rate of phosphorylation of the 122 kD protein is slow compared to that of the 46 kD protein. Its phosphorylation in response to a 1 msec-1 sec burst of light required 1 to 3 min. The time course of its dephosphorylation is not yet clear. We presently believe that this light-stimulated 122 kD phosphoprotein is the same as that phosphorylated in response to the efferent neurotransmotter, octopamine. Thus this protein may be modulated in two ways - by light and by efferent innervation. (Supported by NEI Grant EY05724, NSF BNS 8607660, the Whitehall Foundation, and the Whitney Laboratory.)

386.5 EFFECTS OF FLUORIDE AND GUANOSINE 5'-O-[2-THIODIPHOSPHATE] ON THE EXCITATION OF LIMULUS PHOTORECEPTORS: EVIDENCE THAT R* ACTIVATION OF G-PROTEIN IS A LOW GAIN STEP IN INVERTEBRATE PHOTOTRANSDUCTION A. Kirkwood*, D.Weiner*, J. Lisman. Dept. Biol. Brandeis Univ. Waltham MA 02254.

The involvement of a G-protein in invertebrate phototransduction is supported by previous observations that G-protein activators, such as F^-, can induce quantum bumps in the dark, and that the G-protein blocker, Guanosine 5'-O-[2-thiodiphosphate] (GDP-ß-S), reduces the light response (Corson & Fein, 1983 J. Gen. Physiol 82, 639; Fein, 1986 Science 232, 1543). The bumps induced by F^- are only slightly smaller than the ones induced by light, indicating that F^- affects a very early step of phototransduction, before much of the gain occurs. This raises the possibility that F^- may act at the pigment level, possibly affecting metarhodopsin phosphorylation, the only known biochemical modification of the pigment. However, the results of our experiments in the Limulus median UV photoreceptor do not support this view. We found that the rate of F^- induced bumps is not dependent on the relative amount of rhodopsin and metarhodopsin. Thus, it is likely that F^- acts at a step subsequent to the pigment, probably at the G-protein level. To test this more directly, we injected the G-protein blocker, GDP-ß-S, into a ventral photoreceptor previously treated with F^-. The injection completely suppressed the F^- induced bumps. Since GDP-ß-S is known to block F^- activation of G-protein in the adenylate cyclase system (Eckstein et al., 1979 J. Biol. Chem. 254, 9829) our results strongly suggest that F^- bumps are produced by activation of G-protein. If a F^- activated G-protein induces a bump almost as large as a light induced bump, then it is likely that very few G-protein molecules are activated in the generation of a light induced bump. We further tested this hypothesis by studying the effect of G-protein blockage by GDP-ß-S on the light response. We found that GDP-ß-S injection caused a severe reduction in light sensitivity which was not due to a reduction in quantum bump size, but rather to a reduction in quantum efficiency (the probability that a photon evokes a quantum bump). These results can easily be accounted for with a model in which a single photoexcited rhodopsin normally activates only a few G-proteins. Thus, unlike vertebrates, where it is believed R* activates hundreds of G-proteins molecules, in invertebrates the gain of the first step of phototransduction may be close to one.

386.6 EFFECTS OF PROTEIN KINASE C ACTIVATORS ON PROTEIN PHOSPHORYLATION AND LIGHT-REGULATED CURRENT IN FROG ROD PHOTORECEPTORS. B.M. Binder, G.D. Nicol and M.D. Bownds. Lab. of Molecular Biology and Neurosciences Training Program, Univ. Wisc., Madison WI 53706.

The role of protein kinase C (PKC) activation in rod outer segments that retain their inner segment (OS-IS) was examined by correlating electrophysiological and biochemical measurements. Application of PKC activators, 1-oleoyl-2-acetyl glycerol (OAG, 100 uM) or dioctanoylglycerol (diC8, 10 uM), resulted in a 20% decrease in the light-regulated current after 2 min and a 50% decrease after 20 min. With 10 uM OAG a 20% decrease in the light-regulated current was observed after 20 min. Intensity-response curves showed no change in the light intensity required to half-suppress the maximal photoresponse, indicating no change in the rod's sensitivity to light.

To correlate the decrease in light-regulated current with phosphorylation of specific proteins by PKC, [γ-^{32}P]-ATP was added to suspensions of purified, electropermeabilized OS-IS. OAG enhanced, in a concentration- and time-dependent manner, the phosphorylation of a 55 kD protein and three proteins with molecular weights between 14 and 24 kD. Maximum phosphorylation of these proteins occured within 5 min after OAG addition. In some experiments, illumination caused enhanced phosphorylation of these proteins. These observations suggest that activators of PKC may lead to a reduced photoresponse through phosphorylation of proteins that influence channel closure or proteins that influence ion concentration gradients in the rod. This work was supported by NIH Grant EY-00463.

386.7 **PURKINJE SHIFT IN PHOTORECEPTORS: INTRACELLULAR RESPONSES TO CHANGES OF THE ROD AND CONE DOMINANCE.** Samuel M. Wu and Xiong-Li Yang.* Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030.

Photoreceptors were recorded under visual control with intracellular microelectrodes in flat-mounted isolated retinas of the larval tiger salamander (Ambystoma tigrinum). About 10-20% of the rods (named rod_cs), identified by their morphology while recording, exhibited a mixed response waveform of the rod and the cone under dark-adapted condition: it repolarized immediately after the termination of the light step (like the cone) and it gave a long voltage tail afterwards (like the rod). In the presence of background illumination, rod_cs exhibited a response that closely resembled the waveform of the cone response, but with a smaller amplitude. Under dark-adapted conditions, the maximum spectral sensitivity of the rod_c was approximately 520 nm, which was very close to that of the rods. In the presence of background illumination, rod_c exhibited a spectral sensitivity that resembled that of the cone with a peak around 620 nm. This result demonstrates that the intracellular responses of the dark-adapted rod_c can exhibit Purkinje shift in response to steady background illumination. The strength of electrical coupling between the cones and the rods (or rod_cs) were measured by passing current pulses into the cone while recording the voltage response from the rod (or rod_c). To -1 nA current into an adjacent cone, the voltage response recorded from the rod_cs (9.4 $\pm$ 2.8 mV) is 3.4 times larger than that recorded from the rods (2.3 $\pm$ 1.7 mV). It is likely that the strong electrical synapses between the rod_cs and the cones mediate the mixture of the rod and cone responses in rod_c under dark-adapted conditions. These synapses also enable rod_cs to exhibit a cone response in the presence of background illumination when the rod response is suppressed.

This work is supported by grants from the National Institute of Health (EY04446) and from the Retina Research Foundaion (Houston).

386.8 GEOMETRICAL CONSIDERATIONS PREDICT THE RESPONSE OF BOTH VERTEBRATE RODS AND CONES FOLLOWING A SINGLE ISOMERIZATION. J.P. Raynauld*and S. Gagné* (SPON: F. Lepore). Centre de recherches en sciences neurologiques. Université de Montréal, Montréal, Québec, Canada. H3C 3J7.

An analysis of the fact that the disks of the cones are infoldings of the plasma membrane while the disks of the rods are isolated from the plasma membrane helps to predict the single quantum response of both rods and cones. For the cone, three dimentional analysis of the infolding of the plasma membrane leads to the conclusion that the interactions of an internal messenger are limited to the disk which captured the photon. Assuming that the action of the internal messenger is optimum in the sense that it closes all the open channels located on the disk, the percentage reduction in dark current should be equal to 1/n X 100, where n is the number of disks in the outer segment. For the turtle cone outer segment which contains 800 disks the current reduction should be 0.13%, experimentally the measured value is 0.16% (Baylor D.A. et al. J. Physiol. 242, 685, 1974). For the rods, the area of plasma membrane which contains the closed channels at the peak of the response following a single isomerization is equal to the area of one disk as if the effects of the internal messenger were exactly translocated from the disk to the plasma membrane. The occlusion length along the outer segment can be calculated to be equal to d/2 and the percent reduction in dark curent equal to (d/21) X 100, where d and l are respectively the diameter and the length of the rod outer segment. This formula predicts respectively the values of 6% and 4% for the reduction of dark current in the Bufo and monkey rods following a single isomerization. Theses values are in very good agreement with experimentally obtained values of 5% and 3-5% (Baylor D.A. et al. J. Physiol. 288, 613, 1979 and Baylor D.A. et al. J. Physiol. 3517, 575, 1984).

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386.9 A MODEL FOR ADAPTATION IN VERTEBRATE RODS. K. N. Leibovic, Y. Y. Kim* and Z. H. Pan*. Dept. of Biophysics, SUNYAB, Buffalo, NY 14214.

It is now generally accepted that cGMP controls membrane conductance in photoreceptors and the transduction biochemistry is largely understood. There are remaining questions, especially concerning the response turn off; and the molecular basis of adaptation is unknown. Electrophysiology can provide guidance in this situation. Photoreceptor adaptation is driven by background light I_B and pigment bleaching B. We have shown by recording in Bufo rods that there is an equivalence $I_B \sim B$ with respect to the stationary parameters of threshold elevation and response compression. But this equivalence does not extend to the transient parameters, such as stimulus summation and recovery of sensitivity. To reconcile these and other data with the known biochemistry we propose that: (1) response compression is controlled by the steady state level of cGMP; (2) threshold elevation is controlled by the amounts of activated rhodopsin R^* and activated PDE^* entering the transduction cycle; (3) the processes (1) and (2) are coupled and this is the basis for the $I_B \sim B$ equivalence; (4) arrestin and localized Ca^{++} activity mediate (2) and (1) respectively; (5) response acceleration in the presence of I_B and the nonequivalence of the transient parameters argues for different mechanisms brought into play by I_B and B and the control they exert on R^*, PDE^* and cGMP via arrestin and Ca^{++}. We have modeled photoreceptor adaptation by computer simulation based on the above proposals.

386.10 CIRCADIAN CHANGES IN LIMULUS VENTRAL PHOTORECEPTOR RESPONSE. L. Kass*, Dept. of Zoology, Univ. Maine, Orono, ME, and G.H. Renninger, Biophysics Group, Univ. Guelph, Guelph, ON.

Photoreceptor cells in Limulus polyphemus receive efferent input from a circadian clock in the brain (1). Circadian rhythms have previously been observed in recordings from the lateral eyes and median ocelli (2). Here, we report that ventral photoreceptors also exhibit circadian rhythms in their responses to flashes of light.

The horseshoe crab was inverted, restrained, and maintained in darkness for several days and nights. The tip of a metal electrode was placed within the wart structure housing the ventral eye end-organ. Flashes of light (10 ms) directed onto the end-organ elicited ERG-like responses. The flashes were repeated at a rate of 0.35 Hz and the responses averaged at 4 different intensities of illumination. The average responses were larger at night than during the day for the 2 higher light intensities, but were not significantly different for the lower intensities.

It has been reported that: when the efferent nerve fibers are activated, octopamine is released in the photosensitive membrane region of both lateral and ventral photoreceptors (3); both ventral and lateral eye photoreceptors possess octopamine receptors which, when stimulated, increase intracellular cAMP levels (4); exogenous octopamine, or agents that increase cAMP levels, change fundamental characteristics of photoreceptor function in the lateral eye (5). We have also reported that the same pharmacological agents change ventral photoreceptor function in a similar fashion (6).

We conclude that ventral photoreceptors have circadian rhythms in their response to light which are mediated by biochemical machinery similar to that in lateral eye cells, and that understanding the functioning of ventral photoreceptors will require a delineation of the physiology and biochemistry of efferent neurotransmission in the Limulus visual system.

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CHEMICAL SENSORY SYSTEMS II

387.1 Identification of a Monoclonal Antibody which Reacts with Neuronal Cells in the Rat CNS and Olfactory System Susan L. Warren* and Richard A. Akeson Division of Basic Research, Childrens Hospital Research Foundation, Cincinnati, Ohio,45229

Using the monoclonal antibody(Mab) 6B7, a cell surface component found in adult rat central nervous system membrane preparations and on the surfaces of many neurons in cultures of embryonic rat forebrain has been identified. This Mab was derived from mice immunized with a rat forebrain synaptic plasma membrane preparation. High levels of Mab 6B7 binding are observed with membrane preparations from rat forebrain and olfactory bulb but no detectable binding is observed with membranes from the non-neural adult rat tissues heart, kidney,liver, lung and testes. Binding to dorsal root ganglia preparations was 5 fold lower than to forebrain.In immunofluorescence analyses, Mab 6B7 binds to the surface of a significant proportion of neurons in cultures of embryonic day 14 rat forebrain. However it is absent from GFAP positive astrocytes and fibroblastic cells in rat neural cultures. The distribution of the 6B7 antigen in the olfactory epithelium was characterized in greater detail. In cryostat sections, 6B7 appears to react with a cell population of the basal layer of the adult rat epithelium but is absent from the more mature neuronal population which lies higher in the epithelium. This result suggests that within the olfactory epithelium Mab 6B7 may be useful as a marker for the proliferative basal cells which are the neuronal precursors in the epithelium.In summary the 6B7 antigen may be useful in identifying and analyzing cell subpopulations in both the rat central nervous system and olfactory epithelium. Supported by Grant PO1-NS23348 from the NIH.

387.2 TRANSNASAL TRANSNEURONAL TRANSPORT OF HERPES SIMPLEX VIRUS TYPE I INTO THE RAT BRAIN

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The olfactory epithelium can incorporate macromolecules and transport them into the brain. This phenomenon has been established in the rat with the incorporation of WGA-HRP (Shipley, 1985; Baker and Spenser, 1986) and in the mouse with a virus (Tomlinson and Esiri, 1983). Thus, harmful or potentially beneficial substances may enter the brain via olfactory nerves. The purpose of this study was to determine where in the epithelium a virus is incorporated and whether the virus spreads transneuronally along defined anatomical circuits in the brain.

Live herpes simplex virus type 1 (HSV1)(McIntyre strain) was injected into the nose of anaesthetized adult rats (male, Sprague Dawley). Animals survived three to seven days before perfusion with 4% paraformaldehyde. Cryoprotected sections were cut at 30 um and reacted with an antisera to HSV1. Some additional animals were sacrificed and regions of the brain were cultured for HSV1.

Preliminary results indicate that virus placed in the nasal cavity enters the brain by at least two routes: the trigeminal nerve and the olfactory nerve. Here, we concentrate on olfactory pathways. The results are from animals that were sacrificed 5 days post-inoculation.

Scattered focal regions in the olfactory epithelium were infected with HSV1. The virus was transported to the olfactory bulb and to higher olfactory structures in a retrograde and possibly anterograde direction. In the olfactory bulb, labelled glomeruli and tufted, mitral and granule cells were often concentrated in discrete radial columns. These patterns suggest that the virus passed to successive neurons in local olfactory bulb circuits by a transynaptic mechanism. More distal olfactory-related structures were also labelled including the anterior olfactory nucleus (ipsi- and contralateral), taenia tecta, horizontal limb of diagonal band, nucleus of lateral olfactory tract, piriform cortex, endopiriform nucleus, raphe nucleus and locus coeruleus. These labelled neurons were predominantly ipsilateral to the infected nostral and were so densely stained that individual neurons had a Golgi-like appearance.

These results show that HSV1 can enter the rat brain through olfactory neurons, multiply and spread transneuronally along specific multi-neuronal circuits. Competition studies should be able to determine whether the virus binds to membrane sites similar to those recognized by WGA. We hypothesize that the virus spreads transneuronally in a manner similar to WGA. However, the viral labelling is very much greater than the lectin labelling because the virus multiplies in neurons at each stage of transneuronal transfer.

The nose may thus be an important site of entry for viral infection of the brain. Beyond pathological implications, these findings suggest transneuronal transport of viruses may be useful in delineating multisynaptic neural circuits in the brain. (Supported by BRSG SO7 RR5408-25, PHG AI 23482, NINCDS PO1 23348-01, NS 20643, NS 22053, and DAMD 17-86-C-6005).

387.3 ALL PARTS OF THE OLFACTORY EPITHELIUM RE-MAP INTO A MEDIAL BULB REMNANT. M.T. Shipley, J.H. McLean and W.T. Nickell, Univ. of Cincinnati College of Med., Cincinnati, OH 45267, B.M. Slotnick, American Univ., Wash., D.C. 20016

A central problem in olfactory research is the nature of the relationship between primary olfactory neurons (PONS) in the olfactory epithelium (OE) and their synaptic targets in the main olfactory bulb (MOB). One view is that the epithelium is a mosaic of intermingled, odor-specific PONS; PONS of similar specificity are thought to project to contiguous sites in MOB. This creates a chemotopic map in the bulb. At the other extreme PONS may be arranged to form chemotopic gradients; or the OE may have differential absorptive gradients so that it functions like a chromatograph; in either of these schemes specific sites in OE are selectively activated by different odorants.

Anatomical tracing studies provide evidence that is compatible with either the mosaic or gradient hypothesis; medial OE maps to medial MOB and lateral OE to lateral MOB. There is also some degree of circumferential and longitudinal organization in the OE-MOB projection but the microorganization of the OE-MOB projection is still poorly understood.

For the mosaic hypothesis, a remnant of the OE sheet would suffice to represent a wide range of odor qualities if specific PONS are randomly distributed. In the chemotopic schemes a large part of the OE sheet must be maintained in order to sort odorants along chemotopic gradients. Integrity of the OE sheet is, thus, a higher organizational priority for the chemotopic or gradient than for the mosaic hypotheses. The following results suggest that the OE-MOB projection strives to maintain the integrity of the OE sheet.

The lateral half and most of the dorsal and ventral parts of MOB were surgically ablated in 4-7 day old rats. Medial MOB and its connections with the medial septal OE were left intact. At 60-90 days of age multiple, large injections of WGA-HRP were made in the bulb remnant to retrogradely label PONS in the OE. Similar bulb loading was done either on the non-operated side or in non-operated litter mates. When these control bulb loadings were extensive, there was dense labelling of contigious PONS throughout the OE. Loading of bulb remnants consisting only of the medial part of the bulb produced a similar pattern of OE labelling. Some parts of the lateral OE were less heavily labeled than controls, but there were no major gaps in the labelling pattern. The labelling of the lateral turbinates in medial bulb remnant cases was in stark contrast to the absence of LT labelling in normal animals with injections restricted to medial MOB.

These observations indicate that the OE strives to re-map its entire representation into a drastically reduced bulb remnant. This implies that integrity of the OE sheet is an important organizing principle in the OE-MOB projection and is consistent with the possibility that chemotopic or chromatographic-like gradients are involved in olfactory processing. This suggests that animals with bulb remnants should be able to detect a normal range of different odors but animals with an OE remnant should be anosmic to some odors.

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387.4 PROLONGED INHIBITION OF CONTRALATERAL AND POTENTIATION OF IPSILATERAL ASSOCIATION INPUTS TO THE OLFACTORY BULB BY STIMULATION OF THE DIAGONAL BAND. W.T.Nickell and M.T. Shipley (University of Cincinnati College of Medicine.)

The main olfactory bulb (MOB) receives afferent inputs from several sources: The two bulbs are connected by a large commissural system. There are also strong centrifugal inputs from ipsilateral cortical and subcortical olfactory structures. We report here that these afferent inputs are potently and selectively modulated by the centrifugal projection from the nucleus of the diagonal band (HDB).

Anaesthetized adult male rats were used. Stimulating electrodes were placed stereotaxically in the ipsilateral HDB, in the rostral wing of the contralateral anterior commissure (ACc), and in the ipsilateral piriform cortex (PCi). Stimulation of the ACc or PCi results in a negative field potential in gcl, which reflects an excitatory post-synaptic potential (epsp) in the granule cells. We previously described the field potential in MOB caused by HDB activation. Stimulation of HDB at 10 Hz for several seconds results in a large potentiation of the HDB field potential. We now report that this same HDB stimulation causes a powerful and long-lasting inhibition of the ACc response. After HDB potentiation, the ACc response is reduced to 15% or less of the control value and does not completely recover for more than 20 seconds. During this period of inhibition, a burst of shocks to ACc results in facilitation of response amplitude toward that of the control response.

In contrast to this potent inhibition of the commissural input, the same HDB stimulation potentiated the response to PCi. This potentiation was similar to the potentiation of the HDB field potential by HDB stimulation; it lasted 1-2 seconds and increased response amplitude and duration by factors of 2-3.

At least two mechanisms could account for the inhibition of ACc by HDB. (i) A postsynaptic increase in conductance of granule cells (postsynaptic inhibition) would shunt excitatory synaptic currents. Alternatively, (ii) presynaptic inhibition of ACc terminals synapsing onto granule cells would also inhibit the ACc response. The differential effect of HDB stimulation on the two inputs to the granule cells, and the ability of the ACc response to facilitate to nearly normal size during the inhibition, suggest that the inhibitory effect does not result from conductance changes in the granule cells; these would be non-specific and not reversible by presynaptic effects such as facilitation.

Exogenous application of acetylcholine in hippocampus causes presynaptic inhibition of both afferent inputs and intrinsic inhibitory interneurons. The HDB projection to the bulb contains a cholinergic component and the inhibition of ACc by HDB reported here may represent an analogous mechanism produced by synaptic release of acetylcholine. The potentiation of PCi may result from the same mechanism as the potentiation of the HDB field potential. (Supported by: NINCDS P01 23348-01, NS 20643-01, NS 22053,and DAMD 17-86-C-6005).

387.5 ELECTROPHYSIOLOGICAL AND ANATOMICAL EVIDENCE REVEAL PROJECTIONS FROM PREFRONTAL NEURONS TO THE MAIN OLFACTORY BULB (MOB) AND MEDIO DORSAL THALAMIC NUCLEUS (MD) IN THE RAT. A. R. Cinelli*, H. Ferreyra-Moyano, E. Barragán* and J.S. de Olmos*. Instituto de Investigación Médica M. y M. Ferreyra. C.C. 389. 5000 Córdoba, Argentina.

Although centrifugal projections from allocortical areas to the MOB have been demonstrated beyond doubt, only very limited evidence is available on projections from the frontal cortex (FC) to the MOB (Brutus et al. Brain Res. 1984; Neafsey et al. Brain Res. 1986). Due to the importance attached to FC in odor processing, we sought to explore this matter further. Under Urethane anesthesia, extracellular unit activity was recorded with stainless steel microelectrodes from agranular insular ventral (AIv), lateral orbital (LO) and ventrolateral orbital (VLO) regions. Twenty three neurons were antidromically invaded in these areas following stimulation of the ipsi (N=17; 74%) and contralateral (N=6; 26%) MOB; antidromic driving was confirmed in 47% of cells by collision. Antidromic latency and conduction velocity were 10±1.2 (X ± SE) and 0.47 ± 0.05 (X ± SE) for VLO neurons projecting to the ipsi lateral MOB. Stimulation of sulcal cortex in the dorsal lip of the rhinal fissure inhibited spontaneous discharges of mitral cells in the MOB. FC projections to the MOB were confirmed by anatomical studies. Unilateral injections of solid fluorescent retrograde tracers True Blue, Fast Blue and Nuclear Yellow were placed within the rostral tip of the MOB and in the MD. The MOB halo never encroached upon the AON and was restricted in most cases to the rostral half of the MOB. Following MOB injections, labelled neurons were found in the infralimbic (IL), VLO and AIv cortical regions, as well as in area 3 of the cingulate cortex (Cg3). The densest neuronal labelling occured in the transition cortical zone between the dorsal part of the AON and VLO. Most labelled neurons were found in the supragranular cortical layers with some of them located towards the transition zone of the infragranular layer. Some cells, in particular those in the VLO-AONd transition zone were double-labelled from MOB and MD injections. Tha majority of labelled cells in the opposite hemisphere were located in the IL cortex. These results suggest that prefrontal centrifugal neurons may provide a feed-back signal to the MOB operative in peripheral gating of afferent olfactory information.

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387.6 OLFACTORY PATHWAY AND LOCUS COERULEUS INTERACTION AT MEDIODORSAL THALAMIC NUCLEUS. Luis Pastor Solano-Flores, Maria Olga Legoratti-Sánchez* and Rosalinda Guevara-Guzmán (SPON: H.U. Aguilar-Baturoni). Departamento de Fisiología, Facultad de Medicina, U.N.A.M. Apdo. Postal 70250, 04510 México, D.F.

The noradrenergic nucleus locus coeruleus (LC) projects fibers practically to the whole brain. It has been shown that LC is involved in a number of functions: sleep, stress, learning, aggression. LC also plays a modulatory role in sensory pathways, thus, the expected responses in neurons' firing rates and evoked activity in the visual and in somatosensory pathways are affected by LC stimulation. We have already shown that olfactory tubercle's neural activity is modulated by LC. Additionally, the inhibitory action of LC in the thalamic ventral nucleus has been shown. In the other hand, it is known that the activity of neurons in the mediodorsal thalamic nucleus is modified by olfactory bulb (OB) stimulation. Our laboratory and other researches have pointed out that the functional significance of LC activity upon sensorial pathways might be the modulation of the importance of the sensory information input to central levels in order to adequate the responses to the organism priorities. In order to search for a LC modulatory influence upon the processing of the olfactory input to central levels, this work was performed. In male Sprague Dawley rats, the firing rate of mediodorsal thalamic neurons was conventionally recorded. Stimulating electrodes were set in the LC and OB. Square pulses 0.2 msec, 0.1-0.8 mA, 15-30 Hz during 0.5-2.0 sec were applied to either of these structures. Generally the firing rates of thalamic units were enhanced notably after LC stimulation, this effect was abolished after an OB stimulation and this last effect was sistematically reversed after another LC stimulation. These results suggest that at thalamic levels, the olfactory information is modulated by LC.

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387.7 RESPONSES TO TASTE STIMULI IN THE PARABRACHIAL PONS OF RATS WITH REVERSIBLE LESIONS OF THE GUSTATORY NEOCORTEX. Patricia M. DiLorenzo* (SPON: A. Yozawitz) Dept. of Psychology, SUNY at Binghamton, Binghamton, N.Y. 13901.

Because previous data have revealed differences in taste responses in the parabrachial nucleus of the pons (PbN) in decerebrate vs. intact rats, it is possible that descending forebrain influences play a functional role in the neural code for taste and/or palatability in this structure. This experiment was designed to study the influence of the gustatory neocortex (GN) on the neural code for gustation in the PbN.

Single unit responses to gustatory stimuli in the PbN were recorded in anesthetized rats before and after injections of procaine HCl (10% in saline, 1 µl) into the GN. Sapid solutions of NaCl (.1M), HCl (.01M), Sucrose (.5M), NaSaccharin (.004M) and Quinine HCl (.01M) were individually bathed over the tongue followed by a 20 sec rinse of distilled water.

Preliminary analysis of results suggest several consistent effects: 1) 17% of the taste-responsive PbN units showed significant reductions in spontaneous firing rate after procaine injection into the GN. This effect was accompanied by an attenuation of the responses to all taste stimuli, 2) 40% of the taste units showed attenuated responses to some or all of the taste stimuli without a reduction of spontaneous rate, 3) 35% of the PbN units showed an enhancement of response rate without changes in spontaneous firing rate. In some units responses were present after procaine injection in the GN that were not present before injection. In other units responses to some tastants were enhanced while the responses to others were attenuated, 4) 26% of the taste-responsive PbN units showed a response to the termination of the stimuli (OFF response) after procaine injection in the GN, but not before. This effect was not associated with changes in spontaneous rate. Recovery was observed for all of the affects noted above.

These results suggest that input from the GN to the PbN may be involved in the fine-tuning of the across-unit pattern of firing to gustatory stimuli. Furthermore the suppression of OFF responses in the PbN by the GN may be important for the modulation of the associative salience of gustatory stimuli by the GN.

387.8 CORRELATIONS BETWEEN MULTI-UNIT SPIKES AND EEG IN RAT OLFACTORY CORTEX. F. H. Eeckman and W.J. Freeman. Dept. of Physiology-Anatomy, U.C. Berkeley, Berkeley CA 94720

We performed simultaneous recordings of multi-unit activity and cortical EEG in the primary olfactory cortex of awake and motivated rats. No stimulation was used. Both the EEG and the spike trains were recorded with chronically implanted 100 micron stainless steel electrodes with sharpened tips. The electrodes were positioned at different depths so as to sample activity from all layers of the cortex.

The EEG trace was filtered at 10 and 300 Hz., and digitized at 1 msec. intervals. The unit signal was filtered at 300 and 3,000 Hz., and sent through a window discriminator. For every value within the window a standard 5 V, 1 msec. pulse was stored concurrently with the EEG.

Data were transferred to disk for off-line processing, using a Perkin-Elmer 3220 computer.

We used the two-dimensional conditional pulse probability table (Freeman, 1975) to find correlations between units and EEG.

Last year we reported data on spike-EEG correlations in the olfactory bulb of awake and motivated rats (Neurosci. poster # 370.5, 1986).

The following differences were found between the data obtained from studies in the olfactory bulb (OB) and data obtained in the present study on the olfactory cortex (OC). In the OB, units were found whose firing frequency correlated well with the dominant frequency of the EEG burst at 70- 80 Hz. In the OC, most unit firing is correlated with the 45- 55 Hz. frequency band. In the OB, the percentage of modulation was relatively high (60-90 % of the spikes); in the OC, this percentage is lower (55-75 %). In the OC, some traces were found (approx. 20%) where the spikes correlated with two frequency bands, that are not harmonics of one another. In these traces the pulses have a similar time-reference to the EEG.

In the OB, spikes lead the EEG maximum by approximately 1/4 cycle phaselead; in the OC, two types of unit traces were found: Group A, whose firing probability is in phase with the EEG maxima, and Group B, whose firing probability lags the EEG maximum by a 1/4 cycle (Freeman, 1968).

These data are explained in terms of a model for burst generation in the olfactory cortex (Freeman, 1975).

Freeman W.J. Relations between unit activity and evoked potentials in prepyriform cortex of cats. J. Neurophys: 31: 337-348, 1968.

Freeman W.J. Mass Action in the Nervous System. Academic Press, New York, 1975.

387.9 NONLINEAR SPATIO-TEMPORAL DYNAMICS OF OLFACTORY EEG IN BEHAVIOR. K. A. Grajski* and W. J. Freeman. Grad. Group in Biophysics, Dept. of Physiology. UC Berkeley. Berkeley, CA 94720.

Extended time series of EEG activity (3 seconds @ 1-2 msec. digitizing intervals) from rabbit and rat olfactory bulb and cortex were classified as basal, bursting or epileptiform. Corresponding simulations were generated by a model for olfactory EEG proposed by Freeman. Results of a qualitative analysis using phase portraits and Poincare sections and a quantitative analysis using power spectra and estimation of dimensionality and positive Lyapunov exponents show that low dimensional (3-4) chaotic attractors are manifested in basal, bursting and epileptiform EEG. The significance of these results is that complex activity generated by systems consisting of large numbers of interacting components, such as neurons in the olfactory system, is not random, rather, it is deterministic.

Spatial EEG was analyzed for correlates of behavior in waking rabbits. Habituation experiments demonstrated short- and long-term odor-specific habituation. An aversive differential conditioning procedure showed that subjects could associatively acquire differential response to odors. In all behavioral studies sniff magnitude and probability of sniff responding served as behavioral variables. Oscillatory bursts of 40-80Hz EEG activity (100 msec) were recorded using eight by eight electrode arrays chronically implanted epidurally on the lateral aspect of the olfactory bulb.

In habituation studies, results were found to be consistent with previous studies (Gray, et. al., 1986) in which infusion of norepinephrine into the bulb potentiated the transient spatial pattern change to novel odor stimulation. In the present study, the transient odor pattern averaged over the first four trials differed from that for the last four in the first twelve-trial session of unreinforced odor presentations. Both odor patterns differed from an essentially invariant air pattern. Across habituation sessions the air - odor pattern differences diminished and were statistically indistinguishable by the third twelve trial session. The odor specificity of habituation was revealed by introducing a single novel odor trial in the fifth habituation session. No odor-specific EEG spatial patterning was observed for either the habituated or novel odors.

In a differential conditioning experiment three subjects were presented with twelve reinforced (mild shock) trials of a CS+ odor for three sessions. Beginning with the fourth session, an additional set of twelve trials of an unreinforced odor CS- was introduced. By the sixth and continuing through the eighth session, subjects showed significant differential responding to the CS+ and not the CS- odor (two-sided paired t-test $\alpha \leq 0.04$). Consistent with previous studies (Grajski, et. al., 1986) with appetitive conditioning in rabbits, odor-specific patterns were seen to emerge, stabilize and persist as correlates of differential conditioning.

387.10 A COMPUTER SIMULATION OF A THREE-DIMENSIONAL MODEL OF PIRIFORM CORTEX WITH FUNCTIONAL IMPLICATIONS FOR STORAGE AND RECOGNITION OF SPATIAL AND TEMPORAL OLFACTORY PATTERNS. M. Wilson* and J. Bower (SPON: E. DeYoe). Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Using parameters obtained from anatomical and physiological experiments (Haberly, Chem. Senses 1: 219, 1986), a computer model of piriform (olfactory) cortex has been developed that simulates physiological responses obtained using a variety of recording methods (Wilson et al., Soc. Neuroscience, 370.11, 1986). This one-dimensional model of 70 neurons has now been extended to a two-dimensional array of several thousand pyramidal cells interconnected by a highly divergent/convergent system of associational fibers. Excitatory input is now delivered to the cells along an afferent pathway containing multiple independent fibers. These fibers are capable of carrying complex spatial/temporal patterns with characteristics similar to the lateral olfactory tract projection to piriform cortex from the olfactory bulb. A more detailed dendritic model is now included which allows for spatial interaction of activity generated along different segments of the pyramidal cell dendrite. As in the original model, the two populations of interneurons included are each responsible for different forms of inhibition: a feedforward, long latency, long duration hyperpolarizing potential, which simulates the K^+ mediated potential observed in piriform cortex, and a feedback, short latency current shunting type inhibition simulating the well known Cl^- mediated process. The simulation incorporates the different propagation velocities of the separate fiber pathways, synaptic delays, refractory periods, time constant estimates for inhibitory and excitatory events, and spatial variations in the distribution of different excitatory and inhibitory influences. The previous model has been shown capable of simulating a variety of known intracellular and extracellular responses including odor-like oscillatory bursts, characteristic shock responses, and epileptiform activity. Simulation results have revealed the presence of propagating waves of activity across the cortex which are driven by afferent input and carried by both afferent and association pathways. The current model has been extended to explore the significance of these simulated cortical dynamics in the processing of olfactory information. Using connections consistent with the known circuitry of piriform cortex, and a learning rule which modifies synaptic strengths as a function of physiological variables the model can successfully store and recall spatially and temporally encoded patterns. Discrimination of such patterns is hypothesized to be the basis for olfactory processing in the cortex. Supported by Caltech Presidents Fund and Joseph Drown Foundation.

387.11 DIFFERENTIAL RESPIRATION DURING TRAINING IS NOT REQUIRED FOR EARLY OLFACTORY LEARNING IN INFANT RATS. Jacqueline T. Do*, Regina M. Sullivan* and Michael Leon, (Sponsor - G. Shaw), Department of Psychobiology, University of California at Irvine, 92717

Exposing neonatal rats to an odor while giving them reinforcing tactile stimulation (mimicking maternal contact) produces a behavioral preference and enhanced olfactory bulb focal 14C-2-deoxyglucose (2DG) uptake to that odor. One possible mechanism underlying the development of this neurobehavioral response is an enhanced activation of the olfactory system during odor training produced by a stimulation-induced increase in respiration. Tactile stimulation increases respiration rate in rat pups (Sullivan et al., Dev. Psychobiol, in press). The present experiments determined whether increased respiration during training is required for early olfactory learning, by pairing the odor with a stimulus that mimics an aspect of the dam-litter environment that does not modify pup respiration.

Neonatal Norway rats were assigned to one of the following treatment conditions from day 1-18 postnatal (PN): 1) ODOR-STROKE, 2) ODOR-HIGH HUMIDITY, 3) ODOR-ONLY, and 4) ODOR-STROKE-HIGH HUMIDITY. Additionally, respiration was monitored during training sessions on PN1, PN6, PN12 and PN18. On day 19, pups were either given a two-odor choice test (peppermint vs familiar pine shavings) or injected with 14C-2DG (200 uCi/kg) and given 45 min test exposure to peppermint. Autoradiographs were then developed and analyzed using standard techniques.

The results showed that, similarly to ODOR-STROKE training, ODOR-HIGH HUMIDITY training is sufficient to produce a learned odor preference and enhanced olfactory bulb glomerular focal 2DG uptake. Additionally, ODOR-STROKE-HIGH HUMIDITY pups, which exhibit enhanced respiration during training, do not exhibit the behavioral preference nor the enhanced 2-DG uptake at testing.

These results suggest that various kinds of moderate sensory stimulation, which mimic different aspects of maternal contact, can function to produce the behavioral and neural response modifications associated with early olfactory learning. Moreover, they indicate that enhanced respiration during training is neither necessary or sufficient for the acquisition of the behavioral preference and enhanced olfactory bulb 2-DG uptake.

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387.12 NORADRENERGIC CONTROL OF NEURAL AND BEHAVIORAL CORRELATES OF EARLY OLFACTORY LEARNING. R.M. Sullivan*, D.A. Wilson, J. Do*, and M. Leon, (Sponsor - J. Leon), Dept. of Psychobiology, University of California at Irvine, 92717.

Norway rat pups learn to prefer odors paired with stimulation that mimicks maternal contact. This learned odor preference is associated with an enhanced olfactory bulb metabolic response (14C 2-deoxyglucose uptake) and modified olfactory bulb single unit response patterns to the odor in an odor-specific region of the bulb. This report examined the role of norepinephrine (NE) in the development of these learned behavioral and neural responses.

The olfactory training procedure lasted for 10 min/day from postnatal day 1 to 18, and consisted of either: 1) peppermint odor and vigorous stroking of the pup's body with a brush (Pepp-Stroked), 2) peppermint odor only, 3) stroking only, 4) neither stimulus. Within each training condition, pups were injected with either isoproterenol (NE agonist), or saline. On day 19, different groups of pups were: 1) given a two odor choice test (peppermint vs. a familiar pine odor), 2) injected with 14C-2-deoxyglucose (200 uCi/kg) and given a 45 min test exposure to peppermint, or 3) tested for mitral cell single unit responses to peppermint.

The results indicated that early odor experience paired with either stroking or isoproterenol produced a learned behavioral preference, enhanced focal 2-DG uptake and modified mitral cell response patterns to that odor. These results suggest that NE is sufficient for the acquisition of learned olfactory neural and behavioral responses early in life.

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387.13 ENHANCED OLFACTORY BULB RESPONSE TO LEARNED ATTRACTIVE ODORS IN THE ABSENCE OF ALTERED RESPIRATORY RESPONSE. M. Kim*, D.A. Wilson, R.M. Sullivan*, and M. Leon, Dept. of Psychobiology, University of California at Irvine, 92717.

Norway rat pups learn to prefer odors paired with stimulation that mimics maternal contact. This learned odor preference is associated with an enhanced olfactory bulb metabolic response (14C 2-deoxyglucose uptake) to the odor in an odor-specific region of the bulb. Several mechanisms have been hypothesized to account for this enhanced focal 2-DG uptake to learned attractive odors: 1) structural or physiological changes within the olfactory bulb itself; 2) altered centrifugal input to the olfactory bulb from the rest of the brain; 3) enhanced input from the olfactory epithelium, for example due to altered respiration in response to the attractive odor. This report examined the role of modified respiration in the expression of the enhanced olfactory bulb response.

The olfactory training procedure consisted of 10 min/day from postnatal day 1 to 18 of either: 1) peppermint odor and vigorous stroking of the pup's body with a brush (Pepp-Stroked), 2) peppermint odor only, 3) stroking only, 4) neither stimulus. Experiment 1 - on day 19, pups were injected with 14C-2-deoxyglucose (200 uCi/kg) and given a 45 min test exposure to peppermint in an apparatus which recorded pup respiration patterns. Experiment 2 - on day 19, pups from all 4 groups were anesthetized with urethane and tracheotomized. Tubing was inserted into the trachea to the back of the nose and intermittent suction applied (200 ml/min, 200 ms duration, 2 Hz). The animals were then injected with 14C-2DG and exposed to peppermint odor for 45 min.

Results: Experiment 1 - Pepp-Stroked pups had enhanced focal 2-DG uptake in the glomerular layer, lateral and 1.5-2.2mm for the rostral pole of the bulb. No differences were found between groups in 1) total number of respirations, 2) respiration frequency distribution, 3) respiration rate during consecutive 12 sec monitoring periods. Experiment 2 - While pups in all groups had an equal rate and volume of inhaled peppermint odor during the artificial respiration, Pepp-Stroked pups had significantly enhanced glomerular layer focal 2-DG uptake compared to control pups.

Together, these results demonstrate that modified respiration is not required for the expression of a modified olfactory bulb response to learned attractive odors early in life.

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387.14 POSTNATAL DEVELOPMENT OF NORADRENERGIC MODULATION OF OLFACTORY BULB EXCITABILITY IN THE RAT. D.A. Wilson and M. Leon, Department of Psychobiology, University of California at Irvine, 92717

Noradrenergic centrifugal inputs to the olfactory bulb terminate mainly on inhibitory interneurons, granule cells. In the mature bulb, norepinephrine suppresses granule cell activity, thus increasing the excitability of the primary output neurons of the bulb (Jahr & Nicoll, Nature, 1982, 297:227). However, since granule cells in the rat develop primarily postnatally, the effectiveness of noradrenergic inputs to the bulb during development is unclear. The present report describes the postnatal development of noradrenergic modulation of olfactory bulb function.

On postnatal day 5 (PN5), PN10 and PN20, Wistar rat pups were anesthetized with urethane. A microelectrode was placed in the granule cell layer of the olfactory bulb to record potentials evoked by stimulation of the lateral olfactory tract (LOT) via a bipolar stimulating electrode. A single conditioning pulse was applied to the LOT followed at 10 - 200 ms by an equal intensity test pulse. The amplitude of the response to the test pulse was depressed at 10ms compared to the conditioning response at all ages (Wilson & Leon, Dev Brain Res, 1987, in press). The effects of systemic injections or intra-bulbar infusions of the noradrenergic B-receptor antagonist dl-propranolol, and the B-receptor agonist isoproteronol, on this paired-pulse depressionn were then determined.

At PN10 and PN20, isoproteronol significantly decreased and propranolol significantly increased the magnitude of paired-pulse depression. No significant effects were seen at PN5, although there was a non-significant trend for isoproteronol to decrease and proranolol to increase inhibition at this age also. The results demonstrate that noradrenergic modulation of GABAergic inhibition in the rat olfactory bulb develops toward the end of the first postnatal week.

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387.15 CARNOSINE AND INSULIN INCREASE OLFACTORY BULB GLYCOGEN SYSTHESIS. Robert Coopersmith* and Michael Leon (Spon: J. Conway). Department of Psychobiology, University of California, Irvine, CA 92717

Using histochemical techniques, we have recently shown that glycogen phosphorylase activity is extremely high in the glomerular layer of the rat olfactory bulb, possibly the highest level in the brain. The olfactory bulb also has the highest brain levels both of insulin receptors and the dipeptide carnosine. While the functions of carnosine and insulin in the bulb are uncertain, both substances can affect glycogen metabolism in other organs. We now report that insulin and carnosine increase the incorporation of glucose into glycogen in the olfactory bulb.

We adapted the in vitro tissue slice technique to the olfactory bulb to examine the effects of bioactive compounds and pharmacological agents on glycogen metabolism in this brain region. Briefly, 250 um bulb slices were incubated with tritiated glucose, followed by the addition of drug and further incubation. Following tissue homogenization and extraction procedures to remove glucose, proteins and amino acids, radioactivity corresponding to glycogen was counted. Without addition of drug, glucose incorporation into glycogen increased steadily for 30 minutes followed by a plateau phase. Insulin (1 mU/ml), added at the beginning of the incubation, increased the rate and total amount of incorporaton, with an almost 2-fold increase by 60 minutes. L-carnosine, added after 30 minutes of incubation, caused a 60% increase in glucose incorporation into glycogen at a 100 nM concentration, with smaller increases at both higher and lower concentrations. D-carnosine at the same concentration did not stimulate glycogen synthesis, causing a slight breakdown of glycogen.

It appears that at least part of the action of carnosine and insulin in the olfactory bulb involve modulation of glycogen metabolism. The localization of insulin receptors in the external plexiform layer and carnosine in the glomerular layer suggests that these systems may participate in separate glucose control mechanisms, possibly responding to different olfactory and centrifugal stimulus conditions. We will also report on the effects of other neuroactive substances on olfactory bulb glycogen metabolism.

387.16 OLFACTORY DEPRIVATION INCREASES [^{3}H] SPIROPERIDOL BINDING IN THE RAT OLFACTORY BULB. K. M. Guthrie, J. M. Pullara*, J. F. Marshall and M. Leon, Department of Psychobiology, University of California, Irvine CA 92717

Chemical or surgical afferent denervation of the rat main olfactory bulb decreases the levels of dopamine (DA) and tyrosine hydroxylase (TH) within an intrinsic population of dopaminergic juxtaglomerular cells. Reinervation is accompanied by a return to normal dopaminergic expression. Similarly, interruption of normal afferent stimulation during development by cauterization of the nares reduces bulb DA and TH.

In order to determine whether the effect of early olfactory deprivation on bulb dopamine expression is accompanied by changes in dopaminergic receptors, neonatal rats underwent unilateral naris closure or a sham procedure on postnatal Day 2. TH immunoreactivity and dopamine receptor binding were examined at Day 60. Quantitative autoradiography was used to determine the density of [^{3}H]spiroperidol binding sites in the bulbs of deprived rats and control littermates. Bulb sections were incubated in 0.7 nM [^{3}H]spiroperidol (77 Ci/mmol) in the presence of 40 nM ketanserin with or without 1 uM (+)-butaclamol. Tissue sections and standards were exposed to tritium sensitive film for 19 days. Autoradiographs were examined for [^{3}H]spiroperidol binding using computerized image analysis.

Early olfactory deprivation reduced TH immunoreactivity in the main olfactory bulb ipsilateral to the occluded naris (N=5). Staining of the contralateral bulb appeared similar to bulbs from control animals.

Deprived animals exhibited a 42% increase in [^{3}H]spiroperidol binding in the glomerular layer of the bulb ipsilateral to the lesion when compared to the contralateral bulb (N=5; $P < .01$). Binding in the non-deprived bulb did not differ significantly from that of control animals (N=5).

These results demonstrate that afferent sensory stimulation affects the olfactory bulb dopaminergic system at both the transmitter and receptor levels. The correspondence between the decrease in TH expression seen in deprived bulbs and the increase in [^{3}H]spiroperidol binding suggests that a reduction in DA results in an increase in glomerular D2 receptor density and/or affinity. Studies are now underway to determine the effects of deprivation on the distribution of D1 receptors and DA uptake sites in the olfactory bulb.

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387.17 OLFACTORY DYSFUNCTION AND ODOR MEMORY IN ALZHEIMER'S DISEASE, HUNTINGTON'S DISEASE AND NORMAL AGING C. Murphy, B.R. Lasker* and D.P. Salmon. UCSD, San Diego State University, San Diego, CA 92182.

Neuroanatomical evidence for damage to olfactory areas in Alzheimer's Disease suggests the possibility of selective olfactory dysfunction in Alzheimer's Disease. Although deficits in olfactory-mediated tasks have been reported by Serby and his colleagues in 1985 and by Doty and his colleagues in 1986, such deficits could be ascribed to cognitive impairment and do not necessarily demonstrate selective olfactory deficits. The present study was designed to investigate the possibility of olfactory dysfunction in Alzheimer's Disease and in Huntington's Disease, a dementia in which there is no known selective olfactory involvement. We first investigated both olfactory threshold and memory for odors and visual stimuli in ten patients who met the NINCDS ARDA criteria and were thus diagnosed as either possible or probable Alzheimer's Disease by two different neurologists. Olfactory thresholds were determined on all subjects using a two-alternative, forced-choice, ascending series with butanol and using a criterion of four correct in a row. Results showed significantly higher olfactory thresholds for patients with Alzheimer's Disease relative to groups of elderly and young controls. Results of the threshold testing on the Huntington's patients will be compared to these results. Recognition memory was explored for three types of stimuli: common odors, faces of American presidents and vice-presidents, and electronic symbols. The Alzheimer's patients' performance was compared to that of young and elderly controls whose performance in the same task (with twice as many trials) has been reported (Cain and Murphy, ISOT, 1986). To control for differences in the subjects' criterion bias, ANOVA was conducted on the subjects' Az scores. Young subjects performed equally well with odors, faces, and symbols. The Alzheimer's patients scored significantly lower than the normal controls on all tasks and showed a greater decrement in odor memory than in visual memory. This impairment was distinguished by its severity from that seen for odor memory in normal elderly persons and for visual memory in Alzheimer's patients. The group of patients with Huntington's Disease were subjected to the same recognition memory testing and their results will be compared with those of the Alzheimer's patients. This study suggests significant impairment in both olfactory threshold and olfactory memory in Alzheimer's Disease. Since other primary sensory systems appear to be spared the early neuroanatomical damage and the sensory dysfunction seen in the olfactory system in Alzheimer's disease, selective olfactory dysfunction may serve as a marker for the disease. We suggest olfactory function be assessed to assist in the diagnosis of Probable Alzheimer's Disease.

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388.1 CYCLIC AMP CHEMORECEPTOR OF PARAMECIUM. J. L. Van Houten, J. Zhang, B. Cote, J. Baez, and J. M. Sasner. Dept. of Zoology, Univ. of Vermont, Burlington, Vt 05405

Cyclic AMP is an external attractant for Paramecium tetraurelia. Cells bind ^{3}H-cAMP specifically and saturably (Smith et al., Biochim. Biophys. Acta. in press, 1987) and cyclic AMP induces a hyperpolarization that is characteristic of attractant stimuli. 8-N_3-cAMP in ultraviolet light covalently binds to neighboring proteins and thereby affords a way to specifically label receptors and block chemoreceptor function. Cells treated with 8-N_3-cAMP in ultraviolet light lose attraction to cAMP but not to other attractant stimuli; no comparable loss occurs with photolysis of the parent compound cAMP. Therefore, a surface cAMP binding site, a chemoreceptor, appears to be involved in cAMP chemoresponse.

The cAMP chemoreceptor should be among the membrane proteins. The cell body membrane proteins of Paramecium include several cAMP binding proteins, but one prominent protein specifically elutes from cAMP-agarose affinity columns: A protein of 48,000 daltons (determined by SDS polyacrylamide electrophoresis) elutes from the column with cAMP or 5'AMP, but not with cGMP or 5'GMP. This correlates with the chemoresponse behavior, which is inhibited by 5'AMP, but by neither of the guanylate nucleotides. Binding of cAMP to whole cells likewise is inhibited by 5'AMP (Smith et al., 1987). This 48,000 d protein appears to be heterogeneous: KOAc, an unrealted attractant, does not elute any proteins from the column following the cAMP elution; however, KOAc does elute a 48,000 d protein when it precedes the cAMP elution, which then elutes off even more 48,000 d protein. A mutant, that is defective in its response to cAMP and does not bind cANP, does not appear to have this 48,000 d binding activity as defined by affinity chromatography.

Two dimensional electrophoresis, Affigel blue chromatography, ^{32}P-8-N_3-cAMP labeling, N terminal sequencing, and production of polyclonal antibodies comprise our current approaches to characterize this putative chemoreceptor.

Supported by NSF and Whitehall Foundation.

388.2 THE RESPONSE OF THE RAT GLOSSOPHARYNGEAL NERVE TO BITTER COMPOUNDS: MULTIPLE BITTER RECEPTORS? Pamela E. Scott and Joseph Farley. Program in Behavioral Neuroscience, Department of Psychology, Princeton University, Princeton, N. J. 08544.

Human psychophysical and animal behavioral studies suggest the presence of multiple subclasses of bitter perception (Fox, Proc.Nat.Acad.Sci. 18, 1932; McBurney et al., Per.& Psych., 11(3), 1972; Sugimoto and Sato, Comp.Biochem.Physiol. 69A, 1981; Herness and Pfaffman, Chem. Sens 11, 1986). These have been suggested to reflect multiple peripheral bitter receptor sites: a quinine site, a urea site, and a phenylthiocarbimide (PTC) site. We have attempted to obtain direct evidence for multiple bitter receptors by recording from the glossopharyngeal nerve of the rat, through the use of a cross-adaptation paradigm.

The glossopharyngeal nerve of adult male Sprague Dawley rats was prepared for whole-nerve multiple-unit recording as described by Frank (1965). The circumvallate papillae was stimulated. The control stimulus used was water; test stimuli were urea (0.01M, 0.03M, 0.1M 0.3M, 1M) and quinine hydrochloride (0.0001M, 0.0003M, 0.001M, 0.003M, 0.01M). In the initial experiments, the tongue was adapted with water (30 C) and was then stimulated with one of the two test stimuli. Concentration- response relationships were established for both test stimuli. Threshold concentrations were 0.0003M for QHCl and 0.1M for urea. In adaptation experiments, the tongue was adapted with QHCl (0.003M). The test solution used was urea (1M or 0.3M). After adaptation of the rat tongue to 0.003 QHCL, the response to 1M urea was attenuated but not completely abolished. The results can be interpreted in two ways: a) separate receptors for quinine or urea and b) one receptor site which is only partially adapted by quinine. Further experiments are needed.

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388.3 AMILORIDE SENSITIVE SODIUM CHANNELS ARE REQUIRED FOR THE EXPRESSION OF SODIUM APPETITE IN THE RAT. C.J.Hennessy and I.L. Bernstein. Department of Psychology,University of Washington, Seattle, WA. 98195

Recent evidence indicates that the behavioral and electrophysiological response of the mammalian gustatory system to sodium chloride (NaCl) is dependent upon a Sodium (Na) transport system which is specifically blocked by the lingual application of the sodium transport blocker, amiloride. When rats are subjected to Na deficency they demonstrate avid ingestion of NaCl solutions, a response referred to as sodium appetite. Sodium appetite requires the recognition of solutions containing the sodium ion. The present study examined whether the expression of sodium appetite was dependent upon amiloride sensitive sodium channels in the rat.

Subjects were 8 male Long-Evans rats trained to drink from a spout by providing access to consecutive 2 minute presentations of water, 0.9% NaCl, and 10% sucrose under conditions of water deprivation until they reliably and consistently approached the tube and licked for the solutions presented. Individual licks were detected by photocell and recorded by microcomputer.

The effects of lingual amiloride on licking for 3% NaCl was first tested prior to induction of sodium appetite. Animals licked a 3% NaCl solution for 2 minutes after water or 100 uM amiloride hydrochloride exposure. Animals were then subjected to acute Na depletion by furosemide treatment (10 mg/Kg IP) along with maintenance on Na-free chow and distilled water and licking for 3% NaCl after either water or amiloride was again assessed.

The effects of exposure to amiloride on subsequent licking for 3% NaCl by rats were found to differ as a function of the animal's sodium balance. Licking for 3% NaCl was significantly increased in sodium replete and significantly decreased in sodium deplete rats by amiloride pretreatment. Expression of sodium appetite was virtually eliminated by pretreatment with amiloride. This suggests that the recognition of sodium solutions in animals with a sodium deficit is dependent on amiloride-sensitive sodium transport at the taste bud.

388.4 VOLTAGE-DEPENDENT AND CHEMICALLY-MODULATED IONIC CURRENTS IN ISOLATED TASTE RECEPTOR CELLS OF THE TIGER SALAMANDER. K. Sugimoto* and J.H. Teeter. Monell Chemical Senses Center and Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

In previous studies, we have shown that taste receptor cells isolated from larval tiger salamanders generate whole-cell currents exhibiting both inward and outward components in response to depolarizing voltage steps from a holding potential of -60 mV. Inward currents consisted of a transient component carried by Na^+ and, in some cells, a more slowly activating, persistent component, probably carried by Ca^{2+}. The outward currents were composed of at least two types of K^+ currents. In the present study, we have further characterized these voltage-dependent currents using single-channel recording techniques and examined the effects of taste stimuli on whole-cell currents and single-channel activity.

Taste receptor cells were dissociated from isolated pieces of lingual epithelium by treatment with a mixture of 0.5 mg/ml collagenase and 0.5 mg/ml hyaluronidase in low Ca^{2+} Ringer (20 min), followed by 4 min in Ca^{2+}-free Ringer containing 2 mM EGTA. Taste cells were collected in a pipette and plated on plastic culture dishes in Ringer containing (mM): 111 NaCl, 3 KCl, 5 $CaCl_2$, 3 HEPES and 2-glucose. The pipette solution contained (mM): 110 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 2 EGTA, 10 HEPES and 2 ATP. Chemical stimuli were applied by pressure-ejection from micropipettes placed close to the cell or patch. In voltage-clamped taste cells, salts (500 mM NaCl, 120 mM KCl) and acids (1 mM acetic, 1 mM citric acid) elicited slow, inward currents which lasted as long as the stimulus was applied. The inward currents induced by NaCl were not suppressed by addition of 1 µM TTX to the stimulus solution and reversed at about + 20 mV. The inward currents evoked by KCl were not suppressed by 10 mM TEA and reversed at about 0 mV. The large voltage-dependent outward currents observed in most isolated taste cells were significantly reduced during stimulation with NaCl, KCl and acids. L-arginine and Na^+-glutamate (1-10 mM) both elicited small outward currents. In outside-out patches of taste cell membranes, voltage-dependent K^+-channels (blocked by 5 mM TEA and 5 mM $BaCl_2$ in the bath) were closed by application of 1 mM acetic acid.

These results suggest the presence of stimulus-activated cation channels in taste cell membranes and also indicate that some taste stimuli (e.g., acids and L-arginine) may modulate voltage-dependent channels. Further resolution of the ionic mechanisms involved in taste transduction will require selective stimulation of the apical (receptive) membranes of isolated cells.

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388.5 CELL TYPES AND INTERCELLULAR CONNECTIVITY OF DYE-INJECTED TASTE CELLS OF NECTURUS MACULOUS R.J. Delay*, J. Yang*, and S. D. Roper Department of Anatomy, Colorado State University, Ft. Collins, CO 80523 and Rocky Mt. Taste & Smell Center, Denver, CO 80262.

Taste cells of Necturus are unusually large and thus are amenable to intracellular microelectrode studies. Yang and Roper (Soc. Neurosci. Abst. 12:1351,1986) found that 20% of the taste cells were elecrically-coupled, as revealed by dye couplings. However, there are several cell types within the mudpuppy taste bud and to date it has been extremely difficult to determine which cell types are electrically coupled. In addition, it has been difficult to correlate physiological responses with its taste cell type. To establish this correlation we impaled taste cells, recorded from them and then filled the cell with Lucifer Yellow.We have modifiated the technique described by Moranto (Science 217:952) to form an electon-dense reaction product visible at the electron microscope.

Lucifer Yellow was injected intracellularly into mudpuppy taste cells. Dye-filled single cells and coupled cells were then illuminated with UV light in the presence of diaminobenzidine (DAB) until a dark brown reaction product was formed. The tissues were then processed for electron microscopy. The reaction product formed an electron-dense product within the dye-injected cells that was visible in thin sections. The reaction product did not obscure the identifing granules of the Dark cells nor the smooth ER of the Light cells. It was possible to trace the torturous basal processes of dye-injected cells. Both Light and Dark cells reached the base of the taste bud and spines from the basal cells could be observed projecting into the cytoplasm of the injected cell. All the dye-coupled cells examined to date have been found to be Dark cells.

In the future this technique will allow us to determine more precisely the synaptic connectivity between taste cells, as well as between taste cells and nerve fibers more precisely and correlate ultrastructure with the physiological characteristics of the cells.

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388.6 Characteriztion of a Taste-Stimulus Sensitive Adenylate Cyclase from the Gustatory Epithelium of the Channel Catfish, Ictalurus punctatus. D.L. Kalinoski, V. LaMorte* and J.G. Brand*. Monell Chemical Senses Center, Philadelphia, PA 19104, and Veterans Administration Medical Center, University of Pennsylvania, Philadelphia, PA 19104.

Adenylate cyclase activity has been reported in the gustatory systems of several species (Nomura, Olfaction and Taste, 7: 219, 1980) and has been suggested as a possible second messenger system for taste receptor transduction (Cagan, J. Neurosci. Res. 2: 363, 1976). Recently, the presence of GTP-binding regulatory proteins has been demonstrated in a purified plasma membrane fraction from catfish taste epithelium (Bruch and Kalinoski, J. Biol. Chem. 262: 2401, 1987), shown previously to contain receptors for taste stimuli (Cagan, Biochemistry of Taste and Olfaction, 175, 1981). With the present characterization of an adenylate cyclase system in catfish taste epithelia, the components of a G-protein mediated receptor-transduction process have been identified. We report here the initial characterization of adenylate cyclase activity in the sedimentable fraction (P2) from catfish taste epithelium. Taste epithelia exhibited low levels of adenylate cyclase activity, even in the presence of 1 mM isobutyl methyl xanthine, an inhibitor of cyclic nucleotide phosphodiesterase. Basal activity was stimulated approximately 3-fold by GTP, 8-fold by GppNHp and Forskolin and approximately 25-fold by GTP S. Sodium fluoride increased adenylate cyclase activity in a dose-dependent manner with 20 mM NaF yielding a maximal 10-fold stimulation of basal activity. Adenylate cyclase of Fraction P2 was sensitive to calcium ion. Addition of 0.63 mM $CaCl_2$ to the assay medium suppressed Forskolin activation of adenylate cyclase essentially to zero. Addition of 1 mM EGTA to Fraction P2 prepared in zero-calcium medium causes a 3-fold increase in unstimulated levels of cyclase activity. The taste stimulus L-alanine augmented adenylate cyclase activity in a dose-dependent manner. Stimulation of cyclase activity by low concentrations of L-alanine (0.5-4 uM) was more pronounced in the presence of GppNHp, suggesting a guanine nucleotide dependence for adenylate cyclase stimulation by amino acid taste stimuli. At all concentrations tested, GppNHp and L-alanine displayed a synergistic effect on cyclase activity. L-alanine stimulation of cyclase activity was affected by addition of EGTA or calcium ion. Addition of 1 mM EGTA increased basal and alanine-stimulated activity while addition of calcium ion inhibited amino acid stimulation. Thus, these data support a role for adenylate cyclase in the transduction pathway of the gustatory system and indicate that divalent cations act to regulate both basal and taste-stimulated adenylate cyclase activity.

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388.7 RESPONSE PROPERTIES OF FACIAL LOBE SUBNUCLEI TO AMINO ACIDS IN THE CHANNEL CATFISH, ICTALURUS PUNCTATUS. T. Hayama and J. Caprio. Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA. 70803

The medullary facial lobes (FL) in freshwater channel catfish are the primary taste centers which receive facial nerve input in a somatotopical manner from taste and tactile neurons innervating the external body surface (1). The peripheral facial taste fibers respond best to either alanine or arginine among the amino acids tested (2). A preliminary report indicated that taste neurons in the FL of the same species also responded best to alanine or arginine (1). Previously, we reported that each FL of the channel catfish is divided into five subnuclei, each receiving input from one of the four barbels or the face-flank, respectively. We further showed that taste responses in three of the four barbel subnuclei are generally confined to dorsal areas, while tactile responses are observed throughout the subnuclei (3).

Presently we are examining the response specificity of taste neurons in the FL to amino acids and whether amino acid responses are differentially distributed. Single or multiunit activity in response to amino acid stimulation of three barbels (the medial and lateral mandibular, and the maxillary barbels) is recorded in the corresponding three subnuclei. Multiunit taste activity within the the three barbel subnuclei was greatest to alanine and/or arginine among the amino acids tested. Alanine and arginine responses were observed throughout the entire rostro-caudal extent of the dorsal part of the maxillary barbel subnucleus. The magnitude of the arginine responses ranged from ca. 0.5 to 1.5 times that of alanine responses. Some taste neurons showed suppressive responses to amino acid stimulation.

(Supported by NIH Grant NS 14819)

(1) T. Marui and J. Caprio (1982) Brain Res., 231: 185-190
(2) C. J. Davenport and J. Caprio (1982) J. Comp. Physiol., 147A: 217-229
(3) T. Hayama and J. Caprio (1987) AchemS IX Abstr. Number 86

388.8 ULTRASTRUCTURE OF FACIAL NERVE TERMINALS WITHIN THE GUSTATORY LOBES OF THE CATFISH, ICTALURUS PUNCTATUS. M. Womble* and T.E. Finger (SPON: J. Kinnamon). Dept. Cellular & Structural Biol., U. Colo. Hlth. Sci. Ctr., Denver, CO 80262.

Previously (Finger & Womble, Abst. Soc. Neurosci., 1986), we demonstrated that two distinct types of primary afferent nerve terminals occur within the facial gustatory lobe of the channel catfish, Ictalurus punctatus. These terminal types include fine, vesicle-filled synaptic terminals and large (2-3 μm) bulbous processes replete with mitochondria, vesicles and accumulations of filaments. Since both trigeminal (general somatic) and facial (gustatory) nerve fibers are involved in innervating the barbels, and since both trigeminal and facial nerve fibers terminate within the facial lobe, each type of terminal, bulbous and fine, could have been associated uniquely, with one or the other cranial nerve. The present experiment was undertaken to test this hypothesis.

The body surface of catfish receives two types of innervation: general somatic, from the spinal nerves, and gustatory, from the facial nerve. The gustatory innervation to taste buds along the fish's flank is derived from the recurrent branch of the facial nerve. This branch is a pure facial nerve and contains no admixture of trigeminal or spinal components. Thus, we selected this nerve to fill peripherally with HRP. Following transganglionic transport of the enzyme, the brain was prepared for peroxidase EM-histochemistry. Both bulbous and fine terminal processes were labeled within the facial lobe. Thus, neither terminal type can uniquely be associated with trigeminal innervation of the barbels. Further, the general morphology of the recurrent nerve branch terminals was similar to that of terminals from the barbel nerves. Intriguingly, a common finding in these cases is the prevalence of specialized areas of contact between neighboring filled primary afferent fibers. These areas of contact involve relatively long (1-2 μm) regions of membrane specialization and often occur in association with the bulbous type ending. We have been unable to demonstrate an unequivocal synaptic contact, involving both presynaptic perimembrane vesicle accumulation, and postsynaptic membrane specialization, between two labeled processes. Many profiles, however, are suggestive of functional contact, e.g. presynaptic perimembrane vesicle accumulation, but details of the area of specialization are often obscured by the peroxidase reaction product. Thus, colloidal gold, immunocytochemical techniques will be used to identify labeled processes in future experiments.

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388.9 ANATOMICAL AND ELECTROPHYSIOLOGICAL INVESTIGATION OF THE SUPERIOR SECONDARY GUSTATORY NUCLEUS OF THE JAPANESE SEA CATFISH. C. F. Lamb IV, T. Marui* and Y. Kasahara*. Dept. Oral Physiol., Kagoshima Univ. Dent. Sch., Kagoshima 890, Japan.

The superior secondary gustatory nucleus (nGS) of teleost fishes receives afferent fibers from the medullary taste centers, the facial (FL) and vagal (VL) lobes, and projects to the inferior lobe (LI) of the ventral diencephalon (Herrick, 1905). We used extracellular glass microelectrodes to study electrophysiological responses of nGS neurons of the Japanese sea catfish, Plotosus lineatus (=anguillaris), to mechanical and chemical stimulation of the external body surface and oropharyngeal cavity. HRP was iontophoretically injected into the nGS, as well as the FL, VL, and LI, to hodologically identify the connections of the nGS.

Responses of nGS neurons generally involved large receptive fields (RFs), including the whole body surface and oropharyngeal cavity. Restricted RFs were observed including only the ipsilateral barbels, but these neurons did not appear to be topographically arranged within the nGS. This is quite different from the FL of P. lineatus, where peripheral input is distinctly segregated into five somatotopically organized columns (Marui et al., submitted). Although mechanosensitive units were identifiable throughout the nGS, responses to amino acid solutions and to extracts of commercial feed were rarely encountered and were often unreproducible. This could result from the effects of capture and maintenance of the specimens, but the same recording techniques in the medullary taste centers produce readily identifiable, phasic chemoresponses. These results indicate a functional difference between the processing of information within the primary and secondary gustatory nuclei.

Results from HRP-labelling supported the electrophysiological results, showing overlapping bilateral termination within the nGS of FL and VL efferents. Restricted injections in the nGS typically retrogradely filled cell bodies in both the VL and several columns of the FL. HRP-labelled efferents from the nGS terminate ipsilaterally in the vicinity of the nucleus lobo-bulbaris (nLB), nucleus thalami posterioris (nTP), and nucleus diffusus lobi inferioris (nDLI). Reciprocal connections were found between the nLB/nTP and the VL, FL, and nGS. This provides an anatomical basis for a feedback system between the diencephalon and the primary and secondary gustatory nuclei.

388.10 GUSTATORY MODULATION OF OROMOTOR AND VISCEROMOTOR OUTPUTS IN THE CHANNEL CATFISH, ICTALURUS PUNCTATUS. J.S. Kanwal and T.E. Finger. Dept. of Cellular & Structural Biology, Univ. Colo. Hlth. Sci. Ctr., Denver, CO 80262.

Most animals utilize the gustatory sense to discriminate food from non-food prior to ingestion. Various behavioral and physiological responses to gustatory stimuli suggest the presence of relatively direct, sensorimotor interconnections between the gustatory system and the visceral motor systems within the medulla. We tested this hypothesis by recording from vagal motor neurons while applying taste and tactile stimuli to the oral and extra-oral epithelium of the channel catfish, Ictalurus punctatus. We observed gustatory-related changes in the electrophysiological activity of these motor neurons; this implies that taste and tactile information can modulate visceral functions.

Juvenile channel catfish, obtained locally, were paralysed by an intramuscular injection of Flaxedil and anaesthetized by local application of Xylocaine prior to surgery. Neural recordings from vagal motor neurons were obtained centrally with glass microelectrodes (impedance <1 Mohm; tip diameter, approx. 1 um) or peripherally by means of a silver wire hook electrode on which the central cut end of a branchial or coelomic branch of the vagus nerve was placed. The visceral motor column in ictalurid catfish is morphologically divisible into a lateral vagal motor column (LMC, = nucleus ambiguus of mammals) which innervates orobranchial musculature, and a medial vagal motor column (MMC, = dorsal motor nucleus X of mammals) which innervates coelomic viscera. Neurons in the LMC exhibited rhythmic patterns of activity which were only transiently excited or inhibited by taste and tactile stimulation of oral and peri-oral regions. Liver extract and high concentrations ($>10^{-2}$ M) of amino acid mixtures (alanine, arginine, and proline) were effective stimuli when applied to the oral and extra-oral surfaces, while responses to quinine ($>10^{-3}$ M) were obtained only for oral stimulation. Taste and touch sensitive neurons in the MMC responded differentially to stimulus application over separate areas extending bilaterally from the lips to the caudal flank. Response patterns similar to those of both populations of vagal motoneurons also were obtained from cells in the reticular formation at the level of the facial lobe. The present electrophysiological data, thus indicate medullary convergence of orofacial taste and tactile inputs onto neurons of the visceral motor column. Neuroanatomical tracing experiments, currently in progress, will elucidate further the gustatoreticular pathways mediating orovisceral changes observed at the behavioral and physiological levels.

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388.11 AFFERENT CONNECTIONS OF THE CAUDOLATERAL ORBITOFRONTAL CORTEX TASTE AREA OF THE PRIMATE. Leslie L. Wiggins, Edmund T. Rolls and Gordon C. Baylis, University of Oxford, Department of Experimental Psychology, Oxford OX1 3UD, England

A cortical taste region has recently been identified in the caudo-lateral orbitofrontal cortex of the primate (Rolls, E.T., Yaxley, S., Sienkiewicz, Z.J. and Scott,T.R. (1985) Chemical Senses 10: 443.). In this area, single neurons are sharply tuned to gustatory stimuli, and are influenced by the motivational state of the monkey. In order to determine the afferents to this region of taste cortex, and to determine whether it is a primary, secondary, or tertiary region of cortical taste processing, injections of WGA-HRP for retrograde neuronal tracing were made into this region in three monkeys in which the exact location of this cortical taste region had been identified by recordings of the activity of single neurons. Labelled cell bodies were found in the frontal opercular taste cortex and in the insular taste cortex, both of which are primary taste cortices in that they receive from the thalamic taste nucleus, VPMpc. Further, the caudolateral orbitofrontal cortex taste area did not receive inputs from VPMpc, but instead received projections from the mediodorsal nucleus of the thalamus, the thalamic nucleus which projects to the prefrontal cortex. These results show that the caudolateral orbitofrontal taste cortex is a secondary taste cortical area, and that it receives gustatory inputs from the frontal opercular and insular taste cortices. Afferents were also shown to reach the caudolateral orbitofrontal taste cortex from the more ventral part of the rostral insular cortex, the amygdala, the substantia innominata, the rhinal sulcus, and from the surrounding orbitofrontal cortex. Through some of these pathways visceral information important in modulation of responsiveness by motivational state may reach the caudolateral orbitofrontal taste cortex.

388.12 PERIRECEPTOR EVENTS IN PURINERGIC CHEMORECEPTION BY THE SPINY LOBSTER. H. G. Trapido-Rosenthal, R. A. Gleeson* and W. E. S. Carr*. C. V. Whitney Lab. and Dept. of Zoology, University of Florida, St. Augustine, FL 32086.

Chemosensory transduction is initiated by the interaction of odorant molecules with receptors present on the plasma membranes of chemosensory neurons. Odorant molecules are affected by a variety of events that can directly influence their ability to interact with these receptors; such processes have been termed perireceptor events (Getchell et al., Prog. Neurobiol., 23:317, 1984). We describe here our studies of perireceptor events in purinergic chemoreception by the spiny lobster, Panulirus argus.

The olfactory organ of P. argus consists of a group of aesthetasc sensilla present on the antennule of this marine invertebrate. Each sensillum consists of a chitinous sheath, permeable to odorants, that encloses a volume of approximately 250 picoliters; within this sheath are densely packed dendrites of about 320 primary chemosensory neurons. Electrophysiological data indicate that many of the neurons are differentially excited by purine nucleotides (AMP, ADP, and ATP) when these compounds are present in seawater bathing the sensilla. Responses of the neurons sensitive to purine nucleotides are mediated by several classes of purinergic receptors with different ligand specificities. Adenosine (Ado) is only slightly stimulatory to these neurons.

Biochemical studies have shown that the aesthetasc sensilla contain ectonucleotidases that dephosphorylate the stimulatory purine nucleotides, generating the much less active nucleoside Ado; Ado is then specifically taken up into an intracellular compartment. The best characterized of these dephosphorylating enzymes is the 5'-ectonucleotidase which converts AMP to Ado; this enzyme has characteristics similar to those of ectonucleotidases on membrane surfaces of mammalian cells. It is characterized by a Km of 23 μM, and a Vmax of 89 pmoles/sensillum/second. It is inhibited by ADP and the poorly hydrolyzable ADP analog α,β-methylene ADP (AMPCP). The kinetics of this enzymatic activity are such that a micromolar concentration of AMP in a sensillum is reduced by a factor of 10 in 153 milliseconds. The electrophysiological response of AMP-sensitive chemosensory cells decays in a manner that is consistent with the hypothesis that signal molecules (AMP) are rapidly converted to non-signal molecules (Ado) by this enzymatic activity. The inhibition of 5'-ectonucleotidase activity by ADP or AMPCP can lead to a potentiation of the response of AMP-sensitive cells to this nucleotide. These results indicate that perireceptor events can make a direct and quantifiable contribution to the chemosensory process.

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388.13 INTRACELLULAR STUDIES OF THE IONIC DEPENDENCY OF THE LOBSTER'S OLFACTORY RECEPTOR POTENTIAL. Ingrid Schmiedel-Jakob*, Peter A. V. Anderson and Barry W. Ache*. (SPON: J. Tautz). Whitney Lab. University of Florida, St. Augustine, FL 32086

The olfactory organ of the spiny lobster, Panulirus argus, consists of tufts of hair-like sensillae, which are innervated by bipolar neurons. The somata of the neurons are gathered in clusters at the base of each sensilla and their dendrites project into the lumen. Using the whole-cell configuration of the patch-clamp technique we have made intracellular, current clamp recordings from these receptor cells in situ. These cells have high input impedances, 4.87 (± 1.6 s.e.m.) Gohm, and long time constants (19.5 ± 5.7 ms).

Application of a chemical stimulus (individual and mixed amino acids, or extract of fish food) to the sensillae evoked a slow depolarization which reached peak amplitude in 60-300 ms. The amplitude of this depolarization was dose-dependent and linearly related to the log of stimulus concentration. With some cells chemically evoked depolarizations were associated with a decrease in conductance (up to 40%) while with others an initial increase in conductance was followed by a decrease. Depolarizations produced by current injection alone were associated only with a conductance increase.

To identify the ionic basis of this receptor potential the ionic composition of the medium surrounding the sensillae was changed. When external Na^+ was reduced from 480 mM to 48 mM by substitution with choline or TMA the receptor potential in some cells was eliminated or reduced. The effect was reversible and recovery occurred within 6 min. These Na^+-dependent receptor potentials were TTX-insensitive at concentrations where superimposed action potentials were blocked. Reducing $[K^+]_o$ from 13.4 mM to 1.4 mM led to the elimination or a reduction of the receptor potential in some cells. A similar effect was observed when $[Ca^{++}]_o$ was reduced. Both effects were reversible. On occasions, the receptor potential was reduced or eliminated by the presence of external Cs^+ ions, suggesting that voltage-sensitive K^+-currents may be involved in the transductory process.

The results suggest that at least three different ions, Na^+, K^+, and Ca^{++}, contribute to the generation of the receptor potential, and that the permeability changes evoked by chemical stimulation occur on the dendritic portion of the olfactory neurons.

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388.14 CATION-SELECTIVE ION CHANNELS FROM PRIMARY OLFACTORY RECEPTOR NEURONS OF THE SPINY LOBSTER. T. S. McClintock* and B. W. Ache*. (SPON: C. Leonard). C. V. Whitney Lab. and Depts. of Zoology and Neuroscience, University of Florida, St. Augustine, FL 32086.

Single-channel recording from the primary olfactory neurons of the lobster, Panulirus argus, reveals cation-selective channels on the neurons' somata. Because recent morphological evidence has suggested that the extreme length and small diameter of the branching dendritic process of this neuron might prohibit effective passive spread of a receptor potential (Grunert, U. and Ache, B. W., submitted to Cell Tiss. Res.), we are interested in possible alternative mechanisms of receptor potential propagation. These cation-selective channels have properties which would allow them to aid the spread of a receptor potential.

In inside-out patches, a 320 pS channel is observed which has a Na+/K+ permeability ratio of 1.28. Outward currents through this channel show a rapid, flickering block when 20 mM or 50 mM tetraethylammonium is applied to its intracellular side. The percent of time it spends in the open state is independent of bath calcium concentrations from 0.1 mM to 0.1 nM. The apparent gating chrge of 1.1 reflects the weak voltage dependency of this channel. At -60 mV holding potential, the channel appears to have a single open state with a mean open time of 4.9 msec.

Curiously, we never observe this 320 pS conductance in cell-attached patches. Many of these channels, however, are present in patches excised from cell-attached patches which contained 100 pS channels. This 100 pS channel is present with either KCl or NaCl solutions in the pipette and reversal potentials extrapolated from current-voltage relationships also suggest that it is cation-selective. It is characterized by short openings activated by steps from -60 mV to potentials of -40 mV or more. It inactivates only slowly, if at all, and appears to be weakly voltage dependent. We have never observed the 100 pS conductance in inside-out patches, allowing that both cation-selective conductances may be from the same channel.

We hypothesize that the role of this cation-selective channel, or channels, is to aid in the spread of the odor-stimulated receptor potential. The weak voltage dependency would be necessary to allow the graded nature of the receptor potential to be faithfully transmitted to the spike generating zone, presumably near the somatic-axonal junction.

388.15 NEURAL CODING OF QUALITY OF STIMULI BY THE OLFACTORY RECEPTOR CELLS IN THE SPINY LOBSTER: TOWARDS A UNIFIED MODEL. M.-N. Girardot and C.D. Derby. Department of Biology, Georgia State University, Atlanta, GA 30303.

Behavioral studies show that the spiny lobster, Panulirus argus, discriminates between food-related chemicals, whether of simple composition such as amino acids or nucleotides, or of a complex chemical nature (Daniel and Derby, Fine-Levy and Derby, AChemS Abstracts, 1987). The search for the physiological correlate of this discrimination using multivariate techniques provided evidence that the neural code is the pattern of responses generated by the entire population of chemoreceptors (ANP). This was true whether the stimuli used were of simple chemical nature, such as taurine, betaine, glutamate, glycine and adenosine-5'-monophosphate, or of highly complex composition (artificial mixtures based on the chemical composition of extracts of crab, mullet, oyster and shrimp, which varied only in terms of the relative quantitative contribution of the components). However, when the set of single components was used to stimulate the receptor cells, the resulting cell specificity was high (mean H values, index of breadth of responsiveness = .29), suggesting that the olfactory receptor cells are narrowly tuned and that quality discrimination among types of chemicals results from specific activity in well-defined groups, or types, of cells (Derby and Ache, 1984). While, when the chemicals used were the artificial mixtures, the cells were broadly tuned to the stimuli, revealing a lack of cell specificity (mean H value = .92), and the results failed to provide evidence that discrimination among chemicals results from the specific activation of well-defined types of cells with identical intertype across-stimuli pattern of responses. Various analytical techniques, including multidimensional scaling, will be used in an attempt to provide a unified model for chemodiscrimination by the olfactory receptor cells that will account for the findings resulting from the use of both simple and complex chemical stimuli.

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388.16 SPATIAL-TEMPORAL FILTERING IN OLFACTORY CHEMORECEPTOR CELLS. R. Voigt* and J. Atema. (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543).

The lateral antennular flagellum of the American lobster functions as an olfactory organ, and is of critical importance for orientation in odor plumes. Intermittent sampling by flicking the antennules and the patchy nature of odor plumes make it likely that the receptor cells of this organ detect pulsed stimuli in a chemically noisy background. A major cell population of these receptors is narrowly tuned to taurine. We have started to characterize the time courses of adaptation and disadaptation of receptor cells using a series of standard 1s stimulus pulses with varying signal-to-noise ratios (SNR).

The temporal stimulus pulse profile was determined by measuring the change of conductivity of flowing deionized water (20 ml/min) after injection of 100 ul of 1M NaCl solution over 300 ms. The stimulus chamber allowed 5s interpulse intervals without interference from the previous stimulus. Single cells were identified electrophysiologically with 10^{-4}M taurine. A series of five pulses was applied in 10s intervals for one of several concentrations (10^{-3}-10^{-6}M) in different backgrounds (10^{-4}-10^{-7}M). Combinations of stimulus and noise background concentrations provided similar SNR at different absolute stimulus concentrations and vice versa.

The results suggest that regardless of the background noise levels the same SNR resulted in similar responses, including similar cumulative adaptation. Greater SNR caused stronger responses and showed greater cumulative adaptation while smaller SNR caused weaker responses and less adaptation. Thus, SNR ratios and not absolute stimulus levels were predictive of the responses of receptor cells in different backgrounds. This feature predisposes the receptor cells to extract information on spatio-temporal fine structure of odor plumes and not on the time-averaged concentration gradients.

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388.17 RESPONSE SPECTRA OF MEDIAL ANTENNULAR CHEMORECEPTORS IN THE AMERICAN LOBSTER. Ann Jane Tierney, Rainer Voigt, Bruce R. Johnson and Jelle Atema. Boston Univ. Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

The biramus antennules of decapod crustaceans are olfactory organs which mediate orientation to odor sources. Most physiological studies have focused on the lateral filament which bears chemoreceptive aesthetasc hairs. Cells from this filament are sensitive to low molecular weight compounds, particularly amino acids, and most are narrowly tuned to single compounds (Shepheard, P., Mar. Behav. Physiol., 2:261, 1974; Johnson, B.R. and Atema, J., Neurosci. Letts. 41:145, 1983). The medial filament is also chemosensory (Fuzessery, Z.M., Comp. Biochem. Physiol., 60:303, 1978). However, little is known about the response spectra or tuning properties of individual cells from this organ. In this study we determined the response specificity and sensitivity of medial antennular chemoreceptors to 15 compounds presented singly and within a mixture.

A suction electrode was used to record extracellular activity from small bundles of nerve in ablated medial antennular filaments. Chemosensitive cells were identified with a mixture of the following 15 compounds, all at an injected concentration of 10^{-4}: L-alanine, L-arginine, L-asparagine, L-aspartate, betaine, L-glutamate, L-glutamine, glycine, L-histidine, hydroxy-L-proline, L-leucine, L-lysine, ammonium chloride, L-proline and taurine. These compounds were then tested singly at 10^{-4} to determine the response spectra for individual cells. Injected concentrations were diluted to approximately $7x10^{-6}$ after introduction to the test chamber.

The best stimuli for the medial filament cells included taurine, hydroxyproline, betaine and ammonium chloride, which are also the most effective stimuli for lateral filament chemoreceptors. A significant population of arginine-best cells was also found. Most cells were narrowly tuned to single compounds, but some had a broader response spectrum. Broadly tuned cells which responded best to hydroxyproline, taurine, betaine or ammonium chloride were variably responsive to other compounds, with no consistent second best stimulus. In contrast, arginine-best cells consistently had leucine as the second best compound and lysine as the third best compound. As in lateral filament receptors, mixture suppression was common. In most cells responses to stimulatory compounds presented alone was clearly greater than responses to the same compounds presented in the mixture.

This work was supported by grants from NSF (BNS 85-12585) and the Whitehall Foundation.

388.18 MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERIZATION OF INDIVIDUAL OLFACTORY INTERNEURONS CONNECTING THE BRAIN AND MEDULLA TERMINALIS OF THE CRAYFISH. C.D. Derby and D. Blaustein*. Department of Biology, Georgia State University, Atlanta, Georgia 30303.

Toward understanding the functional organization of the crustacean olfactory system, we are using intracellular, dye-filled microelectrodes to correlate the structure and function of single, odorant-sensitive interneurons in the brain of the crayfish Procambarus clarkii. We report on a subset of interneurons that connect the brain proper with the medulla terminalis, a higher-order integrative center located in the eyestalk. All of the interneurons recorded in this study are assumed to be at least second-order olfactory interneurons, because they do not branch in the olfactory lobe, the neuropile to which primary olfactory receptor cells of the antennules project. The parolfactory lobe probably represents a low- or mid-order chemosensory or multimodal integrative center, since it contains chemosensory or multimodal interneurons that directly output to the medulla terminalis. Areas of the medulla terminalis shown definitively to be involved in integrating olfactory or multimodal (including olfactory) information are neuropile regions III, VI, and XII, and soma clusters D and E). The medullae terminales are connected directly to the nerve cord by chemosensitive interneurons. Morphologically complex interneurons (defined by laterality of branching) were more likely to have complex response characteristics (defined by modal sensitivity). For example, bilateral interneurons were all found to be multimodal (odorants, touch, chemicals), while unilateral interneurons could be either uni-, bi-, or multimodal. Higher-order olfactory interneurons (with soma in the medulla terminalis) showed the most complex response profiles, being multimodally sensitive and having "mixed" responses (e.g., excited by odorants but inhibited by touch and/or light). This correlation indicates that such higher-order interneurons may function in multimodal processing and feature detection in a manner qualitatively different than do lower-order interneurons. The diversity of structural and functional classes of interneurons in the eyestalk nerve indicates that chemosensory and other information is transmitted and processed at this level by parallel arrays of neurons.

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388PO EFFECTS OF UNILATERAL NARIS CLOSURE ON THE OLFACTORY EPITHELIA OF ADULT MICE. J.A. Maruniak* and P.J. Lin.* (SPON: W.S. Stark). Biological Sciences, University of Missouri, Columbia, MO 65211.

This study investigated the effects of unilateral naris closure in adult mice on the histology of the bilateral olfactory epithelia, turnover rates of their receptor neurons, and production of olfactory marker protein (OMP). Adult mice at least 6 months old had either their right or left naris closed by cautery and apposition of the burned areas with 7-0 suture. Controls were untreated. Groups had their nares closed for 1, 3, 6, 8, or 12 weeks.

Our general procedure was to assess: 1) turnover rates, using on-the-slide autoradiography of the sectioned epithelia of mice that had been injected with tritiated thymidine; 2) histology, using H &E stained paraffin sections; and 3) presence and maturational state of the olfactory receptor neurons, using immunohistochemistry for OMP.

In the control group and 3 groups with unilateral closure for 6 weeks or less, no differences could be detected between the 2 sides of the nose in any of the histological or immunohistochemical parameters. In mice with naris closure for 8 or 12 weeks, there were time-dependent differences in both the histology and immunohistochemistry of the 2 sides. The olfactory epithelia on the closed sides displayed no detectable abnormalities compared to controls, but the open sides showed varying degrees of degenerative changes. Beginning after 8 weeks of closure, losses of olfactory receptor neurons could be observed on the open side in most of the mice. Particularly within the 12 week group, there was a range of responses such that in about half of the mice there were minor to almost no losses of receptor neurons on the open side while in the other half there were moderate to virtually complete losses of olfactory receptor neurons.

Data from the tritiated thymidine studies of turnover rates indicated no significant differences in turnover between the 2 sides. This suggested that losses of cells on the open sides were caused by the inability of a more or less constant rate of turnover to compensate for a greater than normal rate of receptor neuron loss.

389.1 IMMUNOCHEMICAL DETECTION OF CAPSAICIN CONGENERS
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Capsaicin (8-methyl N-vanillyl 6-noneamide) is a pungent component of red peppers, which exerts excitatory and toxic actions on a subset of sensory neurones. The strict cellular specificity of capsaicin, together with the well defined structural requirements for activity revealed by an analysis of a series of capsaicin congeners (Szolscanyi and Jancso-Gabor, Arz-Forsch, 25, 1877, 1975) are consistent with the existence of a specific capsaicin receptor. No direct evidence for such a receptor or an endogenous capsaicin-like ligand exists. To help define the putative capsaicin receptor and endogenous capsaicin-like ligands, specific anti-capsaicin antisera have been used to;

1) screen for endogenous capsaicin-like immunoreactive material
2) produce anti-idiotypic antisera which may define a capsaicin receptor
3) detect capsaicin-like photoaffinity probes coupled to proteins and lipids.

8-amino-octylvanillylamine was covalently coupled to thyroglobulin with glutaraldehyde, emulsified with Freunds adjuvant and used to immunise rabbits. Experiments described here were all carried out with a single serum sample from one rabbit. The specificity of the serum was assessed in a competition radioimmunoassay using reductively tritiated dihydrocapsaicin (specific activity 60 Ci/mMol, Amersham). The structural requirements for recognition by the serum lay in the vanillylamine ring and amide bond region with a less stringent requirement for an aliphatic side chain. The serum was highly specific, failing to recognise molecules such as vanillylamine and 3-methoxytyramine which are structurally similar to capsaicin. The detection limit of the assay was 1pmol of capsaicin-like immunoreactive material.

Analysis of inflammed and normal rat and mouse tissue extracts failed to reveal the presence of endogenous capsaicin-like immunoreactive material.

Affinity purified $F(ab)_2$ anti-capsaicin antibodies have been used to generate rabbit anti-idiotypic antisera, which are weak antagonists in a capsaicin-stimulated calcium accumulation assay in rat DRG neurones in culture. Proteins labelled with the photoaffinity probe N-(4-hydroxy, 3 methoxy benzyl)-4'azidophenyl-propionamide, which shows capsaicin-like activity on rat DRG neurones in culture, could be detected with the antiserum on dot blots with a sensitivity limit of 10fmol. A Western and TLC immunoblot analysis of photaffinity labelled rat DRG neurones in culture reveals a number of immunoreactive molecules.

389.2 SENSORY CHARACTERISTICS OF HISTAMINE-INDUCED EXPERIMENTAL ITCH.
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The purpose of this work was to examine the sensory response to controlled patterns of histamine application to skin in normal young adult human volunteers. An area of approximately 0.4 sq cm was lightly stripped by repeated application of adhesive film. Histamine phosphate (HP) dissolved in buffered physiological saline, pH 7.30, was applied to the stripped region using a special perfusion chamber and control system ("pruritometer") which permitted application of any desired concentration pattern. Subjects reported verbally itch intensity at 10 second intervals using a numerical 0-10 analog scale, with 0 = no sensation, and 10 = most intense itch imaginable. Using a ramp concentration pattern, itch threshold averaged 0.159 ± 0.041 mM HP over all subjects tested. Itch intensity increased with increasing HP concentration; at the highest concentration used here, approximately twice threshold, the maximum response was "5". Upon application of a suprathreshold step function stimulus, the typical response pattern was a 40 second delay before the subject reported any sensation, an increase in sensation intensity to maximum over the next 30 seconds, followed by spontaneous adaptation to a lower itch intensity during the following 100 seconds even though HP concentration was held constant. Subsequent perfusion with control solution followed several minutes later by reapplication of HP demonstrated that histamine sensitivity remained low. Sensory evaluation of the test patch immediately following the experiment indicated that subjects retained normal sensitivity to sharp prick (pain), but often had reduced sensitivity to touch-pressure, compared to surrounding control skin. We conclude the following: (1) The "pruritometer" is a useful instrument for investigation of the psychophysics of itch; (2) HP-induced experimental itch differs from natural itch, since the former is characterized by adaptation while the latter is not. (3) Our finding that cutaneous HP application results in eventual reduction of itch sensitivity while pain sensitivity remains normal is surprising, since pain and itch are assumed to be closely related. (Supported by NIH grant NS 22594.)

389.3 STIMULATION OF NASAL TRIGEMINAL RECEPTORS WITH NICOTINE AND TOLUENE.
W.L. Silver and D.B. Walker*. Dept. of Biology, Wake Forest University, Winston-Salem, NC 27109.

Trigeminal receptors in the nasal cavity respond to a variety of chemical stimuli. Although some of these stimuli appear to be non-irritating, trigeminal chemoreception is often considered a part of the common chemical sense whose primary function is to elicit protective reflexes when stimulated with irritating compounds. Compared to olfaction, however, there is still relatively little quantitative information available about the kinds and concentrations of chemical compounds which are effective trigeminal stimuli. In the present experiment we examined the effectiveness of two potential environmental irritants, nicotine (NI) and toluene (TO) as trigeminal stimuli. Multiunit activity was obtained from the ethmoid branch of the rat trigeminal nerve. Respiration was monitored via a thermistor wire placed into a tracheal cannula. Stimuli were delivered in the vapor phase via a microprocessor controlled, air-dilution olfactometer. Dilution ratios were controlled by electronic mass flow controllers. Concentration-response curves and thresholds (defined as the concentration which first elicited a response discernible from baseline) were obtained from 8 rats. The response to NI differed from responses to TO (and other compounds) in that it only gradually returned to baseline. Thresholds for NI and TO were approximately 4 and 2400 ppm, respectively. Respiration was affected at concentrations as low as 7 ppm for NI and 2400 ppm for TO. These results demonstrate that NI and TO are effective in eliciting neural responses from the rat trigeminal nerve. Whether or not they function as sensory irritants in the environment obviously depends on their concentration in the environment and the sensitivity of the organism.

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389.4 AN *IN VITRO* NEURAL NETWORK MODEL OF THE OLFACTORY SYSTEM.
S.P. Fracek, Jr., G.W. Gross and R. Schafer, North Texas State University, Denton, TX 76203.

Mouse embyronic olfactory tissues are being used to to develop an *in vitro* model of information processing. Our goal is to create a tissue culture-based system that responds to natural stimuli and processes stimulus information. The olfactory system offers certain advantages. Both the olfactory neuroepithelium (which detects odors) and the olfactory bulb (where information processing occurs) are anatomically distinct and can easily be removed and cultured. Unlike other neurons, the olfactory receptor cells constantly develop from basal cells, mature, and become functional when their axons form synapses with mitral cells of the olfactory bulb. Thus, it is potentially possible to construct an "artificial nose" consisting of tissue explants of olfactory epithelium, whose axons synapse with mitral cells from cultured disassociated olfactory bulbs. Although the three dimensional structure of the olfactory epithelium and bulb is lost along with most of the normal inputs and outputs of the system, it is hoped that this system will mimic in some respects the natural process. This "artificial nose" can be used to examine the processes of information processing and storage; chemical, electrophysiological, pharmacological, and morphological manipulations are possible. A functional system will allow testing of mathematical models of information processing, such as the Hopfield model.

Several concurrent projects involving microelectronic technology and computer hardware and software will permit full analysis of the "artificial nose." Tissues are currently being cultured on multimicroelectrode plates (MMEPs). MMEPs have 36 or 64 photoetched transparent indium-tin oxide electrodes in a 6X6 or 8X8 array. The surface of the MMEP has been modified to allow cell adhesion and growth. We are designing a recording chamber that maintains sterility, pH, osmolarity, and temperature during recording as well as the introduction of pharmacological agents and odorants. A multichannel amplifier system allows us to simultaneously examine all the channels for activity. We are developing a data analysis system that will allow simultaneous recording and data analysis of 32 channels (generally not all of the electrodes are active) on a MassComp 5700.

The project is divided into two phases. Phase one is the analysis of the olfactory bulb cultures. The data indicate that olfactory bulb neurons form active neural networks in culture with single- and multiunits having tonic and phasic activity. This type of activity including rhythemic bursting is also seen in cultured spinal cord cell networks, although, in general, the activity of olfactory bulb cultures is higher than spinal cord cultures. Although we have yet to fully characterize the olfactory bulb neural network, we have embarked on phase two, which is the addition of tissue explants of olfactory neuroepithelium to a functioning olfactory bulb neural network.

389.5 CONSTRUCTION OF A RAT OLFACTORY NEURONAL CELL LINE. P. Hen,*[1] J. Dodd,[2] C. Cepko*[3] and R. Axel*[1] (SPON: P. Bovolenta). [1]Howard Hughes Medical Institute and [2]Department of Physiology, Columbia University College of Physicians and Surgeons, New York, NY 10032; [3]Department of Genetics, Harvard Medical School, Cambridge, MA 02115.

Olfaction in vertebrates is thought to be mediated by specific odorant receptors located on sensory neurons within the olfactory epithelium. The population of olfactory neurons is renewed throughout life by the differentiation of continually dividing basal cells. In order to study the cellular and molecular events underlying the transduction of olfactory information, we wish to obtain immortal cell lines capable of differentiating into mature olfactory neurons in cell culture. To this end, partially dissociated olfactory epithelia of young rats were exposed to recombinant retrovirus containing the immortalizing oncogene E1A of adenovirus 2, together with the neo R gene which confers resistance to the antibiotic G418. Transformed olfactory epithelial cells resistant to antibiotic were then examined for the expression of olfactory neuron markers. One clone, 13S.1, contained a subset of cells exhibiting a bipolar morphology and reactive with a number of antibodies which label carbohydrate determinants selectively expressed by olfactory neurons in vivo.

When 13S.1 cells were grown on a monolayer of cerebral cortex primary astrocytes, most of the cells extended long bipolar processes and over 50% of these cells expressed olfactory marker protein (OMP), a cytoplasmic protein found exclusively in mature olfactory neurons. These studies suggest that we have constructed an immortal olfactory neuroblast cell line which, upon exposure to astrocytes, undergoes differentiation to express a variety of properties characteristic of a mature olfactory sensory neuron. We are currently examining the response of these cells to odorants. The expression of odorant receptors by 13S.1 cells would support the use of this line as a homogeneous in vitro system with which to examine the molecular and cellular mechanisms of olfaction.

389.6 **A BOVINE OLFACTORY CILIA PREPARATION: SPECIFIC TRANSMEMBRANE GLYCOPROTEINS AND PHOSPHOPROTEINS.** **Rita Kropf*†, Doron Lancet, and Daniel Lazard*. Dept. of Membrane Research, the Weizmann Institute, Rehovot, Israel; †now at Dept. of Chemistry, Amherst College, Amherst MA 01002, USA.**

We have obtained cilia preparations from cow olfactory and respiratory epithelia, using the calcium shock technique previously used for rat and frog. The bovine preparation is advantageous because relatively large amounts of starting material are available, and because it allows one to make a clear distinction between olfactory and control nasal respiratory epithelia. The SDS polyacrylamide gel electrophoresis polypeptide patterns show a remarkable similarity between olfactory and respiratory cilia preparations. The glycoprotein pattern visualized by ^{125}I-Concanavalin A overlays is also very similar for both, including a common 85 kDalton glycoprotein previously thought to be homologous to frog gp95 (Chen et al., J. Biol. Chem. 261:1299, 1986). A notable exception is a prominent glycoprotein at 56 kDalton (gp56) which is found in olfactory, but not in respiratory cilia. gp56 behaves as a transmembrane glycoprotein in Triton X-114 and pH 11.3 extractions. A polypeptide with almost identical properties is also seen in a rat olfactory cilia preparation. Protein gp56 is thus an interesting mammalian olfactory receptor candidate, similar to the frog ciliary glycoprotein gp95. Efforts are now under way to purify it and study its possible function.

The olfactory cilia preparation in cow has a relatively low specific activity of adenylate cyclase, compared to that in rat and frog. Thus the background level of cAMP-dependent protein kinase (PK-A) activity is diminished, and the study of specific substrates for this enzyme is facilitated. We have identified cAMP-regulated phosphoproteins at 24, 40, 52 and 57 kDalton. For phosphoprotein 57 (pp57) [^{32}P]-phosphate incorporation decreased with added cAMP, and this polypeptide is tentatively identified as type 2 regulatory subunit of PK-A. The phosphorylation of the other three polypeptides markedly increased with cAMP addition. Phosphoprotein 52 (pp52) was notable in being both olfactory cilia-specific (compared with control respiratory cilia and deciliated olfactory epithelial membranes), and in behaving as a transmembrane protein in Triton X-114 extraction. Phosphoprotein 40 (pp40) is near to but does not comigrate with rat olfactory G_s α chain. Phosphoprotein 24 (pp24) may be akin to a similar cAMP-regulated phosphoprotein in frog (Heldman et al., J. Neurochem. 47:1527, 1986) and rat olfactory cilia. The role of these phosphoproteins in olfactory adaptation is being investigated (Pace et al., Neurosci. Soc. Abst. 13:000, 1987).

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389.7 DEVELOPMENTALLY REGULATED EXPRESSION IN OLFACTORY STRUCTURES OF REACTIVITY TO A MONOCLONAL ANTIBODY AGAINST FETAL OLFACTORY EPITHELIA. V.McM.Carr and A.I.Farbman.Dept. of Neurobiol. & Physiol., Northwestern Univ., Evanston, IL 60201.

The Mab 1 A-6 was initially raised in mice against fetal rat olfactory epithelia. 1 A-6 reactivity was examined in embryonic and neonatal rats using immunohistological techniques at light and electron microscopic levels. In the olfactory epithelia (OE) 1 A-6 reactivity appeared at the luminal surface of the nasal cavity. The intensity of this reactivity increased to a peak at E19 and then declined. Its initial appearance was quite early, at embryonic day 14 (E14). This luminal reactivity occurred on the surface membranes, microvilli, and cilia of olfactory dendritic knobs and supporting cells. Reactivity was also present in the respiratory epithelia (RE), as a punctate staining of some cell surfaces and the basal membrane. In both the OE and RE reactivity through at least E19 was noticeably greater on the medial than on the lateral walls of the nasal cavity.

In the olfactory bulb (OB) 1 A-6 reactivity also first appeared at E14. This initial OB reactivity was confined to the ventrolateral wall of the rostral cerebral vesicles, the site of the developing OBs. Little or no reactivity was present in the medial or dorsal walls. The most intense staining in the lateral wall was associated with radial processes that traverse the entire thickness of the wall. By the following day (E15), however, these reactive processes disappeared, and only short lengths of randomly oriented fibers showed reactivity. The lateral and medial walls were similar in appearance. As the OB assumed a more adult organization, reactivity subjacent to the olfactory nerve layer declined. Olfactory glomeruli showed reduced reactivity relative to the neighboring olfactory nerve fibers.

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389.8 ION CHANNEL MODULATION BY CAMP AND PROTEIN KINASE INHIBITOR.
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We reported (Soc. Neurosci. Abstr., Vol.12, Part 2, p.1353, 1986; Neurosci. Lett., 73, 253, 1987) a model system to study the initial electrochemical membrane events in chemoreception by the mammalian olfactory epithelium: membrane from rat olfactory epithelial homogenates (ROH) incorporated into planar bimolecular lipid membranes (BLM's). The steady-state conductance of BLM modified with ROH became sensitive to very low concentrations of odorant in the presence of adenosine triphosphate (ATP) and guanosine triphosphate (GTP). Adenosine 3',5'monophosphate (cAMP) mimicked the effect of the odorant. It was not known if changed levels of cAMP in our reconstituted system activated a protein kinase which phosphorylates the ion channel to modulate its activity or whether the cAMP directly gates the channel.

In this work we study the electrochemical properties of bilayers treated with ROH by the tip-dipping method. The bare BLM was first formed by the successive transfer of two phospholipid monolayers upon a tip of a patch pipet (about 1 u), and then the ROH was added to the *cis*-side of the membrane. The membrane unitary currents were measured with Yale Mk V Patch-clamp system. The signal from the clamp was filtered at low pass 3 kHz and recorded with PCM/VCR DASS System (Unitrade, Inc).

Analysis of single channel fluctuations indicate the existence of an ion channel of about 70 pS in 30 mM KCl, 30 mM NaCl, 2 mM $CaCl_2$, pH 7.4, activated by addition of cAMP (no ATP, GTP). The mean open time is about 1 sec. Subsequent addition of ATP to the *cis*-compartment of the system does not change the unitary amplitude, but causes a significant decrease of the mean open time to 6 msec. This activity was completely antagonized by protein kinase inhibitor. A change in the mean open time of single channel events in response to the presence of odorants was previously demonstrated (Vodyanoy and Murphy, Science, 220, 717, 1983). We believe that phosphorylation does not mediate the opening and closing of the channel, but is likely to modulate the mean open time in our reconstituted system. This suggests that cAMP regulates this channel activity in two ways: (a) directly, (b) *via* protein kinase system. These data are consistent with the hypothesis that cAMP is a second messenger in the initial steps of olfaction and a protein kinase can be involved in the ion channel modulation.

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389.9 OPTICAL RECORDING OF ELECTRICAL ACTIVITY IN SLICES OF MAMMALIAN OLFACTORY STRUCTURES: EXTRINSIC SIGNALS FROM OLFACTORY BULB AND PYRIFORM AND SULCAL CORTICES OF THE MOUSE. A.R. Cinelli* and B.M. Salzberg. Dept of Physiol., Univ. of Penn., Phila., PA, 19104.

Optical methods provide an opportunity to study the spread of activity in large numbers of neurons. Sagital or tranverse slices from the mouse brain were stained with a pyrazo-oxonol voltage sensitive dye (RH155 or RH482), and a system for multiple site optical recording of transmembrane voltage (MSORTV) was used to monitor changes in the light absorbance following focal electrical stimulation (1 to 3 mAmp, 300-500 usec) of the slice. All the brain structures exhibited both fast and slow depolarizing optical responses, whose relative magnitudes depended on the site of recording. The two dye signals had characteristic wavelength dependencies and disappeared in high KCL. No significant intrinsic signal was observed.

The fast component of the signal recorded from the olfactory bulb appeared to arise from action potentials propagating in the lateral olfactory tract and the mitral cells. Increased stimulus intensity resulted in the spread of the fast signal in the glomerular and external plexiform layer, and may indicate the genesis of action potentials in the dendritic tufts of the mitral or granule cells. Stimulation of the pyriform cortical slice ellicited fast and slow components in the sulcal region. Observation of the fast signal suggests a direct projection between these two cortical regions.

Both the slow component and the field potential recorded from the olfactory bulb were partially suppressed when tested by paired volleys or trials delivered at 1 Hz. The magnitude and spread of the fast and slow components evoked orthodromically and antidromically depended on temperature in a complex fashion. Cd^{++} and low Ca^{++}-high Mg^{++} solutions substantially reduced the size of the slow component. GABA (1 mM) reduced the magnitude of the slow component ellicited by orthodromic or antidromic stimulation. Bicuculine (10 uM) reversed the effect of GABA.

The slow component of the optical signal may derive from several sources: synaptic activity and/or electrotonic spread of action potentials in dendrites; and/or glial cell depolarization secondary to K^{+}-efflux.

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389.10 *IMPROVED VISUALIZATION OF THE SALAMANDER OLFACTORY BULB FOR OPTICAL RECORDING.*

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Recently, Kauer, Senseman and Cohen (Brain Res., *in press*) demonstrated the ability of potentiometric probes to optically monitor changes in neuronal activity in 124 anatomical regions of the salamander olfactory bulb in response to odor stimulation of the olfactory mucosa. For these experiments, Kauer *et al.* viewed the bulb, *in situ*, from its dorsal aspect. Since the laminae of the salamander olfactory bulb are arranged as a series of plates stacked along the rostral-caudal axis, they could readily observe differences in odor-evoked activity between bulbar layers but not regional differences across the laminar faces.

Since spatial differences in intra-laminar activation might be important for encoding odor quality information, we have developed a minimum preparation of the salamander olfactory system that provides improved visualization of the olfactory bulb for optical recording. The *in vitro* preparation consists simply of the anterior 2/3's of a single cerebral hemisphere attached via the olfactory nerve to its ipsilateral olfactory mucosal chamber. The transected hemisphere is mounted vertically in the recording chamber, parallel to the optical axis of the microscope, so that the bulb is viewed along its rostral-caudal axis. Mounting is accomplished by slipping the cut rostral end of the hemisphere over a vertical 1mm dia clad quartz rod that fits within the large cerebral ventricle. The olfactory bulb itself rests on top of the clad rod which also serves as a light guide for illumination.

We are currently using this preparation to map the central projections of the primary olfactory receptors to their post-synaptic bulbar targets. A miniature concentric bipolar electrode is being used to electrically activate small patches of primary olfactory receptor neurons within various regions of the dorsal and ventral olfactory mucosa while optically monitoring evoked responses in the olfactory bulb with a 124-element photodiode array. In accord with earlier histological investigations, our initial studies show that focal electrical stimulation of the receptor mucosa evokes relatively widespread, but not spatially uniform, activity in the olfactory bulb. Moreover, the widespread distribution of post-synaptic responses is not significantly altered by a 100-fold change in stimulating voltage. This suggests that our electrode current is, in fact , restricted to a small patch of mucosa membrane so that the widespread effects observed in the olfactory bulb are not likely the result of activating nerve fasicles passing through the stimulated mucosal region.

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389.11 COMPARISON OF MITRAL/TUFTED CELL RESPONSES TO ODOR AND ELECTRICAL STIMULATION IN THE OLFACTORY BULB OF THE TIGER SALAMANDER. K.A. Hamilton and J.S. Kauer. Depts. of Neurosurgery, Anatomy and Cell Biology, Tufts- New England Medical Center, Boston MA 02111.

When a defined odor pulse is applied to the olfactory mucosa of the tiger salamander, the action potential activity generated in mitral and/or tufted (M/T) cells in the olfactory bulb often can be classified as an excitatory (E) or suppressive (S) response type. Using intracellular recording techniques, we have previously shown that periods of depolarization and hyperpolarization underlie the E- and S-type odor response patterns (Hamilton, K.A and J.S. Kauer, Brain Research, 338:181, 1985). In the present study, the contributions of synaptic connections to the generation of response patterns in 47 M/T cells were investigated, by examining the responses both to odors and to electrical stimulation of the olfactory nerve and olfactory tract.

Most odor responses began with hyperpolarization. In E-type responses, this initial hyperpolarization was followed by a period of excitation, which in turn was followed by a prolonged period of hyperpolarization and suppression of spontaneous action potentials. The latencies of hyperpolaration and excitation decreased with increases in odor concentration. Action potentials evoked in E-type responses tended to occur earlier, however, than any action potentials evoked in S-type responses. In response to high odor concentration, action potentials preceded the onset of hyperpolarization in E-type responses, but not in S-type responses.

In response to electrical stimulation, any evoked excitation preceded hyperpolarization, unlike most odor responses. As in the odor responses, latencies of depolarizing and hyperpolarizing components of the responses decreased with increases in stimulus intensity. The peak amplitude and duration of hyperpolarization also changed. An analysis of response components indicated that increases in intensity affected responses to stimulation of the olfactory nerve and medial or lateral tracts somewhat differently.

Together with results of tests conducted while hyperpolarizing cells with anodal current and using paired stimuli, these results indicate that the distribution of excitatory and inhibitory synapses on the M/T cell dendrites and soma in the tiger salamander is comparable to the distribution which appears to exist in other vertebrate species. The results further suggest that these synapses are activated differently by orthodromic and antidromic electrical stimulation, and also in E-type and S-type responses to odors. The patterns of activation evoked by odor stimulation generally do not appear to resemble the patterns evoked by electrical stimulation in the salamander olfactory bulb.

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389.12 ODOR INDUCED RESPONSES OF MORPHOLOGICALLY IDENTIFIED RAT OLFACTORY BULB NEURONS David P. Wellis and John W. Scott. Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

While the basic circuitry of the rat olfactory bulb has been established, how this circuitry participates in sensory coding of odorant information remains to be determined. Extracellular electrophysiological techniques fail to identify all olfactory bulb cell types during studies of odor responsiveness. Therefore, we are using intracellular recording and marking to study sensory processing in the olfactory bulb.

Recordings were made with KCl-horseradish peroxidase (HRP) filled microelectrodes bevelled to resistances of 60-130 MOhms. Ten cells have been recorded for periods of 15 minutes to over 4 1/2 hours with membrane potentials of -40 to -75 mV. Antidromic activation of output neurons from the lateral olfactory tract, olfactory tubercle and posterior piriform cortex defined projection patterns. Responses to electrical stimulation of the olfactory nerve layer were observed and compared with odor induced responses. Odors were presented in random series of 6 concentrations spanning 2 log units.

Several output cells (mitral and tufted cells) as well as interneurons (a superficial granule cell and a superficial short axon cell) exhibited odor responses and were filled with HRP. All output cells exhibited complex temporal patterns to different odors over a range of concentration. These responses were stable over time and were systematically correlated with odorant concentration by our measure (Harrison and Scott, J. Neurophysiol. 56:1571, 1986). Previously, cells not antidromically activated were presumed to be interneurons. The two interneurons we have filled so far in this study unequivocally show that certain interneurons do respond to odor and in fact exhibit steeper stimulus-response functions than output neurons in this study. In one middle tufted cell, the response pattern changed with hyperpolarization but without disrupting its graded nature across odorant concentration. This suggests that tonic inhibition by olfactory bulb local circuits has significant effects on olfactory coding.

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389.13 OLFACTORY BULB REMOVAL IN THE RAT RESULTS IN INCREASED BASAL CELL MITOTIC ACTIVITY. M.A. Schwartz*, D.M. Chikaraishi and J.S. Kauer. Neuroscience Program, Tufts-NEMC, Boston, MA 02111.

The olfactory system has the unique ability to regenerate receptor cells from undifferentiated stem cells in adult animals. Biochemical and morphological evidence for regeneration of the olfactory neurons following axotomy or bulbectomy is well established, with regenerating axons reestablishing synapses with their target site, the olfactory bulb (OB) (Graziadei, P.P.C. Br. Res. (1980)186:289-300). In both normal turnover and experimentally induced regeneration, the replacement elements are the basal cells residing in the olfactory epithelium (OE). In the present study, it has been shown that removal of the olfactory bulb in adult rats gives rise to a significant increase in the level of mitotic activity in the ipsilateral basal cell layer of the OE.

Right olfactory bulbs of adult (2-3 months) male, Sprague-Dawley rats were removed by aspiration. The left side served as an intact control. Four days after bulbectomy, each animal received an injection of ^{3}H-Thymidine (^{3}H-TdR) for one hour, was deeply anesthetized with ketamine, and was perfused. The OE and OB were sectioned at 8um and 10um, and prepared for autoradiography. Histological sections through the OB confirmed a complete right bulbectomy. The OE on the bulbectomized side was half the thickness of the control side, due to a marked decrease in the receptor cell layer as assessed using morphological characteristics. Counts of ^{3}H-TdR labelled basal cells revealed a significant increase ($p<.01$) in the mitotic activity on the bulbectomized side compared to the control side. In each animal counts were taken from representative homologous regions of epithelium along the septum, 500um in rostral/caudal extent. In this area, a range of 45-100% increase of mitotic activity over the control side was observed. Counts of labelled cells on the control side of bulbectomized rats gave a basal level of turnover not significantly different from age-matched, unoperated control animals. On the experimental side, the cells that incorporated the radioactive label were not randomly distributed, but were grouped in patches. Mitotically active contiguous cells, separated by at least 60um, constituted a patch. The size of the patches varied along the septum and contained as few as 3 or as many as 10 labelled cells.

These results demonstrate that removal of the olfactory bulb results in an increase in ^{3}H-Thymidine incorporation by the basal cells over that found in control regions. These observations suggest that the patches of active mitosis may represent groups of functionally related cells.

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389.14 THE MORPHOLOGY AND DEVELOPMENT OF DENDRITIC SPINES MEDIATING RECIPROCAL DENDRODENDRITIC SYNAPSES. Charles A. Greer. Sec. Neurosurgery and Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

Reciprocal dendrodendritic synapses occur between the output neurons of the olfactory bulb, mitral and tufted cells, and a population of interneurons, granule cells. As part of an ongoing effort directed at elucidating the properties of these dendritic microcircuits, the present study was undertaken to examine the morphological features of the granule cell dendritic spines. In general, postsynaptic spines exhibit a wide range of shapes and sizes which in turn, influence the extent to which an individual spine attenuates the effectiveness of apposed synapses. The current study sought, in part, to establish if comparable morphological variation occurs in the functionally more complex spines involved in reciprocal synapses.

Granule cell spine morphology was initially studied in Sprague-Dawley rats with a developmental series of Golgi stained material processed for light microscopy (LM). In subsequent studies Golgi-EM material was gold-toned and processed for either conventional transmission electronmicroscopy (TEM) of thin (70nm) sections or high voltage electronmicroscopy (HVEM) of thick (1 - 4um) sections.

The LM and EM analyses revealed the presence of both sessile and pedunculated spines although the latter were predominant. Morphometric analyses demonstrated that spine dimensions increased between 3 and 21 days postnatal. The largest increase (27%) was in the length of the neck while smaller increases were found for the major and minor axes of the spine head (14% and 12%, respectively). By 21 days postnatal spine necks had a mean length of 1.96um (±0.1), but often exceeded 4um. The HVEM analyses demonstrated that spine necks often followed tortuous courses parallel to the parent dendrite. Of particular interest, the HVEM analyses also revealed complex spinous appendages in which the spine neck bifurcated 1 or more times. Serial reconstructions of the TEM material provided a further confirmation that the asymmetrical afferent synapse and symmetrical efferent synapse of the spines occur as reciprocal pairs. The TEM analyses also show that a single spine can sustain more than 1 topologically segregated reciprocal pair.

The data reveal a complexity of granule cell dendritic spines not previously recognized. Morphometric analyses suggest that their biophysical properties may change significantly during postnatal development, possibly reflecting postnatal increases in olfactory acuity previously documented. In addition, the data suggest that some spines may be effectively isolated from the parent dendrite and thus may function as segregated units.

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389.15 THE CHANGES IN THE TRIPARTITE OLFACTORY SYSTEM OF XENOPUS LAEVIS (ANURA, AMPHIBIA) DURING METAMORPHOSIS: AN ELECTRON MICROSCOPICAL AND TRACT TRACING STUDY

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The olfactory organ of the South African clawed frog, Xenopus laevis, was studied in late larval and postmetamorphic stages by means of electron microscopy.

The olfactory organs consist of three parts: the medial diverticulum (MD), the lateral diverticulum (LD) and the vomeronasal organ (VNO). Sensory epithelia occur in all three parts. Receptor cells are lacking in the LD of terrestrial anurans. Their presence in the aquatic Xenopus is regarded as an adaption for smelling underwater.

The ultrastructure of the sensory epithelia corresponds to the general vertebrate scheme. However, the sensory epithelia show specialisations depending upon the medium above (air or water). Their receptor cells have either cilia or microvilli, and their supporting cells either secretory granules or motile cilia. The presence or absence of intraepithelial glands is another characteristic feature.

No substantial changes occur in the sensory epithelia of the LD and the VNO during metamorphosis. In the MD, however, the larval water-flooded epithelium changes to a postmetamorphic air-flooded epithelium.

In tadpoles and postmetamorphic animals the olfactory bulb consists of two parts: the main olfactory bulb (MOB) and the accessory olfactory bulb (AOB). As in terrestrial anurans, the MOBs of the left and right side are fused in the midsagittal line.

The termination of the axons of the receptor cells within the glomerular layer of the olfactory bulb was studied by injections of horseradish peroxidase into the sensory epithelia. Clear topographical relationships were observed. In larval stages, the axons of sensory cells of the MD project to all regions of the MOB; those of the LD run to the ventrolateral region of the MOB, and those of the VNO terminate exclusively in the AOB. In postmetamorphic stages, however, the axons of the sensory cells of the MD project to the medial, dorsolateral and dorsal regions of the MOB, those of the LD again terminate in the ventrolateral region of the MOB.

A neurogenesis of receptor cells is proposed as a mechanism to segregate fibers of the MD and LD in the glomerular layer of the MOB during metamorphosis.

390.1 ELECTROPHORETIC CHARACTERIZATION OF CARBON-14 BOUND TO MOUSE BRAIN TISSUE AFTER THE IN VIVO ADMINISTRATION OF [^{14}C]L-DEPRENYL. S. L. Dewey, A. P. Wolf*, R. R. MacGregor* and J. S. Fowler* Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973

Recently, we reported that carbon-11 labeled clorgyline and L-deprenyl and positron emission tomography could be used to map the distribution of monoamine oxidase A and B in living human brain (Science 235: 481-485, 1987). For both tracers the binding of carbon-11 was significantly reduced by prior inhibition with an MAO inhibitor and for [^{11}C]L-deprenyl the binding was shown to be stereoselective. Since PET is a non-invasive procedure which does not involve the direct sampling and analysis of tissue, we have designed a series of experiments to more fully characterize the binding of L-deprenyl to brain tissue. In the present study, we have used [^{14}C]L-deprenyl and SDS polyacrylamide gel electrophoresis to identify the proteins which are labeled with carbon-14 after its in vivo administration in adult mice.

N-[^{14}C-Methyl]L-deprenyl was injected into a lateral tail vein of adult female mice (30-35g). Following a 30 minute survival period, brains were excised and homogenized in 10.0 vols. of 0.25 M sucrose (4°C). Mitochondria were isolated according to Laduron, et al., J. Neurochem. 41: 83-94, 1983. Pellets were washed by resuspension in 7.0 ml, 50 mM Tris-HCl, pH 7.5 and centrifuged. The final pellet was resuspended in 50 µl of a solution containing 3% SDS, 0.15M sucrose, 7.0% 2-mercaptoethanol, 0.05% bromophenol blue and 0.03M Tris-HCl, pH 7.5. Electrophoresis was carried out in 10% polyacrylamide slab gels in the presence of SDS. Gel slices (2.5 mm) were incubated overnight at 25°C in 5 ml of a mixture containing Aquasol and 4% Protosol (NEN) (Edwards and Pak, Biochem. Biophys. Res. Comm. 86: 350-357, 1979). One band of radioactivity was detected by this method. It had a molecular weight of 58,000 daltons, as estimated in linear 10% gels by the migration of known molecular weight proteins. This is consistent with the molecular weight of MAO as determined using [^{3}H]pargyline (Costa and Breakfield, Mol. Pharm. 16: 242-249, 1979).

In summary, the results of this study show that [^{14}C]-L-deprenyl can be used to specifically identify MAO B from crude mitochondrial preparations on SDS-polyacrylamide gels and support the use of [^{11}C]-L-deprenyl and positron emission tomography to determine the regional distribution of MAO B in human and primate brain.

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390.2 ^{14}C-DEOXYGLUCOSE MAPPING OF COTURNIX QUAIL BRAIN: RESPONSE TO VARIOUS PHOTOPERIODS. M. M. Beck and K. E. Borg*, Dept. of Animal Science, Univ. of Nebraska; B. D. Schambacher*, USMARC, Clay Center, NE; and K. M. Eskridge*, Biometrics and Information Center, Univ. of Nebraska, Lincoln, NE 68583-0908.

Male Japanese quail, raised on an 8L:16D photoperiod, were separated into 3 groups at 10 wks of age: longdays (16L:8D), shortdays (8L:16D), or skeleton longdays (8L:4.5D:2L:9.5D). Additional treatments within photoperiod were pinealectomy, sham-pinealectomy, antimelatonin, control serum, or no treatment. Testicular weights and serum testosterone were used as markers of reproductive response. In all responses, longday photoperiod had significant effects (P<.05), with shortday responses always less, and skeleton longday responses intermediate. Autoradiographic analysis, using Kodak SB-5 x-ray film, and the Bio-Quant microdensitometry system, showed both tectofugal and thalamofugal visual pathways labeled by the deoxyglucose. Photoperiod did not stimulate either pathway preferentially, but longday brains had densest labeling (highest metabolic activity), with shortday brains least (lowest activity), and skeleton longday brains intermediate. At 6 mos of age, an additional 12 quail, maintained on 16L:8D, were divided, with one group receiving 8L:16D and the rest remaining on longdays. Testicular weights and cloacal gland atrophy were used as indicators that shortday photoperiod had reversed reproductive condition. Analysis of autoradiographs of the 2 groups, 10 days after initiation of photoperiod, showed that metabolic activity of visual nuclei was significantly reduced by shortday exposure. The consistent lack of treatment effects within photoperiod confirms earlier research that the pineal does not mediate stimulatory photoperiod response in the quail. Residual extra-pineal melatonin apparently is also not involved in the response. In no birds was the SCN stimulated metabolically.

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390.3 CEREBRAL GLUCOSE UTILIZATION AFTER REPEATED STRESS AND DURING CONDITIONED FEAR IN THE RAT. R.A.W. Lehman and R.M. Bryan. Departments of Surgery (Neurosurgery) and Physiology, The M.S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033.

Regional cerebral glucose utilization (rCMRglu) was studied in rats after repeated stress and during conditioned fear. Four groups of rats were studied. Two groups of rats were aversely conditioned by placing them in a shock chamber (conditioned stimulus) where they received random footshocks. The two remaining groups were placed in the shock chamber but not conditioned. Regional CMRglu and systemic parameters (heart rate, blood pressure, blood gases and pH, plasma catecholamines, and plasma glucose) were measured in unconditioned and conditioned rats in the presence and in the absence of the conditioned stimulus. The changes in rCMRglu described below appeared to be global and not limited to specific regions. Results are as follows: (1) Transferring unconditioned rats to the shock chamber had no significant effect on rCMRglu even though the systemic parameters indicated a stress response. It appears that stress capable of inducing changes in heart rate, MABP, and plasma catecholamines is not necessarily accompanied by increases in cerebral glucose utilization. (2) Conditioned rats not exposed to the shock chamber at the time rCMRglu was measured had decreased rates of rCMRglu compared to rats that were not conditioned. Except for plasma epinephrine, which increased after conditioning, systemic parameters were not affected. (3) Conditioned fear, elicited by transferring conditioned rats to the shock chamber, increased rCMRglu when compared to a control group that was conditioned to footshock using the same paradigm but not exposed to the shock chamber at the time rCMRglu was measured. The systemic parameters indicated a stress response in conditioned rats transferred to the shock chamber. We conclude that past experience of shock can manifest itself by altering cerebral glucose utilization. Aversive conditioning acted to suppress basal rCMRgl and the conditioned fear paradigm was associated with an increase in rCMRgl superimposed on the suppressed basal rate. [Supported by a Grant-in-Aid from the American heart Association (RMB) and with funds contributed in part by the American heart Association, Palm Beach County Chapter, Florida and PHS grant NS 19341 from the National Institute of Neurological, Communicative Diseases, and Stroke (RMB).]

390.4 CEREBRAL METABOLISM AND EEG: RELATIONS TO COGNITIVE TASK. J. T. Metz, N. J.Yasillo*, and M. Cooper*. PET Center, University of Chicago, and Illinois State Psychiatric Institute, Chicago, IL 60637.

EEG alpha activity has long been known to decrease as a subject's attention to a task increases. Cerebral metabolism has also been presumed to change as the neuronal processes mediating cognition change. The relationships between these physiological measures, however, have not been directly examined in a behaviorally controlled experiment. In this study, we simultaneously recorded regional cerebral metabolism and multi-channel EEG while varying complexity of a cognitive task.

Six normal males, aged 20-28, were studied on two occasions each. Metabolic rates during 40 minutes of performing each of two tasks were determined with ^{18}fluoro-2-deoxyglucose and a PETT VI tomograph. EEG during task performance was recorded on analog tape from 16 leads referred to the forehead. Both tasks were presented on a computer screen. The computer also measured accuracy of response and reaction time to each stimulus. The complex cognitive task was a variation of the Wisconsin Card Sorting Test (WCST), a task which involves memory and the ability to adapt to changing conditions. Lesion studies have shown that this task requires functioning of the frontal cortex. The control condition was a simple match-to-sample task which had sensory, motor, and instructional demands similar to the WCST. Half of the subjects received the control task first, half received the WCST first.

A researcher who was blind to the task condition identified 44 regions of interest from three PET slices parallel to the orbital-meatal plane (the slice through the plane of the basal ganglia and the slices immediately above and below the basal ganglia). Metabolic rates in these regions were averaged and z-score normalized for each session. The EEG from each channel was digitized (200 hz) in 2.56 second epochs. Ten artifact-free epochs per subject, sampled during the first 10 minutes of each task, were transformed by the fast Fourier technique and averaged to provide quantification of power in five spectral bands.

The average time to respond to stimuli was significantly longer on the WCST than on the control task (1942 vs. 989 milliseconds), confirming that the WCST was a more demanding task. Average metabolic rate across all regions was significantly higher when the subjects were performing the WCST (mean of 7.69 vs. 5.95 mg/100 grams/minute, $t = 3.86$, $p < 0.02$). The change in average metabolism between the control task and the WCST was significantly correlated with change in alpha power ($r = -0.90$, $p < .05$).

None of the normalized regional metabolic values showed a statistically significant difference between the two tasks. The decrease in alpha power was found in all areas, but was most prominent in temporal and parietal leads. Across subjects and within each task condition, no EEG parameter was strongly related to metabolism.

These results demonstrate that as the complexity of a cognitive task increases, reaction time increases, average cerebral metabolic activity increases and average alpha power decreases. Alpha power was found to be strongly reflective of changes in cerebral metabolism. We were not able to demonstrate that specific brain areas were uniquely involved in the performance of either task.

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390.5 POSSIBLE EVIDENCE OF A FUNCTIONAL DISCONNECTION BETWEEN ORBITAL CORTEX AND CAUDATE NUCLEUS IN UNIPOLAR DEPRESSION USING PET. J.M. Schwartz*, L.R. Baxter, Jr., *, M.E. Phelps, J.C. Mazziotta, B.H. Guze*, C.E. Selin* (SPON: D.X. Freedman). Depts. of Psychiatry, Neurology and Div. of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles, CA 90024.

A substantial body of evidence demonstrates a close functional connection between prefrontal cortex (PFC) and the head of the caudate nucleus (Cd) (e.g. I. Divac, in Functions of the Basal Ganglia, Ciba Fdn. Symp. 107, Pitman Pr, 1984, pp201-215). Previous work by our group (Baxter, et al, Arch Gen Psychiat 42:441-447, 1985), using PET, has shown a decrease in local cerebral metabolic rates for glucose (LCMRGlc) in the Cd relative to the ipsilateral hemisphere in patients with unipolar depression, mainly accounted for by a decrease in LCMRGlc of the Cd with respect to PFC (Schwartz, et al, JAMA, in press).

We extended this work, looking at PFC-Cd relationships in a group of age and sex matched normal controls (NC, n=14,31.6+4.5 yrs), unipolar depressed (UD, n=10, 36.0+11.0) and bipolar depressed (BD, n=10, 38.4+14.0) subjects. Depressed patients were carefully diagnosed and showed comparable scores on the 21-item Hamilton Rating Scale for Depression (UD=22.1+4.9; BD=25.0+5.8). We calculated correlation coefficients and performed linear regression analyses between LCMRGlcs for each Cd and the ipsilateral middle frontal gyrus (MFG), inferior frontal gyrus (IFG) and orbital gyrus (OG) for each group.

As expected, NC showed high correlation coefficients between LCMRGlcs for each of these prefrontal regions and the Cd, with all rs>.9 ($p<10^{-5}$). Coefficients of determination ranged from .81 to .88. Furthermore, the BD group also showed high correlations on these measures: all r >.9 (p<.0005) and coeff of deter >.82, except for the right OG and Cd where they were .84 (p<.002) and .70, respectively. In the UD group, there was a marked discrepancy in the lack of any significant correlations between LCMRGlcs for OG and Cd: on the left r=.49 (p=.15), coeff of deter =.24; on the right r=.42 (p=.22) coeff of deter = .18. In contrast the MFG and IFG showed high correlations with Cd activity, with all r>.85 (p<.002) and all coeff of deter >.72. These data may indicate a loss of functional interaction between the OG and Cd in unipolar depression.

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390.6 LOCAL INJECTION OF TETRODOTOXIN DECREASES METABOLIC ACTIVITY IN DISCRETE BRAIN REGIONS: A 2-DEOXYGLUCOSE AUTORADIOGRAPHY ANALYSIS L.Cahill, R.M.Coopersmith, M.Leon, and J.L.McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717

The production of reversible brain "lesions," with local injections of drugs such as tetrodotoxin or procaine, is a powerful method of analyzing brain function. With such procedures, however, the extent and duration of the treatment is often unknown. In this study we have used 14C-2-deoxyglucose autoradiography (2DG) and the Fink-Heimer stain for degenerating axons to determine the effects of an intra-cranial injection of tetrodotoxin (TTX).

Male Sprague-Dawley rats were implanted bilaterally with guide cannulae terminating just above the amygdaloid complex. After recovery from surgery, the rats received an injection of TTX in one amygdala and vehicle in the other, allowing for within rat comparisons of the TTX effects. In the first phase of the experiment, rats received 0.1, 0.4, or 1.0 μl of a 10 ng/μl TTX solution, followed five minutes later by an intravenous injection of 2DG (150 μC/kg). Forty-five minutes later, the rats were decapitated and the brains frozen in freon. After cryostat sectioning (20 μm), the tissue was exposed for 10 days and the autoradiographs analyzed with a computer-based digital image processor. In the second phase, delays of 2, 4, 8, and 12 hours were placed between the TTX and 2DG injections. Finally, the brains of some rats receiving unilateral TTX (but no 2DG) were stained for degenerating axons by the Fink-Heimer method.

The results show that: 1) Intra-amygdala TTX injections produce significant reductions in 2DG uptake in specific regions, with the largest and most consistent effects seen in the basolateral amygdala; 2) This effect was not seen with 2DG injections delayed 8 and 12 hours after TTX; 3) No degeneration is seen in areas receiving TTX compared to those receiving vehicle. It is concluded that TTX reversibly slows metabolic activity in discrete regions following local injection, and produces no neuronal death.

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390.7 DIFFERENTIAL SENSITIVITY TO COCAINE IN LEWIS AND FISCHER-344 RATS AS INDICATED BY LOCAL CEREBRAL GLUCOSE UTILIZATION. G. Wilkerson*, S.R. Goldberg*, M. Risner, and E.D. London (SPON: B.E. Hackley). Neuropharm. Lab., NIDA Addiction Res. Ctr., Baltimore, MD 21224.

It is well-established that cocaine is a reinforcer with a high abuse potential in humans. However, little is known about genetic factors which may contribute to cocaine sensitivity and, perhaps thereby, to susceptibility for cocaine abuse. We therefore assessed the sensitivity to cocaine, as indicated by rates of local cerebral glucose utilization (LCGU), in two commonly available inbred strains of albino rats.

Rates of LCGU were measured in partially restrained, 4-6-month-old, male rats of the Lewis (L) and Fischer-344 (F) strains, which were treated acutely with cocaine HCl (1, 10, or 30 mg/kg, i.p.) or saline (control) 5 min before the i.v. injection of the radiotracer (100-125 μCi/kg 2-deoxy-D-[1-^{14}C]glucose, DG). Timed arterial blood samples were obtained, and LCGU was determined autoradiographically in fifty-nine brain regions, as described by L. Sokoloff et al. (J. Neurochem. 28:897, 1977).

F rats showed no overt behavioral effects or LCGU alterations with 1 mg/kg cocaine. The 10 mg/kg dose produced stereotypies (head weaving, sniffing) in 17% of the animals and decreased LCGU in the lateral habenula. At 30 mg/kg cocaine, 80% of the rats showed stereotypies and LCGU stimulation in several components of the extrapyramidal motor system (caudate-putamen, globus pallidus, substantia nigra reticulata, subthalamic nucleus (n.), cerebellar vermis), but reduced in the lateral habenula. No significant effects in several limbic areas implicated in drug-induced reward (e.g., accumbens n., ventral tegmental area) were noted.

L rats showed a greater cocaine sensitivity, indicated by a tendency for LCGU stimulation by 1 mg/kg cocaine in several areas, including the ventral tegmental area, accumbens n., and several neocortical areas. At 10 mg/kg, 75% of the rats showed stereotypies. Also at this dose, extrapyramidal areas affected only at 30 mg/kg cocaine in F rats showed stimulation, and LCGU was decreased in the lateral habenula. All L rats treated with 30 mg/kg cocaine showed marked stereotypies and a suppression of LCGU in many areas.

The greater sensitivity of L as compared to F rats to cerebral metabolic effects of cocaine is consistent with a previous report that L rats are more responsive to open field activating effects of cocaine (George, F. R. et al., Fed. Proc. 46:402, 1987). Taken together, these findings support the view that there are genetic differences in the sensitivity to cocaine, which may be related to susceptibility to cocaine abuse. Further studies are indicated to determine whether the observed strain differences in response to cocaine reflect genetic differences in metabolism or CNS sensitivity.

390.8 BRAIN SLICE GLUCOSE UTILIZATION USING ^{14}C-2-DEOXYGLUCOSE. G.C. Newman, F.E. Hospod and P. Wu. Department of Neurology, SUNY, Stony Brook, New York 11794.

Measurement of glucose utilization using the 2-deoxyglucose tracer kinetic model of Sokoloff et al[1] is one of the fundamental means of assessing brain activity in vivo. The ability to measure actual glucose utilization in vitro, especially in brain slices, would facilitate a wide variety of experiments including pharmacological studies, determination of physiological events associated with glucose utilization and studies of brain slice preservation. Most importantly, the ability to measure glucose utilization in vitro would permit the direct comparison of in vitro and in vivo results. To calculate in vitro glucose utilization, it is necessary to modify the equation for glucose utilization and to remeasure the kinetics constants that apply, including the lumped constant.

We have performed the necessary modifications, remeasured the constants and tested the model using the hypothalamic brain slice containing suprachiasmatic nucleus (SCN). Since SCN has a spontaneous cycle of metabolic activity in vivo that is not dependent upon neural input and that persists in vitro,[2] this slice is well-suited to developing methods. The kinetics constants were remeasured for whole hypothalamic slices by incubation with ^{14}C-2-deoxyglucose (^{2}DG), separation of 2DG from 2-deoxyglucose-6-phosphate (2DG6P) by anion exchange chromatography and rinse times were used to study influx or efflux process. The lumped constant was estimated by using the hexokinase Michaelis-Menton constants for 2DG and glucose, assuming small glucose-6 phosphatase activity and measuring the volumes of distribution for 2DG and ^{14}C-glucose in the brain slice. The in vitro system permits phosphatase activity to be measured directly from long term efflux experiments and the small phosphatase activity can thus be confirmed.

Using these constants and the modified equation, we have measured glucose utilization in the SCN and adjacent anterior hypothalamic area during subjective daytime and nighttime. SCN glucose utilization reaches a high of 68 and a low of 48 umole glucose/g tissue/min throughout the day while AHA remains uniformly low at about 30 umole glucose/g tissue/min. These results are entirely consistent with prior in vivo measurements.[3,4] Studies with hippocampus, cerebral cortex and thalamus are in progress.

We have demonstrated the feasibility of measuring actual glucose utilization in vitro using the ^{14}C-2-deoxyglucose method with modifications for in vitro incubations and anticipate wide application for this method in brain slice research.

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390.9 MCPP, a central serotonin agonist: its effects on cerebral metabolism, in relation to behavior and neuroendocrine response in the rat.

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1-(m-chlorophenyl) piperazine (MCPP) is a metabolite of trazodone, a drug used clinically as an antidepressant, and a potent 5-HT agonist in the central nervous system. Its metabolic formation from trazodone has been suggested to account for the antidepressant activity of trazodone.

We measured local cerebral glucose utilization (LCGU), using the quantitative [14C]-2-deoxy-D-glucose method, in awake 3 mo old male Fischer-344 rats at 5, 15, 30 or 60 min after MCPP 2.5 mg/kg or saline i.p. Behavior was evaluated at fixed time intervals after MCPP administration.

The drug produced behavioral and neuroendocrine changes. Sedation, with a decrease of spontaneous locomotor activity and hunched posture, increases in plasma prolactin and corticosterone levels, and a decline in growth hormone concentration, were observed in the animals. These effects were evident within 5-10 min after administration and peaked at about 15 min, thereafter decreasing to reach pre-treatment levels after 1 h. LCGU was decreased significantly ($p<0.05$) at 15 min after MCPP in 24% of the 71 brain regions examined, with a 20% decline of mean cerebral metabolism, as compared to controls. At 1 h after injection of MCPP, LCGU values were similar to pre-treatment values. Cerebral metabolism declined in most neocortical areas (layer IV), the olfactory system, basal ganglia, thalamic and hypothalamic nuclei and hippocampus.

The topography of changes in LCGU after MCPP suggests a preferential action of the drug in areas where 5-HT receptors are present in high density. The peak decline of cerebral metabolism, coupled temporally with hypomotility and peak neuroendocrine effects, is consistent with the role of 5-HT in these functions. The effect of MCPP in some brain regions which have been reported to be involved in the mediation of anxiety (Lader, 1983) also is consistent with the reported anxiolytic properties of trazodone in the clinical use.

390.10 EFFECTS OF ADENOSINE ON ATP AND TOTAL ADENYLATE CONTENT OF RAT HIPPOCAMPAL SLICES.

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Previous results have shown that a 3 hr exposure of hippocampal slices to 25 mM creatine in the incubation medium greatly increased the phosphocreatine content of those slices and also significantly prolonged their ability to maintain electrical function during transient anoxia. The creatine effect may be attributed to increased neuronal energy reserves at the onset of the anoxic insult, or by increased hydrogen ion buffering afforded by the creatine kinase system. If creatine exposure is prolonging synaptic transmission during anoxia by maintaining local ATP levels, then a similar protective effect should be provided by directly increasing slice ATP content.

Hippocampal slices (500 µm) were prepared and incubated in standard bicarbonate medium (125 mM NaCl, 3.0 mM KCl, 1.4 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2.24 mM $CaCl_2$, 26 mM $NaHCO_3$, 10 mM glucose, and equilibrated with 95% O_2/5% CO_2 to adjust pH to 7.4) in a heated shaker bath.

The addition of 8 mM adenosine, an adenylate precursor, following a 1 hr pre-incubation in standard medium, increased hippocampal slice ATP and total adenylate (ATP+ADP+AMP) levels over a period of 5 hr. ATP levels rose from about 10 nmol/mg protein to 50 nmol/mg protein, and total adenylates from 15 to 19.5 nmol/mg protein during these incubations. Slice energy charge increased slightly (from 0.782 to 0.851) in both control and adenosine-treated slices during a 5 hr incubation, indicating that the slices maintained a normal distribution of the adenylate pool in the presence of adenosine. The increase in the adenylate pool was time-dependent, and the presence of adenosine during slice preparation and the initial hour of incubation did not appear to significantly enhance the metabolic effects. Initial results indicate that the enhanced adenylate pool remained elevated after removal of adenosine from the incubation medium, though some loss appears to occur over two hours in normal medium. Recent experiments also suggest that phosphocreatine and total creatine levels may be decreased by the adenosine incubation. This would tend to minimize the energetic benefits of accumulated ATP.

The apparent gain of ATP and loss of phosphocreatine may make it possible to delineate between the energetic and pH effects by which creatine incubation may have acted to provide functional protection during transient anoxia.

390.11 PALMITATE INCORPORATION FOLLOWING HYPOGLOSSAL AXOTOMY IN ADULT FISCHER 344 RATS.

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The effects of axonal regeneration and degeneration on palmitate incorporation into the hypoglossal nucleus were studied using autoradiography applying the method of Kimes et al(1983). Three mo. old male Fischer-344 rats were axotomized on the left side using one of two operations. The 'R'-operation was to transect the hypoglossal nerve and remove a 3 mm fragment. The "D"-operation was to pack the transected proximal end in silicone glue after removing a 1.0 cm segment of nerve. One to 84 days after axotomy, [U-14C]palmitate (450 µCi/kg) was injected i.v in awake animals. Animals were killed after 4 hrs, and brains were sectioned into 20 um at -20 C. Sections were exposed against Kodak SB5 film for 5 weeks. Optical densities of 5 regions, including the hypoglossal nucleus, were determined and the percent differences between operated and unoperated sides were calculated. In the "R"-operation group, palmitate incorporation into axotomized hypoglossal nucleus showed increases of 6%, 9% and 21% against the unaxotomized side ($p < 0.01$) on days 4, 12, and 24 days, respectively, after axotomy. The difference declined gradually thereafter, disappearing on day 70. In the "D"-operation group, at 24 and 35 days after axotomy, there was a 7% and 7.5% decrease ($p < 0.01$) respectively on the axotomized side. Reestablishment of nerve connections was examined histologically using horseradish peroxidase, which was injected into the tongue and whose appearance in the hypoglossal nucleus indicated whether axonal connections were intact. Among animals in the "R"-operation group, axonal regeneration was first demonstrated 20 days after axotomy. After then, axonal connectivity was shown consistantly. No axonal connectivity was shown in the group after the "D"-operation. After axonal injuries, disappearance of synaptic junctions, disintegration of organelles and proliferation of neuroglia occur in the hypoglossal nucleus. These degenerative changes can disappear following return of the functional connections between muscle and neuron cell bodies. If the axons are prevented from regenerating, these phenomena remain and result in the atrophy of the nucleus. The results in the "D"-operation group are attributed only to degenerative changes, as they also show only decreased palmitate incorporation into the region. In the "R"-operation group, the increase in palmitate incorporation from 4 to 24 days after axotomy is ascribed to the regeneration of axolemma, and from 24 to 49 days after axotomy to the reconstitution of organelles and synaptic junctions. Those results indicate that the palmitate method(Kimes et al, 1983) can be used to examine plastic degenerative and regenerative neuronal changes following axotomy.

390.12 METABOLIC EVIDENCE FOR A RED AND WHITE BRAIN.

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In previous studies we have found that capillary density in rat brain does not correlate with mitochondrial density [quantitative cytochrome oxidase (CO) histochemistry] as it does in muscle (Wagman and Collins, submitted). Furthermore, we found no correlation between the distribution of regional rates of glucose utilization [quantitative [^{14}C] deoxyglucose (DG) autoradiography] and regional rates of CO activity. The regional density of capillaries did, however, correlate well with regional rates of glucose utilization ($r = 0.78$). These studies suggest that the separation of aspects of oxidative and glycolytic metabolism in brain may follow some of the principles governing the separation of these activities in muscle.

To explore this idea further we have used lactate dehydrogenase (LDH) histochemical staining and quantitative microdensitometric analysis to compare regional differences in glycolytic and oxidative enzyme activities . Ten um brain sections were cut in a cryostat and stained for LDH by a modification of the technique of Jacobsen (Histochemie, 20:250, 1969). An intermediate electron carrier, phenazine methosulfate, was used to bypass the endogenous NADH tetrazolium reductase. The reaction was found to be linear with time. Sections stained without lactate or without NAD showed only a trace of reaction. Regional optical densities were measured in 19 gray and 4 white matter areas for 4 rats. The highest activities of LDH among gray areas were found in stratum radiatum and oriens of the hippocampus. The lowest activities were in the spinal nucleus V, red nucleus and white matter. There was a 2 fold range of values for gray areas. When the regional distribution of LDH was compared against the regional distribution of CO, [^{14}C]DG and capillary density among these areas, no statistically pleasing relationship was found.

When the pattern of enzyme activity was examined in greater detail in the hippocampus, a striking hodological reciprocity was found between LDH and CO. Terminal zones of the perforant path from the entorhinal cortex showed high levels of CO but low levels of LDH. Terminal zones of the commissural and associational fibers showed high levels of LDH but low levels of CO. These findings suggest that afferent input may control levels of certain enzymes of energy metabolism within brain as within muscle. We are testing this hypothesis.

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390.13 METHOD FOR MEASURING SDH ACTIVITY IN SINGLE NEURONS
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A histochemical technique for quantifying succinate dehydrogenase activity(SDH) in single motoneurons has been developed. Previous measures of enzymatic activities of single neurons(1,2,3) have been based on the optical density(OD) of a region of cytoplasm after a specific period of staining in an incubation medium containing the appropriate substrate, sometimes(3) using fixed tissue. In these assays reaction product not specific to the substrate of interest is included in the OD measurements (1,2,3,). In the present study SDH rates were measured using a video camera interfaced with a light microscope. The camera was calibrated in absolute OD units divided into 256 grey levels for each pixel (480x640). The data were processed using a PDP11-34 computer. The rate of change in OD was determined from video scans repeated every 30s. In 10um fresh-frozen sections of cat lumbar spinal cord placed in an incubating medium with succinate a linear rate of change in OD was observed for 1.5 min followed by a slower, but linear, rate for 6 min. The rate was then reduced and non-linear. Placement of the tissue in an incubation medium without substrate resulted in a high rate of product formation for 2 min subsequently reaching zero. Therefore, the 1.5 min slope with succinate substrate reflects a combination of SDH specific activity and non-specific staining. The subsequent 6 min slope represents the substrate specific activity. Due to the magnitude of the nonspecific staining the endpoint OD was poorly correlated with SDH activity. The OD of tissue sections reacted in the incubation medium with succinate was linearly related to tissue thicknesses ranging between 6 and 20um. Two repeated measures on the same cell(n=45) resulted in a mean difference in the measures of 14.4%. Within one 10u section neighboring regions exhibited as much as an 8.5% variability in SDH activity. This intracellular variability is consistent with the intracellular heterogeneity of cytochrome oxidase observed using electron microscopy(3).

1.Donselaar,Y. et al.Brain Res.385:22-29,1986.
2.Sickles,D.W. et al.Histochem.79:205-217,1983.
3.Wong-Riley,M.T.T. et al.J.Comp.Neurol. 245:41-61,1986.

390.14 ETHANOL EFFECT ON MOLECULAR AGREGATION STATE OF ALCOHOL-NAD-OXIDOREDUCTASE (ADH EC 1.1.1.1) AND ALDEHYDE-NAD-OXIDOREDUCTASE (ALDH EC 1.2.1.3) IN RAT CNS: A COMPARATIVE STUDY BETWEEN NORMAL AND "A.G. RATS".
E. Egaña, P. Grez* &**J. Aldunate*. University of Chile - Faculty of Medicine - Institute of Experimental Medicine - Laboratory of Neurochemistry and **Department of Biochemistry the same University & Faculty - Santiago 7 - Chile.

In previous work communicated by our Institute it was found that the molecular agregation state of CNS ADH and ALDH of rat drinking ethanol 12% v/v as unique fluid intake (permanent and generational "A.G./12"), was different from normal CNS. Based on this result and others such as the increased in CNS enzymic activity in drinking rats (acute and chronic experiments), we were interested knowing the exact molecular agregation state of these enzymes as also the relationship between the different enzymic activity in both experimental groups (Normal and "A.G./12" rats)

Adult albino Wistar rats ♂ & ♀ by separate. Normal (control) and "A.G./12" drinking exclusively a 12% v/v ethanol solution (86 generations). 4 CNS' areas: brain cortex, hypothalamus, cerebellum and midbrain. Electrophoresis was run in 20.000 x g supernatant of the above mentioned areas. Molecular subunits weight were estimated by calibrating the gels against bovine serum albumin dimer and monomer (mol. wt. 132,000 and 66,000 respectively), carbonic anhydrase (mol. wt. 29,000), ∝-Lacto albumin (mol. wt. 14,200) and ovalbumin (mol. wt. 45,000) standards. The enzymic activity was detected in poliacrylamide gels of different concentrations by McRobbie et al. method (1985).

Differences in molecular agregations state of both enzymes were found, which could explain the increase in enzymic activity of alcoholic rat's brain demostrated by some authors in other models of experimental alcoholism (acute and/or chronic) run in one single generation. In "A.G. rat" the enzymatic change through genetic mechanism is supposed to be involved (enzymic mutation).

390.15 THE BRAIN PI POOL INVOLVED IN DEACYLATION-REACYLATION MECHANISM IS INACCESSIBLE TO PHOSPHOLIPASE C.
Meena Navidi* and Grace Y. Sun (SPON:F.S. Vomsaal). Biochemistry Dept. and Sinclair Research Farm, University of Missouri, Columbia, MO 65203.

Although phosphatidylinositol (PI) in brain is a minor phospholipid constituting only 4% of the total lipid phosphorus, it is metabolically active and is involved in the cyclic event related to receptor-mediated turnover of the poly-phosphoinositides. Besides synthesis by the de novo pathway, PI is also actively engaged in an deacylation-reacylation mechanism which is highly selective for turnover of arachidonoyl group. Using [^{14}C]-arachidonic acid and in the presence of Mg^{2+}ATP, CoA, and lyso-PI, the label is actively incorporated into PI and phosphatidylcholine (PC) of somal plasma membranes and synaptosomes of rat cerebral cortex. The washed-prelabeled membranes were further incubated in the presence of Ca^{2+} and/or deoxycholate in order to observe phospholipase A_2 and PI-phospholipase C activities in the prelabeled membranes. Under this condition, Ca^{2+} modulated some decrease in labeled PI and PC with concomitant increase in labeled free fatty acids, suggestive of phospholipase A_2 activity. However no labeled diacylglycerol was released, indicating the absence of phospholipase activity towards the prelabeld PI in the membrane. Phospholipase C activity specifically towards PI was only observed when prelabeled membranes were incubated in the presence of Ca^{2+} and deoxycholate. In another experiment in which [^{14}C]-arachidonoyl-PI was used for incubation with the same membrane fractions, both labeled diacylglycerols and free fatty acids were released as a result of incubation in the presence of Ca^{2+} (1 mM). EGTA (2 mM) blocked the diacylglycerol release completely, but only partially inhibited labeled free fatty acids formed. Deoxycholate further enhanced PI-phospholipase C activity but partially inhibited the free fatty acid release. Results thus indicate that the PI pool in brain subcellular membranes that is involved in the deacylation-reacylation mechanism is inaccessible to reaction by phospholipase C. (Supported in part by NSF 8419063)

390.16 IN VITRO ACYLATION OF ENDOGENOUS PROTEOLIPID PROTEIN IN MYELIN SUBFRACTIONS DURING DEVELOPMENT.
Oscar A. Bizzozero*, James F. McGarry*, and Marjorie B. Lees. Biochemistry Dept., E.K. Shriver Center, Waltham, MA.

Myelin proteolipid protein (PLP) contains covalently bound fatty acids. We have been studying the mechanism of the acylation reaction and have recently shown that incubation of isolated myelin membranes with fatty acid-CoA leads to PLP acylation. Thus, the protein pool available for acylation and the acyl transferase activity are both present in myelin. These observations are consistent with an autoacylation process. The present study was designed to examine the acylation reaction in myelin subfractions during development. Rat brain myelin subfractions obtained from animals of 10, 15, 17, 20, 25, 34, and 90 days of age, were incubated with [^{3}H] palmitoyl-CoA for 15 and 30 min. Proteins were analyzed by SDS-PAGE and radioactivity was determined by fluorography. At all ages and in all subfractions, PLP and DM-20 were the only proteins labelled. Treatment with hydroxylamine showed that the fatty acid was attached to the protein by an ester linkage. Pulse-chase experiments showed that the acylation reaction was a net addition of fatty acids. In vitro acylation of endogenous myelin PLP was observed as early as 10 days and increased with age. However, the amount of label was always proportional to the amount of PLP in the fraction and, therefore, no differences were obtained in the relative specific radioactivities. Similarly, acylation of PLP in different myelin subfractions was proportional to the amount of PLP in each fraction. These results indicate that PLP acylation is a dynamic process that is occurring actively at all ages, regardless of the expression of the protein which is maximal at 17-20 days of age. The findings are in agreement with in vivo experiments showing that acylation occurs in adult animals and in all myelin subfractions. They also provide further support of our hypothesis that PLP acylation is an autocatalytic process. Supported by NIH grants HD05515 and NS 13645.

390.17 THE STRUCTURE AND ANTIGENICITY OF METALLOTHIONEIN-LIKE PROTEIN IN RAT BRAIN. M. Ebadi, D. Babin,*† and S. Swanson*. Dept. of Pharmacol., Univ. of Neb. Coll. of Med., and Dept. of Biochem.†, Creighton Univ. Sch. of Med., Omaha, NE 68105.

We have identified a metallothionein-like protein in the rat brain with an elution volume (ve/vo) of 2.06 and an Mr of <10000 daltons. The synthesis of this protein is stimulated following intracerebroventricular (icv, 0.20 μmol zinc/μl/h, 48 h), but not intraperitoneal (ip), administration of $ZnSO_4$. Furthermore, chronic ip administration of $ZnSO_4$ (5.0 mg/kg/d/10 d) does not alter the level of the metallothionein-like protein in the brain. However, the hepatic metallothionein is induced following icv administration of $ZnSO_4$ (see Ebadi, Biol. Trace Ele. Res. 11:111-127, 1987). The chromatofocusing of metallothionein-like protein isolated by gel permeation chromatography on Sephadex G-75 exhibits three zinc-binding peaks, which focus at pH 6.8, 6.2, and 5.3, respectively. It is expected that the protein peak focusing at pH 5.3 is a metallothionein-like protein. Purification of the zinc-stimulated metallothionein-like protein on ion exchange chromatography on DEAE-Sephadex A-25 columns, using a linear gradient elution procedure, produces two isoforms, eluting at 75 and 137 mM of Tris-acetate buffer, pH 7.5, respectively. The comparative high performance liquid chromatographic (HPLC) profiles of the zinc-induced hepatic metallothionein isoforms I and II (retention times 17.39 and 18.73 min) and those of the zinc-stimulated metallothionein-like protein isoforms I and II (retention times 17.32 and 18.64 min) are very similar. The amino acid compositions of metallothionein-like protein isoforms I and II, which resemble those of the hepatic metallothionein, are:

AMINO ACIDS	ISOFORM I		ISOFORM II	
	Residues	%Total	Residues	%Total
Aspartic Acid	5	8.4	4	6.5
Threonine	3	5.0	3	4.9
Serine	8	13.3	9	14.8
Glutamic Acid	3	5.0	4	6.6
Proline	2	3.3	2	3.3
Glycine	7	11.7	5	8.2
Alanine	4	6.7	5	8.2
Valine	2	3.3	1	1.6
Cysteine	17	28.3	18	29.5
Methionine	1	1.7	1	1.6
Isoleucine	-	-	-	-
Lysine	8	13.3	9	14.8

These two isoforms are devoid of histidine, arginine, leucine, tyrosine, and phenylalanine. A preliminary study completed by Professor J. Garvey (Dept. of Biol., Syracuse Univ.), using as a reference rat hepatic metallothionein isoforms I and II with ^{125}I label on isoform I, is indicative of a complete cross reactivity between the metallothionein-like protein in the brain and the hepatic metallothionein. The function(s) of the metallothionein-like protein isoforms in the brain remains to be elucidated (supported by a grant from USPHS ES 03949).

SPECIFICITY OF SYNAPTIC CONNECTIONS I

391.1 THE PERIPHERAL NERVOUS SYSTEM-CENTRAL NERVOUS SYSTEM INTERFACE: GANGLION CELL ARRANGEMENT AND AFFERENT FASCICULATION IN THE ELECTRORECEPTIVE SYSTEM OF APTERONOTUS LEPTORHYNCHUS (GYMNOTIFORMES). M.J. Lannoo*, L. Maler, and B. Tinner*. Dept. of Anatomy, Univ. of Ottawa, Ottawa, Ont. K1H 8M5 (SPON: W. Staines).

To understand the organizational principles underlying the peripheral electrosensory nervous system of weakly electric gymnotiform teleosts we labelled each of the four afferent nerves to the lateral line ganglion with HRP. We determined the position of labelled cell bodies within the ganglion and followed anterograde fibers to their termination sites in one of the four topographic maps in the electroreceptive lateral line lobe (ELL). Within the ganglion, cell bodies exhibit a general topography based on afferent nerve position: trunk electroreceptors have their cell bodies located in the caudal ganglion, cell bodies to head receptors are rostral. Cell bodies to the head exhibit a rough dorsal-ventral polarity: supraorbital nerve ganglion cells are located in the dorsal ganglion, infraorbital centrally, and mandibular ventrally. Despite this general ordering there was substantial overlap of cell bodies within the region of any particular nerve. There was no rostro-caudal polarity obvious within regions of the ganglion representing the head. There was also no clustering of cell bodies based on receptor function: ampullary and tuberous electroreceptor soma are randomly distributed. Peripherally, axons from the cell bodies fasciculate to form afferent nerves. However, within an afferent nerve there does not appear to be any topographic arrangement. Centrally, axons from the cell bodies to the ELL retain the position of their cell body until they reach the distal border of the deep fiber layer (DFL). Fibers in the DFL are fasciculated and reorganize themselves mediolaterally and rostrocaudally prior to terminating in one of the lateral three segments (tuberous organs) or the medial segment (ampullary organs). Thus the axons from ampullary receptors form a distinct fascicle separate from the axons innervating tuberous organs. Fibers in the DFL run horizontally then turn vertically to terminate in appropriate topographic order; there are few oblique fibers. Horizontal DFL fibers from dorsal receptors seem to run ventrally and vice versa. Developmentally, we view the portion of the peripheral electrosensory system outside the ELL as organized in terms of afferent nerve position, perhaps as a result of axonal guidance involving embryonic lateral line placodal migration. Centrally, the electrosensory system is organized in terms of both receptor function and topographic position. We view fiber reorganization in the DFL as a problem of selective fasciculation and target selection, perhaps mediated by chemical cues.

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391.2 COMBINATORIAL RULES IN SYNAPTIC ORGANIZATION. A. Fröhlich, Mt. St. Vincent University, Halifax, N.S., Canada, B3H 2J6.

The rules determining the combination of postsynaptic elements at a multiple-contact synapse have been studied under conditions where one of the neurons normally postsynaptic is missing.

In the first optic neuropile of the fly's visual system photoreceptor terminals form divergent tetrad synapses at which four postsynaptic elements cluster. Normally a receptor terminal synapses upon two monopolar cells, L1 and L2, which are always situated side by side in the so-called medial positions of the tetrad, flanking which are two other postsynaptic elements in the polar positions. These polar elements are most often both alpha-processes of amacrine cells or both glial cell processes. Alternatively, processes of either an amacrine cell or a glial cell may pair with a process of a third monopolar neuron, L3.

The combination of postsynaptic elements and their geometric arrangement at the synaptic site were analyzed under conditions where one of the normally obligatory postsynaptic contributors, L1, was missing. Under these conditions various findings might be expected: (a) synapses might not be found at all, (b) the synapses formed might lack postsynaptic elements, (c) the elements normally postsynaptic at the photoreceptor tetrad synapse might contribute more than their usual share of postsynaptic processes at any one synapse, (d) a cell not normally contributing to the synapse might become postsynaptic.

(a) Synapses exist despite the absence of L1. Analysis of 140 such synapses identified in consecutive electron micrographs shows: (b) in addition to the tetrads, synapses are found with three (triads) and two (dyads) postsynaptic elements. (c) The monopolar L2 still always provides one but never more than one postsynaptic element at each synapse. At synapses where L3 is a postsynaptic element it never contributes more than once, as in normal synapses. Up to three amacrine processes may be found at a synaptic site instead of the normal maximum of two. Amacrine processes may be postsynaptic together with glial processes at the same synapse, a condition normally not found. (d) In addition, beta-processes of T1 cells may occasionally contribute to a synapse. These have been suspected to contribute to "normal" synapses only rarely. This beta process or any of the other processes normally taking up a polar position may be found in the medial position otherwise occupied by the missing L1.

Further analysis should allow predictions as to the role of differential cell recognition during synaptogenesis.

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391.3 REFINEMENT OF THE GOLDFISH RETINOTECTAL PROJECTION IN THE ABSENCE OF ACTIVITY AND IN THE DARK. M.D. Olson and R.L. Meyer, Developmental and Cell Biology, Developmental Biology Center, University of California, Irvine, California 92717

During optic nerve regeneration in goldfish, retinal fibers initially form a roughly ordered tectal projection which subsequently becomes more refined. Tetrodotoxin (TTX) impulse blockade has been shown to inhibit the refinement of this topographic projection. Whether any refinement can occur under impulse blockade is unknown because of the limitations of previously used mapping techniques. To address this question we have utilized spot injections of wheat germ aggulutinin conjugated to horseradish peroxidase (WGA-HRP) into the retina to reveal the tectal projection of a small area of retina.

In a previous study it was found that these spot injections in normal animals produced a sharply defined patch of product 200-300um wide in the appropriate tectal region. During optic nerve regeneration the product is initially dispersed over about one third of the tectum. This widely dispersed product subsequently becomes condensed into two or more distinct patches separated by an unlabeled interdigitating space resembling ocular dominance columns.

In the present study the optic nerve was crushed and activity was blocked by periodic injections of TTX into the vitreous. At selected time points between 30 and 120 days after nerve crush, a spot injection of WGA-HRP was made into the dorsal and ventral peripheral retina. After 18 hours the fish were perfused, the tectum serially sectioned and the WGA-HRP was visualized with tetramethyl benzidine (TMB) histochemistry.

Animals treated with TTX did not exhibit the normal sequence of projection refinement. The label remained widely dispersed in a large continuous patch for as long as 120 days of regeneration. The projection, however, did not remain as widely distributed as it was at 30 days of regeneration. A progressive topographic refinement was observed in which the label gradually condensed over the appropriate tectal region. Thus substantial topographic refinement occurs without activity but the formation of discrete clumps appears to be activity dependent. In other fish TTX activity blockade was stopped after 60 or 125 days and the fish were allowed to recover for 74 to 130 days. Normal refinement and the formation of clumps was found, indicating the TTX effect was reversible.

We also examined animals kept in total darkness during optic nerve regeneration since there has been contradictory claims of whether this disrupts refinement. These animals exhibited the normal refinement and clumping, though it may have been delayed by about two weeks.

This work was supported by NIH grant 9R01 EY06746

391.4 INHIBITION OF OCULAR DOMINANCE PATCH FORMATION IN GOLDFISH OPTIC TECTUM BY INTRAOCULAR COLCHICINE. R. E. Davis, Neurosci. Lab., Univ. of Michigan, Ann Arbor, MI 48104-1687.

To examine whether their formation of functional synapses with optic tectum neurons inhibits regenerating optic axons from forming ocular dominance patches (Meyer, R.L., Brain Res. 155:213, 1978), goldfish (8-10 cm) kept at 30°C were administered right optic tectum ablation followed by io injection of colchicine (Sigma; 0.1 or 0.5) in the right eye at 2 wks postaxotomy WPA. The extent of patch formation was measured at 8, 10 and 12 WPA using autoradiography to trace the projections of the foreign axons (Schlumpf, B.E. and Davis, R.E., Brain Res. 386:305, 1986). Normally, following the tectum ablation, regenerating axons from the left eye grow into the optic layers of the ipsilateral tectum and their terminal arbors are distributed among the intact resident optic axons in a crude retinotopic map within 4 weeks (Easter, S.S. and Schmidt, J.J., J. Neurophys. 40:1245, 1977). At ca. 6-7 weeks postaxotomy (WPA) the endings of the foreign and resident optic axons variously segregate forming eye-specific patches of innervation. Patch formation appears to be complete at ca. 12 WPA (Springer, A.A. and Cohen, S.M., Brain Res. 224:23, 1981). The formation of distinct ocular patches, as demonstrated by autoradiographic localization of [^{3}H]-labeled foreign or resident axons, preceeds or accompanies synaptogenesis in the foreign optic axons, based on EM results (Airhart, M.J. and Norden, J.J., Brain Res. 325:307, 1985) and the recovery of visual function which occurs between 7-15 WPA (Davis, R.E., Schlumpf, B.E. and Klinger, P.D., Behav. Brain Res. 13:287, 1984). This suggests that optic axons may aggregate in patches prior to establishing terminals on tectum targets. The aggregation of unconnected axon endings is proposed as an alternative to the synaptic stabilization theory of patch formation (Boss, V.C. and Schmidt, J.T., J. Neurosci. 4:2891, 1984). Intoxicating the resident axons by io colchicine facilitates recovery of visual function in the foreign axons; vision with the left eye is restored within 3-5 WPA and prior to patch formation. The colchicine was found to inhibit fast axonal transport of [^{3}H]-labeled retinal protein to the tectum. In the present experiments, fish given the io colchicine treatment showed decreased patch formation up to 12 WPA. Lumicolchicine, which does not inhibit axonal transport, had no significant effect on patch formation. These results suggest that patch formation is a result of forces of axon aggregation and exclusion and the tendency of optic axons to synapse with suitable, available targets. Both connected and unconnected axons exert exclusionary forces. Aggregation forces are seen to operate only on unconnected axons. Colchicine could decrease patch formation by inhibiting axon exclusionary forces in the residents thereby enabling foreign axons to become connected before being greatly affected by the forces of axon aggregation.

391.5 RESTORATION OF VISUOTOPIC MAPS IN THE GOLDFISH TECTUM AND TORUS LONGITUDINALIS AFTER OPTIC NERVE CRUSH. D.P.M.Northmore. Dept. of Psychology & Institute for Neuroscience, University of Delaware, Newark, DE 19716.

Following crush of the goldfish optic nerve and its subsequent regeneration, the first visual multiunit receptive fields (MURFs) that can be recorded electrophysiologically in tectum are abnormally diffuse. Presumably, such MURF characteristics reflect the widespread branching of regenerating optic fibers, but not necessarily the pattern of retinotectal connections. If connectivity were more specific than tectal MURFs imply, receptive fields with relatively normal properties might be found in the torus longitudinalis (TL) which receives a topographic synaptic input from the adjacent tectal lobe.

At various times after intraorbital crush of one optic nerve, the projections of both visual fields to tectum and TL were mapped electrophysiologically using objective methods. The eyes were corrected for vision in air by contact lenses. Two-dimensional maps giving tectal MURF size, shape and strength were obtained by recording multiunit activity to red LEDs flashed in a grid pattern. Because TL responds with sustained activity only to dark objects in the contralateral field, it was mapped one-dimensionally by recording activity profiles during horizontal rotation of a vertical black stripe. The same method was also useful for mapping newly regenerated retinotectal projections.

In the normal visual system, two-dimensional mapping of tectum yielded nearly circular MURFs, 5-10° across. One-dimensional mapping of tectum typically gave double-peaked response profiles indicating the nasal and temporal limits of the MURF, while TL gave bell-shaped profiles 30-40° wide.

In the regenerating system, the earliest visual responses that were recorded in tectum around 21 days post crush (24 °C) were weak multiunit bursts to moving black objects. Flashing LEDs evoked no consistent responses. In both tectum and TL, one-dimensional mapping revealed broad, low amplitude profiles (60-90° across), often with multiple peaks, but roughly normal topography. After about 30 days, tectal responses to LEDs reappeared but MURFs were abnormally large, irregular and weak. Although these abnormalities declined over time, they were still noticeable at 200 - 250 days post crush. Meanwhile, TL response profiles returned to a normal unimodal shape.

Conclusions: The first recordable tectal MURFs are excited by dimming, and are closely related in time and space to the dimming responses of TL, suggesting that the initial diffuse retinotectal MURFs represent a similarly diffuse connectivity with the tectal cells that project to TL. (Supported by NIH grant EY2697).

391.6 NAVIGATION AND TARGET RECOGNITION OF REGENERATING RETINAL AXONS IN LONG-TERM DENERVATED TECTA IN GOLDFISH. U. Busse* and C.A.O. Stuermer (SPON: W. Harris). Friedrich-Miescher-Lab., Max-Planck-Institute, D-7400 Tuebingen, FRG.

To test whether the re-establishment of the retinotopic projection is governed by "tectal cues" induced by retinal fibers (Schmidt 1978) or by markers inherent to tectum (Meyer 1987), we pursued the path of regenerating axons in long-term denervated tecta.

The right optic tectum was deprived from retinal afferents by enucleation of the left eye. Axons from the right eye were induced to regenerate into the denervated ipsilateral right tectum by removal of the left tectum (T-rem) 0, 1, 2, 3, 4 or 5 months after eye enucleation. 20 - 150 d post-T-rem small populations of axons were labeled by intraretinal application of HRP to dorso (D)temporal (-nasal) or ventro(V)temporal (-nasal) retina and viewed in tectal whole mounts.

Despite T-rem, axons grow contralaterally and only 10 - 20 % reach the ipsilateral tectum. To evaluate whether ipsilaterally projecting (IP) axons show a preference for retinotopically appropriate regions, labeled axons were counted at the brachial bifurcation of the ipsilateral optic tract and at rostral, mid-tectal and caudal levels in the tectum (N=39). With similar label sites in retina, the relative fiber distribution over the regions examined varied little after different denervation periods so that the data were pooled.

At the brachial bifurcation of the optic tract normal axons from D and V retina segregate almost completely. IP-axons make pathway mistakes, but 78 % of D(V) axons pass through the retinotopically appropriate V(D) brachium. In tectum 82.5 % of temporal axons were found rostrally, 17 % at midtectal levels and 0.5 % caudally. In contrast, the distribution of nasal axons over rostral and midtectal regions was 53.1 % and 41.6 % resp. and 5.3 % caudally. Thus, in tecta denervated for up to 5 mo - very similar to axons regenerating immediately into contralateral tecta (Stuermer 1987) -, temporal axons show a strong preference for rostral and nasal axons for their more appropriate caudal tectum. Likewise, IP-fibers in incorrect tectal regions correct their routes and form terminal arbors 30 - 50 d after their arrival in tectum at retinotopic sites.

In tecta denervated for several months, IP-fibers still grow preferentially to the correct hemitectum and terminate retinotopically. These findings are consistent with the idea that axons are guided by long-lasting positional markers intrinsic to tectum.

391.7 TECTAL PATHWAYS OF REGENERATING GOLDFISH OPTIC AXONS AFTER HALF-NASAL OR HALF-TEMPORAL RETINAL REMOVAL. M.F. Humphrey* and C.A.O. Stuermer (SPON: J. Bolz). Max-Planck-Institut für Hirnforschung, D-6000 Frankfurt, FRG (M.F.H.) and Friedrich-Miescher-Lab., Max-Planck-Institute, D-7400 Tuebingen, FRG (C.A.O.S.).

The pathways of regenerating goldfish optic axons through the tectum are abnormal but not random. 65 % of temporal (temp) axons course through rostral tectum, 31 % in midtectal and 4 % in caudal tectal regions, whereas nasal (nas) axons proceed into caudal tectum (Stuermer et al '86). In this study we tested whether temp axons were confined to rostral tectum by the presence of nas axons in caudal tectum or whether they preferentially course within rostral tectum regardless of other axons. We similarly tested whether nas axons would grow preferentially into caudal tectum in the absence of temp axons.

Either the nasal or temporal half retina was removed at the time of optic nerve section (ONS). At 35 and 70 days after ONS regenerating optic axons were labeled with HRP and their pathways and distribution determined in DAB reacted tectal whole mounts.

In the absence of nas axons the relative density of temp axons in rostral, mid and caudal tectum was 70 %, 28 % and 2 %, respectively. The corresponding values for nasal axons were 30 %, 40 % and 30 %, respectively.

In the Stratum opticum both nas and temp axons formed fascicles similar to those of whole retinal regenerates (Stuermer et al '86). Most nas axons exited from fascicles in caudal tectum and branched preferentially there in the synaptic layer. Those which had entered the synaptic layer in rostral tectum coursed in caudally oriented routes.

The majority of temp axons exited from fascicles in rostral tectum where they branched in the synaptic layer. Near the boundary between rostral and caudal tectum, temp axons made bends to avoid entering caudal tectum. In the absence of nas axons, temp axons formed numerous mediolaterally oriented fascicles which crossed the tectal equator in the synaptic layer. Axons misrouted into the incorrect dorsal or ventral hemitectum often take such pathways (Stuermer et al '86).

Thus, nas and temp axons obviously discriminate between rostral and caudal tectum despite pathway disorganization and absence of axons from the other hemiretina. This is consistent with axonal growth being under the influence of positional markers in tectum.

391.8 MINOR REORGANIZATION OF THALAMOCORTICAL PROJECTIONS FOLLOWING LARGE NEONATAL THALAMIC LESION IN THE GOLDEN HAMSTER B.Miller*, M. S. Windrem*, L. Anllo-Vento* and B. L. Finlay. Department of Psychology, Cornell University, Ithaca, New York 14853

Previous studies on developing thalamocortical connections have demonstrated that thalamic fibers make initial contact with the developing cortex in the same tangential pattern as seen in the adult (Crandall and Caviness, 1984). This specificity is in contrast to corticofugal (callosal, intracortical and subcortical) systems which make transient initial projections to multiple targets. These corticofugal systems demonstrate considerable reorganization after early damage (see review by Fish, Mooney & Rhoades, 1985). This experiment addresses the question of whether the thalamocortical system,with its more specific patterns of early connectivity, can also reorganize its connections after early thalamic lesion.

Golden hamster pups were given electrolytic lesions on the day of birth in posterior thalamus, damaging principally either the ventrobasal (VB) or the dorsal lateral geniculate (LGd) thalamic nucleus, but also the posteromedial (Pom), lateral posterior (LP) and lateral (L) nuclei. At 30 days of age, horseradish peroxidase was implanted in either the somatosensory or visual cortex matching the area of implant with the intended thalamic lesion. The thalamus was reconstructed to determine remaining nuclei and the distribution of retrogradely labeled cells was plotted. These animals were compared to a group of normal adult animals which established which thalamic nuclei normally project to somatosensory and visual cortex.

Animals with lesions to the primary visual nuclei (LGd, LP, L) showed some retrogradely labeled cells in anomalous nuclei: Medial dorsal (MD), submedial (S), anterodorsal (AD), anteroventral (AV) and medial geniculate (MG). The projections from these nuclei were weak, however,with only a few cells per nucleus sending projections to the deafferented visual cortex. One experimental animal with a lesion of the ventrobasal nucleus had anomalously labeled cells in MD and AD, with a substantial fraction of the AD cells projecting to the deafferented somatosensory cortex.

In summary, thalamocortical projections show very limited reor ganization after early thalamic lesions.

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391.9 THE ULTRASTRUCTURAL ORGANIZATION OF SEROTONIN AXONS IN ADULT RAT HYPOTHALAMUS AND SUBTHALAMUS AFTER 5,7-DIHYDROXYTRYPTAMINE-AXOTOMY INDUCED REGENERATION. M. Frankfurt and A. Beaudet. Lab. of Neuroanatomy, Montreal Neurological Institute, Montreal, Quebec H3A 2B4.

It has been shown, at the light microscopic level, that serotonin (5-HT) axons reinnervate the adult rat hypothalamus following 5,7-dihydroxytryptamine (5,7-DHT)-induced axotomy (Frankfurt and Azmitia, Brain Res., 298, 273, 1984). In the present study, the ultrastructural organization of regenerated 5-HT axons was examined in the dorsomedial hypothalamic area (DMH) and medial zona incerta (ZI), 30 (DMH) and 50 (DMH and ZI) days, after unilateral injection of 5,7-DHT or vehicle solution into the dorsolateral hypothalamus. 5-HT terminals were identified by electron microscopic (EM) radioautography following prolonged intraventricular infusion of (^{3}H)5-HT. For studies of the ZI, tyrosine hydroxylase (TH)-immunoreactive neurons were visualized in the same sections using peroxidase anti-peroxidase immunocytochemistry.

In the DMH of sham rats, (^{3}H)5-HT-labeled axon terminals were small, contained many small clear vesicles (30-50 nm), one or more large, granular vesicles (70-90 nm) and showed very few synaptic specializations. Both 30 and 50 days after 5,7-DHT injection, the internal organization and microenvironment of (^{3}H)5-HT-labeled profiles in 5,7-DHT-treated rats resembled that of sham-treated rats. However, a slight increase in synaptic frequency was found for (^{3}H)5-HT-labeled terminals in the 5,7-DHT treated group fifty days post-lesion as compared to sham. Also, at both time points there was an increase in the number of perikarya contacted by (^{3}H)5-HT-labeled terminals.

In the ZI of both sham and 5,7-DHT-treated rats, (^{3}H)5-HT-labeled axon terminals resembled those in the DMH and were observed abutting TH-positive dendrites, dendritic spines and occasionally TH-positive perikarya. Our results also indicate that, 50 days after 5,7-DHT treatment, (^{3}H)5-HT-labeled terminals in 5,7-DHT-treated rats may establish synaptic relationships with TH-positive elements as they occasionally do in both sham-treated and normal (Bosler et al., Neurosci. Lett., 48, 279, 1984) rats. These results indicate that 5-HT fibers, which have regenerated following 5,7-DHT induced axotomy, reoccupy a cellular environment comparable to that observed normally. Furthermore, some of the targets of regenerated 5-HT axons are chemically identical to those observed normally. Taken together these observations suggest that regeneration of 5-HT axons occurs with a great deal of cellular specificity and could, therefore, provide a morphological substrate for functional restoration.

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391.10 FUNCTIONAL SIGNIFICANCE OF THE HYPERINNERVATION OF STRIATUM BY SEROTONIN NEURONS AFTER NEONATAL 6-HYDROXYDOPAMINE (6-OHDA) LESIONS. H.A. Tilson, K.P. Nanry*, S.J. Li, J.-S. Hong and M.K. Stachowiak. Lab. Behav. Neurol. Toxicol., NIEHS, Research Triangle Park, NC 27709.

Neonatal lesions of the nigrostriatal bundle (NSB) result in the ingrowth of serotonergic neurons into the striatum (Stachowiak et al., Brain Res. 291: 164, 1984). The purpose of the following experiment was to determine whether or not hyperinnervating serotonin terminals could substitute for dopamine in the control of striatal substance-P (SP) and enkephalin (EK)-containing cells. Three-day old male rats were injected with 6-OHDA (150 ug, ivt.) or vehicle. To protect noradrenergic neurons, rats were pretreated with desmethylimipramine (DMI, 25 mg/kg). When rats were sacrificed at 45 days of age, striatal DA and DOPAC contents were reduced by more than 95%. As previously reported, striatal serotonin levels were increased 2.5 fold. Changes in monoamine levels were accompanied by 50% increase in [Met^5]-enkephalin-like immunoreactivity (ME-LI) and 40-50% decreases in SP-like immunoreactivity (SP-LI). To examine the role of serotonin in the control of striatal peptides, rats treated neonatally with 6-OHDA were given bilateral injections of 5,7-dihydroxytryptamine (5,7-DHT, 16 ug/side, 30 min after pretreatment with 25 mg/kg DMI) at 30 days of age. Neurochemical measurements performed two weeks later found that 5,7-DHT decreased striatal serotonin by 50-80%. 6-OHDA did not alter the striatal ME-LI in the neonatal vehicle group, but produced an additional 50% increase of ME-LI in the 6-OHDA treated group. In contrast, 6-OHDA-induced decrease in the striatal level of SP-LI was not affected by 5,7-DHT treatment. These data are consistent with the hypothesis that heterotypic serotonergic sprouting may lead to establishment of new functional contacts between serotonin terminals and striatal EK neurons.

391.11 MONOCLONAL ANTIBODY G8 IDENTIFIES AN ANTIGEN PRESENT ON A SUBSET OF DENDRITES IN THE RAT CNS. J.M. Gossels and V.M. Ingram. Dept. of Biology, Mass. Institute of Technology, Cambridge, MA 02139.

The specificities of synapses in the CNS are ultimately determined by the protein and chemical compositions of both pre- and postsynaptic extensions. An investigation of proteins present in a subset of axons or dendrites, therefore, might help to identify or explain similarities between these synapses and possible relationships in neuronal function. A monoclonal antibody, G8, has been isolated which identifies an antigen present on a subset of dendrites in the adult rat CNS. This antibody has been used to characterize its antigen both anatomically and biochemically.

Immunohistochemistry was performed on 30 um floating cryostat sections of paraformaldehyde fixed rat brains of various ages. In the adult, G8 stains pyramidal cell cytoplasm and dendrites in the hippocampus. Staining is somewhat stronger in CA1 than in CA3. Dentate gyrus granule cell somas and dendrites also stain darkly. The G8 antigen is present in a subset of Purkinje cell bodies and their dendrites. The stained Purkinje cells are clustered and are usually localized on the dorsal region of each folium. In P1 and P5 rat brain sections G8 recognizes processes almost exclusively. The antigen is prominent in processes in the cortex, deep cerebellar nuclei, olfactory bulb, and regions of the brainstem. Staining is also evident in the hippocampus. At embryonic ages there is very little antigen present. Preliminary results in tissue culture suggest immunofluorescent staining of filaments in extensions and somas of a subset of P1 cerebellar and cortical cells.

On immunoblots of SDS PAGE G8 recognizes a single somewhat diffuse band of M.W. 52,000. This protein is more prominent in adult than newborn brain homogenates. In a comparison of brain, heart, kidney, and liver homogenates, G8 recognizes a band only in brain. The antigen recognized by G8 does not comigrate with GFAP or vimentin. The G8 antigen is actually enriched in the supernatant of a 100,000x g spin, indicating that it is a soluble component of the cytoplasm.

We are continuing to characterize the protein recognized by G8. The localization of the protein to diverse regions of the brain suggests that cells in these areas share a common function, perhaps involving a second messenger system.

391.12 MONOCLONAL ANTIBODIES DIRECTED AT THE SURFACES OF EMBRYONIC BRAIN CELLS. H. Rayburn*, H.M. Wu*, D. Stainier*, E. Bennett*, and W. Gilbert. Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138.

In a search to identify antigens expressed on the surface of localized groups of cells in the embryonic CNS, monoclonal antibodies were raised to cell suspensions of rat E15 mid and hind brain. Antibodies were screened on cultures of E15 cells. Hybridomas that yielded antibodies that stained rare cells (1 in 1000 or fewer) of neuronal morphology were cloned. Six antibodies will be described. Group I consists of two antibodies which stain patches on and between cells in culture. The stain is not reduced by neuramidase treatment. Group II consists of four antibodies that stain cell surfaces and processes solidly and brightly in culture, and is neuramidase sensitive. The localization of these antibodies is being studied on 15 micron frozen sections taken from day E15 through birth. At E15 all antibodies stain cells of the germinal layers, radial fibers, and patches on the pial surface of the brain. Group I antibodies, however, are expressed primarily in small regions of the prosencephalon, whereas Group II antibodies are localized in discrete patches of cells throughout the embryonic brain. Later in development, the antibodies are expressed in fewer places. The characterization of the antibodies and the chemistry of the antigens will be presented.

391.13 MONOCLONAL CELL SURFACE ANTIBODIES DEFINING MESENCEPHALIC NUCLEI. D. Stainier*, H.M. Wu*, H. Rayburn*, E. Bennett*, and W. Gilbert (SPON: M. Livingstone).
Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138.

Monoclonal antibodies (mAbs) were generated against surface antigens of brain cells. Cell suspensions from embryonic rat or mouse brains, whole or partial, were injected up to four times into the peritoneum of Balb-c mice. Hybridomas were screened via immunofluorescence on fresh mouse brain, either on small chunks in squashes or on 150 micron vibratome sections. Those showing a localized pattern of surface staining were cloned by limiting dilution. Double immunofluorescence in conjunction with a mAb against a neurofilament protein indicated the neuronal nature of the antigens recognized.

Of the mAbs isolated this way, two, B30 and B53, have been studied extensively. Around birth, they outline the neurons and the axons of what corresponds to the location and description of the deep mesencephalic and the mesencephalic trigeminal nuclei. The stained neurons include both small and large (35μ) globular or oval pseudounipolar cells and variably sized multipolar cells. Axons can be seen projecting to the cerebellum, where small cells in the granule cell layer are prominently stained. Fibers from the caudalmost part of the mesencephalic trigeminal nucleus proceed caudally to reach the ipsilateral spinal trigeminal nuclei which are also stained.

On renatured immunoblots of SDS gels, mAb B53 reacts with a 40,000 MW band which has been enriched by appropriate dissection.

391.14 IMMUNOHISTOCHEMICAL VISUALIZATION OF GABAERGIC SYNAPSES IN FROG SPINAL CORD. S.L. Stewart*, D.L. Glanzman, S.A. Hoffman and A. Narendran*. Depts. of Psychology and Microbiology, Arizona State University, Tempe, AZ 85287.

Immunohistochemical techniques were employed to visualize axo-axonal synapses within the lumbosacral region of frog spinal cord (Rana pipiens). Application of anti-GABA (gamma-aminobutyric acid) antibodies allowed detection of synapses formed by GABAergic interneurons upon terminal elements of descending lateral column (LC) fibers.

Production of anti-GABA antibodies utilized a protocol in which GABA was conjugated to a series of protein carrier molecules. This circumvented the haptenic limitation afforded by small molecules (such as amino acids) and also increased the magnitude of the specific antibody response to the common immunogenic constituent (i.e., GABA)(Seguela, P., M. Geffard, R.M. Buijs and M. Le Moal, Proc. Natl. Acad. Sci. USA, 81:3888, 1984). Rabbits were sequentially immunized with antigens prepared by glutaraldehyde-induced conjugation of GABA to bovine serum albumin (BSA), to ovalbumin, and to poly(L-lysine); subsequent reduction of immunoreactive double bonds with sodium borohydride increased the specificity of the antibodies thus produced. Standard enzyme-linked immunosorbent assay (ELISA) provided quantitative and qualitative characterization of these anti-GABA antibodies.

Positive identification of LC fibers and terminals was achieved by transection of the lateral funiculus at the level of spinal segments 5-6 approximately 7-10 days prior to sacrifice. Alternate horizontal sections of spinal cord were then stained with 1) reduced silver procedure to clearly visualize axonal and terminal degeneration, or 2) rabbit anti-GABA antisera, using a conventional indirect immunoenzymatic assay, with peroxidase conjugated goat anti-rabbit IgG antisera as the secondary antibody. Direct comparison of adjacent tissue sections provided an estimation of the association between GABAergic interneurons and LC terminals.

392.1 BIOPHYSICAL AND IMMUNOLOGICAL STUDY OF GAP JUNCTIONS IN NORMAL AND REGENERATING GIANT AXONS IN *Lumbricus terrestris*. A.W. Lyckman and G.D. Bittner. Department of Zoology, University of Texas, Austin, TX, 78712.

We are studying the cellular and molecular mechanisms in *Lumbricus terrestris* which contribute to the regeneration of cell-specific connections between uniquely identifiable neurons, the medial and lateral giant axons (MGA and LGA). The MGA and LGA are segmental interneurons which form two anatomicaly and functionally distinct giant fiber systems, the MGF's and LGF's, respectively. Septa between the giant axons of one fiber contain gap junctions. Regenerating neurites sprout from the cut ends of transected giant axons and rapidly reestablish bidirectionally transmitting connections between appropriate giant axons (Balter et al., *J. Exp. Zool.* **211**:395, 1980; Birse and Bittner, *J. Neurophysiol.* **45**:724, 1981). Injection of Lucifer yellow CH (LYCH) into regenerating giant axons has demonstrated that transected giant axons become appropriately dye-coupled across the lesion (Lyckman and Bittner, *Soc. Neurosci. Abs.*, 1986). Thus, regenerating giant axons can reform appropriate electrotonic connections.

Intracellular injection of LYCH into normal giant axons followed by epiflourescent examination showed that LYCH diffuses more rapidly and completely through the LGF than through the MGF. LGA septa, but not MGA septa, are detectably permeable to Lucifier yellow 37. In collaboration with Dr. P.R. Brink (SUNY at Stonybrook), septal permeability coefficients (P_s) were determined by intracellular injection of carboxyflourescein (CFL). Dye spread in axoplasm and across septa was measured flourometrically and analyzed by computer modelling (Brink and Ramanan, *Biophys. J.* **48**:299, 1985). P_s is 8.5×10^{-6} cm/s for the LGF and 8.6×10^{-7}cm/s for the MGF, i.e., CFL permeance is approximately ten-fold greater for the LGF than for the MGF. The difference in CFL permeance is not attributable to differences in the axoplasmic or plasma membrane diffusion coefficients, which were equal for both giant fibers. MGF and LGF septal resistances were determined by measuring trans-septal currents under double voltage clamp conditions (Verselis and Brink, *Biophys. J.* **45**:147, 1984). Septal resistance is 50kΩ for the MGF and 15kΩ for the LGF. We conclude that the LGF septa are more permeable and have a higher conductance than the MGF septa. This macroscopic conclusion has two microscopic explanations: 1) LGF septa have more gap junctions than MGF septa, and/or 2) the gap junctions of the LGF have greater single channel conductance and permeability than MGF gap junctions. In the latter case, the LGF and MGF channels might differ in protein structure.

To decide which of these two microscopic parameters may account for our macroscopic observations, we are determining the density of gap junction channels and single channel conductances in the MGF and LGF by patch-clamping septal membranes. In addition, we are determining if MGF and LGF gap junctions differ structurally by measuring differences between affinities of monoclonal antibodies to MGF vs. LGF gap junctions. It is possible that the specificity of giant fiber regeneration occurs because MGA's and LGA's are incapable of forming functional electrotonic junctions with each other because of differing and incompatible molecular specificities in their respective gap junction proteins.

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392.2 THE FORMATION OF TERMINAL FIELDS BY IDENTIFIED MOTONEURONS IN THE ZEBRAFISH. Dennis W. Liu and Monte Westerfield, Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

In some parts of the nervous system, individual neurons innervate many postsynaptic cells. The size and shape of these fields appear to be precisely regulated, although the underlying mechanisms are unknown. To learn how the terminal fields of motoneurons are established, we examined the growth of individual axonal branches of two identified motoneurons in the zebrafish that innervate adjacent territories.

In adult zebrafish, muscle fibers on each side of every body segment are innervated by three motoneurons which can be identified on the basis of their cell body positions within the spinal cord. One of these motoneurons, CaP, innervates muscle fibers in the most ventral part of its body segment while the RoP motoneuron innervates fibers just dorsal of this region in the central part of the segment. There is an abrupt boundary between the terminal fields of these two motoneurons with no obvious overlap between their adjacent territories.

To understand how this boundary is established, we made direct observations of single, labeled motoneurons in live embryos throughout the first few days of development. We found that during initial development, the CaP growth cone, which pioneers the peripheral nerve, extends from the spinal cord and grows straight to the ventral part of the segment, the region that it will ultimately innervate. Consistently (13 of 16 neurons examined), however, we found that the CaP axon subsequently formed varicosities and small side branches along its proximal trunk in the region that would be innervated subsequently by the RoP motoneuron. We followed the fates of these proximal side branches on individual CaP motoneurons and found that they stopped growing during the second day of development and usually were retracted even though more distal branches located in the ventral part of the segment continued to grow.

These results suggest that the terminal fields of CaP motoneurons are established by two processes: exuberant axonal growth within the appropriate territory and selective pruning of side branches located in inappropriate regions. Supported by NS21132.

392.3 THE PROBABILITY OF QUANTAL SECRETION ALONG TERMINAL BRANCHES OF COMPETING AMPHIBIAN NERVE TERMINALS DURING SYNAPSE ELIMINATION. Nickolas A. Lavidis* and Max R. Bennett, Neurobiology Research Centre, University of Sydney, Sydney, Australia, 2006.

At amphibian motor endplates most mature and juvenile terminal branches possess a high level of quantal secretion for release sites near the branch origins; this declines exponentially for release sites further along the branch (Bennett, M.R. and Lavidis, N.A. Dev. Brain Res. 5: 1-9, 1982; Bennett, M.R., Jones, P. and Lavidis, N.A. J. Physiol. 279: 157-174, 1986). About 20% of muscle fibers in the amphibian glutaeus muscle receive a dual innervation from segmental nerves 8 (N8) and 9 (N9), (Bennett, M.R. and Lavidis N.A. J. Physiol. 375: 303-325, 1986; Malik, R. and Bennett, M.R. Dev. Brain Res. in press) during post metamorphic development, A comparison is made of the probability of quantal secretion along simple primary nerve terminal branches belonging to N8 and N9 nerve terminals on single and dually innervated muscle fibers.

On muscle fibers innervated either by N8 or N9 the total quantal content (mi) per 100μm of terminal length decreased exponentially with an increase in the total length of the terminal; the length constant for N8 was 400μm (correlation coefficient 0.79) and for N9 it was 430μm (0.81). The release of quanta along individual terminal branches measured with an extracellular electrode (me) decreased exponentially with distance from the point of branch origin for 62% of N8 terminal branches and 64% of N9 terminal branches (n=25); the remaining short branches in each case did not show a decline in release. (Table 1)

TABLE 1. Quantal release from terminals for single and dually innervated muscle fibers.

Innervation	mi/100μm of terminal length ±SD	Terminal Size (μm)	me along the length of terminal branches
Single N8	1.00±0.18	375-800	62% decreased exponentially
Dual N8	0.88±0.24	375-800	100% decreased exponentially
Single N9	2.35±0.80	50-250	64% decreased exponentially
Dual N9	0.28±0.12	50-250	100% uniformly low

There is a decrease in mi per 100μm of terminal length, as the terminals increase in length, for fibers singly innervated by N8 or N9 and for N8 on dually innervated fibers. This occurs as a result of the decline in the probability of secretion of release sites (me) added to the distal parts of terminal branches. N9 terminals on dually innervated fibers show a significantly low mi per 100μm of terminal length when compared to the larger N8 terminals. The probability of secretion at release sites of N9 terminals is suppressed by the larger N8 terminals on dually innervated muscle fibers prior to their possible elimination.

392.4 THE POSSIBLE ROLE OF ALPHA-MELANOCYTE STIMULATING HORMONE IN THE REGULATION OF SYNAPSE ELIMINATION IN THE FROG. N. Oren,* P.E. Micevych, and M.S. Letinsky. Depts. of Physiology and Anatomy, Ahmanson Laboratory of Neurobiology, University of California, Los Angeles, California 90024.

Motor nerve terminals in the frog cutaneous pectoris (CP) muscle have been shown to contain α-MSH-like immunoreactivity (Soc. Neurosci Abstr. 12:547, 1986) and to respond to in vitro α-MSH by increasing transmitter release (Johnson et al., Science 220:1071, 1983). Since the augmented release outlasted the presence of the peptide, it is possible that α-MSH modulated the nerve terminals' functional state. In order to determine whether α-MSH can modulate synaptic function in vivo we tested the effect of a potent and long lasting analogue [Nle^4, D-Phe^7]- α-MSH on synapse elimination in the frog. Tadpoles and postmetamorphic bullfrogs (Rana catesbeiana) were injected twice weekly with 0.1 ml of 10^{-6} mM analogue in 0.1% BSA-NFR or vehicle solution. Animals received injections throughout the period of rapid synapse elimination for the CP muscle beginning at premetamorphic stage XXII until the end of the second week postmetamorphosis. Motor nerve terminals were stained with nitroblue tetrazolium salts and the amount of polyneuronal innervation and synaptic organization determined for various development stages (Letinsky and Morrison-Graham, J. Neurocytol. 9:321, 1980). Administration of [Nle^4, D-Phe^7]- α-MSH, significantly accelerated the appearance of single innervation. This was especially apparent at premetamorphic stages where analogue injected tadpoles had 30-35% single innervation compared to the 12-23% found in the vehicle injected animals. With further development the amount of single innervation increased in both groups, but the [Nle^4, D-Phe^7]- α-MSH treated muscle had 57-59% single innervation compared with 41-42% in the control. Terminals innervated by overgrown branches from terminals or neighboring muscle fibers (serial-innervation) were the first to be eliminated (Morrison-Graham, Develop. Biol. 99:298, 1983). α-MSH analogue treatment also accelerated this process. The number of serially-innervated nerve terminals/muscle was significantly decreased during metamorphosis in analogue (56-61%) compared to vehicle (79-82%) treated muscles. These results suggest that α-MSH may be a factor involved in the regulation of innervation and synaptic organization in the frog. These experiments were supported by USPHS grants NS13470 (ML) and NS23468(PM).

392.5 **DIFFERENTIAL LOSS OF NEUROMUSCULAR CONNECTIONS ACCORDING TO SPINAL POSITION OF MAMMALIAN SKELETAL MOTOR NEURONS.** E.M. Callaway and D.C. Van Essen. Div. of Biol., Caltech, Pasadena, CA 91125.

We have examined the distribution of motor unit sizes as a function of spinal position in soleus muscles of rabbits at different postnatal ages. Motor unit twitch tensions and rise times were measured *in vitro*. The majority of the soleus motor axons (about 65) exit through the S1 root, while a minority (mean of 4-6 per root) leave through the L7 and/or S2 roots. Units were separated into fast and slow groups based on rise times, and twitch tensions were normalized to the median fast or slow value (as appropriate) from the S1 population for that animal to elucidate differences in size between L7/S2 and S1 root motor units. Motor unit sizes were measured from animals in 3 age groups: 4-5 days, when the rabbit soleus is heavily polyinnervated (seven animals); 8-9 days, when elimination is about half completed (six animals); and 11-15 days, when the soleus muscle is singly innervated (nine animals).

Results from 4-5 day animals indicate that when polyinnervation is substantial, L7/S2 neurons on average innervate at least as many muscle fibers as S1 neurons. For the slow population, L7/S2 units were larger than S1 units (129 ± 9 % of median, mean $\pm$ S.E.M., versus $106 \pm 5, p < 0.05$, Mann-Whitney U-test); for the fast population there was no significant difference (108 ± 7 versus $105 \pm 3, p > 0.5$). However, by 8-9 days L7/S2 units were significantly smaller than S1 units for both the slow population (84 ± 6 versus $109 \pm 5, p < 0.001$) and the fast population (79 ± 5 versus $108 \pm 4, p < 0.001$). This difference persisted in 11-15 day animals where L7/S2 motor units were smaller than S1 units for the slow population (86 ± 7 versus $107 \pm 4, p < 0.05$) and for the fast population (84 ± 7 versus $103 \pm 4, p < 0.05$). No significant differences were observed between motor units from L7 versus S2 extremes at any age, confirming the absence of a rostro-caudal bias reported by Gordon & Van Essen (*J. Physiol.* **339**, 591-597).

We conclude that motor neurons from extreme positions in the rabbit's soleus motor pool lose significantly more synapses than those from the middle. The differential loss of synapses appears to occur primarily during the earlier stages of synapse elimination, day 4 to day 9, when the rate of loss by extreme neurons is 50-80% faster than for middle neurons.

Bennett and Lavidis have shown that a differential loss of synapses by rostral versus caudal motor neurons contributes to topographic ordering of inputs within the the lateral gastrocnemius of the rat (*J. Neurosci.* **4**, 2204-2212). No topography has been demonstrated for the soleus muscle. It will be of interest whether our result is observed in other muscles or species since previous work has not made comparisons among extreme versus middle populations of motor neurons.

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392.6 **REINNERVATION OF NEONATAL RABBIT SOLEUS LACKS SPECIFICITY BY FIBER TYPE.** J. M. Soha, E. M. Callaway and D. C. Van Essen, Division of Biology, Caltech, Pasadena, CA 91125.

Neonatal rabbit soleus muscle contains both fast-contracting and slow-contracting muscle fibers, in about a 2:1 ratio. Previous physiological and anatomical studies have suggested a pattern of innervation which is highly specific by muscle fiber type, even while polyinnervation remains widespread. Chemospecific recognition of appropriate muscle fibers by motor neurons during synaptogenesis is one possible mechanism by which this specificity could be established. To study this possibility, we have looked for specificity during the reinnervation which follows experimental crush of the muscle nerve in neonatal rabbits, using the distribution of single motor unit twitch tension rise times in an *in vitro* assay.

Soleus nerves of rabbits were crushed just proximal to their insertion into the muscle at postnatal day 4 (N=7, 162 units) or 1 (N=3, 72 units). To minimize the opportunity for conversion of muscle fiber contractile type, muscles were analyzed *in vitro* 6 days after nerve crush, within 1-2 days of the start of reinnervation and when reinnervation averaged only about 30% complete. Single motor units were isolated by graded stimulation of teased ventral root filaments, and twitch tension traces recorded at room temperature. Histograms of twitch tension rise times in normal muscles were broad and bimodal. In contrast, histograms of twitch tension rise times in all 10 reinnervated muscles were narrow ($\sigma = 36$ ms vs. $\sigma = 76$ ms for normal units) and unimodal, suggesting that all motor units were composed of a similar combination of fast and slow contracting fibers. Rise times for reinnervated units were intermediate to those of normal control fast and slow motor units, indicating that the unimodal distribution did not arise from specific reinnervation by only one population of motor neurons.

To control for a possible dedifferentiation of muscle fiber contractile properties during the interval of denervation, a second set of muscles was analyzed following a transmission blockade for an equal interval induced by botulinum toxin poisoning. Following bath application of 4-aminopyridine to restore synaptic transmission, motor units from toxin treated muscles exhibited a distinctly bimodal distribution of twitch rise times. Hence, short-term inactivity did not markedly affect muscle contractile properties.

From these experiments, we conclude that there is little if any specificity by fiber type during reinnervation of early postnatal rabbit soleus. Our findings do not rule out chemospecific recognition during initial synaptogenesis. Differences in our results and those of Soileau and Thompson (Soc. Neurosci. Abstr. 11:101, 1985), who reported evidence of fiber-specific reinnervation of neonatal rat soleus, remain to be reconciled.

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392.7 TOPOGRAPHICALLY SELECTIVE REINNERVATION OF ADULT MAMMALIAN MUSCLES. M.B. Laskowski[1] and J.R. Sanes[2]. [1]Department of Physiology, St. Louis University School of Medicine, St. Louis, MO 63104; and [2]Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

In two rat muscles, serratus anterior and diaphragm, the rostrocaudal axis of the motor pool is systematically mapped onto the rostrocaudal axis of the muscle's surface (Laskowski and Sanes, J. Neurosci. 7: 252, 1987). One explanation for this orderly topography is that motor axons and muscle fibers might bear positionally varying labels that bias synapse formation between positionally matched partners. To test for the existence of such labels, we asked whether axons would selectively reinnervate topographically appropriate portions of the muscles following nerve transection in the adult.

Motor nerves supplying diaphragm or serratus anterior were cut near the muscle's edge; proximal and distal stumps were separated to avoid passive guidance of axons by their endoneurial sheaths. Four to 11 weeks later, following reinnervation, topography was assessed by recording intracellularly from muscle fibers while stimulating motor axons in individual ventral roots or rootlets. In both muscles, axons from the rostral portion of the motor pool reinnervated more fibers in the rostral half of the muscle than in the caudal half, while caudally-derived axons selectively reinnervated the caudal half of the muscle. Thus, while several previous studies have reported nonselective reinnervation of adult mammalian muscle (Landmesser, Ann. Rev. Neurosci. 3: 279, 1980), selectivity is demonstrable when rostrocaudal position is evaluated.

In a separate series of experiments, the serratus anterior was denervated in neonates (1 day postnatal), and reinnervation assessed 1-2 months later as above. Reinnervation was somewhat more topographically selective following neonatal than following adult denervation, although in neither case was the normal adult map faithfully reproduced. Thus, positional cues can be expressed after injury in motor neurons and muscle fibers of neonates as well as of adults. Together with previous evidence that preganglionic sympathetic axons from various levels of the spinal cord preferentially reinnervate intercostal muscles from matching segments (Wigston and Sanes, J. Neurosci. 5: 1208, 1985), the present results argue for the existence of a system of positional cues that play a role in organizing neuromuscular topography. (Supported by NIH and MDA.)

392.8 ABSENCE OF COMPETITIVE INTERACTIONS AMONG REGENERATING AXON TERMINALS OF MOTOR NEURONS. J.L. Denburg, B.F. Murphy Jr.*, S.L. Powell*. Biology Department, Univ. of Iowa, Iowa City, IA 52242.

The formation of patterns of connections between neurons and their targets often proceeds through a transient stage of multiple innervation in which the targets form synapses with both appropriate and inappropriate neurons. The elimination of the inappropriate connections is usually taken as an indication of competitive interactions among the axon terminals. In adult cockroaches, Periplaneta americana, axotomized motor neurons regenerate axons and eventually reform synapses only with the leg muscles to which they were originally connected. However, the reformation of this innervation pattern is preceded by the functional innervation of the muscles by excess motor neurons. This is followed by the elimination of the inappropriate synapses. The experiments reported here indicate that this process occurs without competitive interactions occurring among the axon terminals.

Leg muscle 178 is normally innervated only by the identified motor neuron, D_f. When the nerve containing the axon of D_f is crushed, this neuron and 30 other motor neurons are axotomized. At early stages of regeneration all of these motor neurons send axon terminals into muscle 178, as detected by the retrograde transport of wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP). During the interval of 40 - 60 days after nerve crush the inappropriate axon terminals are eliminated and only those of the appropriate neuron, D_f, remain.

In order to test for competitive interactions among the axon terminals this experiment was repeated in insects in which D_f had been killed by intracellular injection of pronase. The destruction of this motor neuron was confirmed by the disappearance of its cell body in toluidine blue stained whole mounts of the ganglion. The destruction of D_f's axon terminals was confirmed by the loss of uptake and retrograde transport of WGA-HRP, absence of binding of a monoclonal antibody specific for axon terminals and the loss of synaptic junction potentials evoked in the muscle by nerve stimulation. All these changes occurred by 5 days after injection of D_f and at this time the nerve was crushed. Inappropriate axon terminals grew into muscle 178 and were eliminated with an identical time course as during axonal regeneration when D_f was present.

These results indicate that competition does not play a role in the reformation of the original innervation pattern. A specific cell-cell recognition is required such that each motor neuron eliminates its inappropriate axon terminals only after it synapses with its original target muscle. (Funded by NIH grant 14295).

393.1 SELECTIVE LOSS OF NON-SYNAPTIC AND CLUSTERING OF SYNAPTIC 5- HT RECEPTORS DURING INNERVATION OF AN IDENTIFIED LEECH NEURON IN VITRO. P. Drapeau and S. Sanchez-Armass*, Neurosciences Unit, Montreal General Hospital Research Institute and McGill University, Montreal, Canada, H3G 1A4.

Identified Retzius neurons reform an inhibitory serotoninergic (5-HT) synapse with pressure-sensitive (P) neurons when the somas are removed from the leech and placed in tissue culture. We have shown previously (Biophys. J. 51:63a; manuscript submitted) that single P cells in culture have both Na channels (gNa) and Cl channels (gCl) that are activated by 5-HT. When a P cell is innervated by a Retzius cell, only gCl is activated by synaptic 5-HT release. These results are consistent with previously reported observations of differences in synaptic and somal responses to 5-HT for P cells in vivo. We have measured, using the voltage clamp, the pre- and postsynaptic ionic currents induced by 5- HT in order to examine the redistribution of receptors during synapse formation in this model culture system.

Application of saturating (>100 μM) 5-HT onto single P cells elicited a gCl of 27 +/- 4 nS (n=12). Synaptic release or application of 5-HT onto innervated P cells induced a gCl= 12 +/- 3 nS (n=22) and 3.6 +/-0.4 nS (n=5), respectively. A previous electron microscopic study (J. Comp. Neurol. 256:515-527,1987) demonstrated that the area of synaptic contacts is <0.1% of the total P cell surface area. This suggests a >400 fold increase in postsynaptic gCl density.

Synaptic release of 5-HT failed to activate gNa in P cells; 5- HT application resulted in a much lower gNa (3.5 +/- 1.2 nS; n=5) than was observed in single P cells (14 +/- 3 nS;n=18). gNa was reduced in P cells paired with Retzius cells prior to synapse formation (3.0 +/- 0.7 nS; n=19) whereas gCl was unaffected (23 +/- 4 nS; n=12).

gCl and gNa were observed in presynaptic Retzius cells but were unaffected by pairing with a P cell.

It is concluded that synapse formation is preceeded by the selective loss of counter-effective gNa and results in clustering of gCl specifically in the postsynaptic neuron.

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393.2 THE DEVELOPMENT OF EVOKED TRANSMITTER RELEASE AND THE PRESYNAPTIC CALCIUM CURRENT IS INDEPENDENT OF INTERNEURONAL COMMUNICATION. P.G. Haydon, E.M. Cawley*, and R.G. Wong. Dept of Zoology, Iowa State University, Ames, IA 50011, and Dept. of Biology, Carleton College, Northfield, MN 55057.

We previously reported that pairs of isolated identified neuronal somata of Helisoma reliably form a cholinergic chemical synapse in cell culture in the absence of neurite extension(Haydon, P.G. Neurosci. Abst.12: 188). Since both the pre and postsynaptic elements of the synapse are large spherical somata (100 um diameter) which are devoid of neurites, this system permits direct electrophysiological access to the synaptic terminal.

We now report that the initial development of synaptic transmission is independent of interneuronal communication. Using the somata of neurons B5 (presynaptic) and B19 (postsynaptic) we have determined whether maintained contact between B5 and B19 is required i) to gain the ability to release neurotransmitter, and ii) for the development of a specific calcium current of the presynaptic membrane.

Single identified neurons were isolated from adult specimens of the pond snail Helisoma and cultured alone in 1% hemolymph in defined medium for upto 3 days. To detect transmitter release from B5, this neuron was transferred to a recording chamber and the soma of B19 was manipulated into contact with B5 to act as a bioassay for released acetylcholine. On days 1-3 the spontaneous release of transmitter from B5 was reliably detected in the form of transient depolarizing potentials in B19. Action potential evoked release of neurotransmitter was readily detected only after a culture period of 3 days; at earlier times (days 0-1) evoked release was rarely present. Thus, maintained contact with a postsynaptic cell is not required to develop the ability to release neurotransmitter.

Given the importance of an influx of Ca^{++} in the transmitter release process, we determined the characteristics of the presynaptic calcium current when evoked transmitter release was reliably detected. A voltage clamp study of neuron B5 was performed using patch pipettes in the whole-cell mode of recording. Calcium currents were selectively detected using a combination of standard ion-substitution and pharmacological manipulations. The membrane of B5 contains at least 2 pharmacologically separable calcium currents on day 0-1 of culture. However, by day 3, when action potentials in B5 can evoke transmitter release, only one calcium current was present. This voltage-dependent current is characterized by its slow rate of decay, activation threshold of -20 mV, is blocked by cadmium, and barium readily replaces calcium as a charge carrier. Furthermore, the appearence of this specific calcium current occurs irrespective of whether the presynaptic cell (B5) contacts the postsynaptic neuron (B19). Thus, interneuronal communication is not required for the initial development of the presynaptic calcium current nor is maintained contact required to develop the ability for action potentials to evoke transmitter release

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393.3 DEVELOPMENT OF PROPERTIES OF TRANSMITTER RELEASE DURING INITIAL STAGES OF SYNAPTOGENESIS. I.Chow, S.H.Young, J.Cheng* and A.D. Grinnell. Jerry Lewis Neuromuscular Res. Ctr. and Dept. of Physiol. University of California, Los Angeles, CA 90024.

Previous studies have shown that after a muscle cell is manipulated into contact with an isolated cholinergic neuron MEPP-like depolarizations can be recorded from the muscle cell within a few minutes (Chow & Poo, J.Neurosci.5:1076, 1985). We have investigated the possibility that this acetylcholine (ACh) release is specific to muscle contact and that it may be a result of cell-cell surface interaction rather than that of a secreted factor. Whether such a manipulated muscle contact will also cause changes in the neuron, such as increase in the number and preferential localization of presynaptic ACh vesicles was also investigated. In addition, experiments testing the presence of an excitation-transmitter release mechanism at the manipulated soma-myoball contacts were carried out. Both intracellular microelectrode and patch electrode recording techniques were carried out on 1-day old Xenopus nerve-muscle co-cultures. The presence of presynaptic vesicles was detected by using monoclonal antibodies to vesicle membrane proteins labelled with peroxidase-anti-peroxidase (Ab-PAP).

Excised outside-out muscle membrane patches were used to detect the extent of ACh release from growth cones of isolated neurons,and after masses of "freshly killed" muscle cells or neurons were pressed into cotnact with the region of neurite just proxinal to the growth cone. Within minutes of muscle mass contact bursts of ACh channel activity were detected, whereas no or only slight increase in single ACh channel activity was detected after neuron mass contact. Since the cell masses consisted of membranes of cells stabbed successively, these results suggest that the triggering molecules for ACh release are found on the muscle cell membrane surface.

After 5-6 hours of manipulated contact between myoball and soma Ab-PAP reaction product was found in 80% of the somata and 100% of the neurites when contact was made between muscle and neurite. In isolated uncontacted neurons about 55% of the somata and 27% of the neurites had the reaction product.

Using the tight seal patch clamp technique, which allows the detection of action currents (see Cohan & Kater, Science 232: 1638, 1986), we stimulated the soma manipulated into contact (up to 1hr) with a myoball from which continuous recording with an intracellular microelectrode was maintained. No evoked EPPs were detected even when action currents were induced in the soma, while MEPP-like depolarizations continued to be present. This suggests that, at least during the initial stages of nerve-muscle contact, ACh release cannot be easily evoked by depolariaing the somatal membrane, and that some "maturation" process takes place for development of the excitation-tansmitter release machinery.

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393.4 MORPHOLOGY OF DEVELOPING NEUROMUSCULAR JUNCTION DURING THE FIRST FEW HOURS OF FUNCTIONAL CONTACT. J. Buchanan*, Y-a. Sun, and M-m. Poo. Section of Molecular Neurobiology, Yale University School of Medicine, New Haven, Ct 06510.

The structure and function of early synaptic contacts between Xenopus spinal neurons and myotomal muscle cells were studied in cell cultures. Isolated spherical muscle cells (myoballs) were manipulated into contact with the growth cone of the co-cultured neuron at a defined time and the development of synaptic transmission was monitored by tight-seal whole-cell recording of the nerve-evoked and spontaneous synaptic currents in the muscle cell. In the majority of cases, functional neuromuscular transmission was established within the first minute of contact. To assess changes in synaptic function, two separate recordings (each about 3 min duration) were made at the beginning and the end of the contact period (ranging from 20 min to 6 hrs) and immediately prior to fixation. These functional cell pairs were then fixed and processed for thin-section transmission electron microscopy.

The morphology of isolated (non-contacting) nerve and muscle cells and that of naturally-occurring neuromuscular synapses in 2-3 day cultures were used as the basis for comparing synaptic specialization in the manipulated contacts. We found that during the first few hours of contact, when functional transmission had already become highly reliable, few specializations that characterize mature synapses were detectable. Some nerve processes in contact with the muscle cells showed clear and dense-core vesicles in a distribution similar to non-contacting growth cones. Coated pits and vesicles were frequently observed in both nerve and muscle cells. Interestingly, the intercellular spacing between the nerve and muscle membranes, or cleft, at the contact sites was quite narrow (less than 20 nm) and sometimes absent. Little basal lamina-like material was seen on the cell surface or in the cleft. The earliest specialization (observed in 20 min contacts) was a fuzzy appearance of the muscle membrane at the contact sites. Thickening of muscle membrane at the contact sites was also observed in some cases (as early as 3 hrs). Out of 18 cell pairs, for which serial sections have been examined, only in one case (40 min contact) did we find aggregations of clear vesicles in a configuration similar to that of the active zone. We concluded that early functional neuromuscular contacts are in general morphologically undifferentiated and the first sign of specialization appears to be an accumulation of undefined muscle membrane associated substance at the site of contact. The narrow cleft between the nerve and muscle membranes during the first few hours of contact may allow direct interactions of cell surface molecules. Supported in part by NIH grant NS-22764.

393.5 ELEVATED POTASSIUM INCREASES SYNAPSE FORMATION IN SPINAL CORD - MUSCLE COCULTURES. J.M. Thompson and S. Ruch*. Department of Anatomical Sciences, School of Life Sciences and College of Medicine, University of Illinois, Urbana, IL 61801

One force which has been implicated in stimulating the formation and stabilization of synapses is activity of either the presynaptic and/or the postsynaptic cell. As a model of increased activity, in tissue culture the application of depolarizing agents such as increased extracellular KCl will produce a chronic depolarization of neural and muscle cells which mimics constant activation of the cells. Thus, cultures of spinal cord neurons and muscle cells were grown in 5.4 mM, 13 mM and 38 mM KCl (osmolarity balanced by reducing NaCl, normal KCl concentration of medium is 5.4 mM). To assure that the components of the culture medium do not change, the medium was changed every other day. After 1 and 5 days of coculture in these conditions, one hour prior to recording, the culture medium is removed and replaced with fresh medium at the normal extracellular potassium concentration (5.4 mM KCl). The cultures were then examined for the presence of nerve-muscle synapses by intracellular recording of the muscle cells. Previous studies have demonstrated that spinal cord - muscle synapses are formed within the first 48 hr of coculture.

We observed an increase in the percentage of muscle cells innervated by spinal cord neurons with increasing concentration of KCl at both 1 and 5 days in vitro as shown in the following table:

	5.4 mM KCl	13 mM KCl	38 mM KCl
	(Percent of muscle cells innervated)		
1-Day in Vitro	26 ± 4.0 (5)	39 ± 7.5 (5)	47 ± 13.3 (5)
5-Days in Vitro	16 ± 4.0 (5)	58 ± 6.5 (6)	78 ± 8.0 (5)

The frequency of spontaneous muscle responses also increased with potassium concentration. There was not a selective increase in the survival of neurons in the higher potassium conditions, nor was there a selective increase in neurite outgrowth from the spinal cord neurons. Addition of cytosine arabinoside, a mitotic inhibitor, to the high potassium medium did not inhibit the increase in synapse number seen at 5 days. Addition of d-tubocurarine (100 uM) partially reversed the increase in synapse number produced by the high potassium medium. These results indicate that chronic depolarization by increased extracellular potassium stimulates the number of spinal cord - muscle synapses.

393.6 AN ACETYLCHOLINE RECEPTOR INDUCING FACTOR FROM CHICK BRAIN INCREASES THE LEVEL OF mRNA ENCODING THE RECEPTOR α-SUBUNIT. D.A. Harris, D.L. Falls, and G.D. Fischbach. Dept. of Anatomy and Neurobiology, Washington Univ. School of Med., St. Louis, MO 63110.

We have recently purified a glycoprotein from chick brain that stimulates the rate of insertion of acetylcholine receptors (AChRs) into the surface of cultured chick myotubes (Usdin and Fischbach, J. Cell Biol. 103:493-507, 1986). This protein, called 42kD ARIA (for AChR inducing activity), may play a role in the nerve-induced accumulation of AChRs at developing neuromuscular synapses. The increased rate of insertion of AChRs produced by ARIA might result from enhanced synthesis of receptor subunits, or from more efficient post-translational processing. We report here that ARIA increases the level of α-subunit mRNA, and thus may increase insertion by stimulating subunit biosynthesis.

The preparation of 42kD ARIA used in our experiments consisted of the most active fraction from a Vydac C_{18} column eluted with an acetonitrile gradient in heptafluorobutyric acid. The material used is purified more than 40,000 fold compared to a saline extract of chick brain. ARIA was assayed in 35mm plates that had been seeded 5 days earlier with $1.5x10^6$ mononucleated muscle cells. The rate of new receptor insertion was measured with ^{125}I-α-bungarotoxin after blocking all preexisting receptors with unlabeled toxin. The amount of α-subunit mRNA was quantitated in the same cultures by a nuclease protection assay. ^{32}P-labeled anti-sense RNA was synthesized using a template constructed from px2, a genomic clone which contains exon 7 of the chick α-subunit (kindly provided by M. Ballivet, Universite de Geneve). Total cellular RNA (2-10 ug/plate) was hybridized in solution with the probe and then digested with RNase and analyzed on urea-polyacrylamide gels. Northern blots demonstrated that α-mRNA is approximately 3kb in both control and ARIA-treated cultures.

We have found that 42kD ARIA produces a dose-dependent increase in the amount of α-subunit mRNA, which parallels the increase in the rate of AChR insertion. A 1.5-fold stimulation can be produced by as little as 5ng/ml of the partially purified preparation, and the maximal effect was approximately 3-fold at 110ng/ml. A 3-fold increase in α-subunit mRNA levels and in AChR insertion rate was also produced by treating muscle cultures for 24 hrs with 1uM tetrodotoxin. ARIA does not alter the amount of mRNA encoding β-cytoplasmic actin.

Experiments are in progress to assess the effect of ARIA on the mRNAs encoding the γ and δ subunits of the AChR, and to determine the time course over which the factor acts.

393.7 ELIMINATION OF DISTRIBUTED SYNAPTIC ACETYLCHOLINE RECEPTOR CLUSTERS FROM DEVELOPING AVIAN FAST-TWITCH MUSCLE FIBERS ACCOMPANIES LOSS OF POLYNEURONAL INNERVATION. William D. Phillips* and Max R. Bennett, (SPON: J. Stone). Neurobiology Research Centre, University of Sydney, Sydney, Australia, 2006.

In the adult chicken, the fast-twitch fibers such as those of the posterior latissimus dorsi (PLD) muscle are each innervated at a single (focal) synaptic site. Depolarization of the fiber depends upon the propagated action potential. However nerve evoked contraction of the muscle is underway by embryonic day 6-8 (E6-E8) several days before the development of the action potential mechanism. In order to investigate the distribution of synaptic acetylcholine receptor clusters (AChR-C) on developing fast-twitch fibers, embryonic muscles were ultrasonically dissociated into single fiber fragments and were stained with fluoresceine conjugated α-bungarotoxin. Presumptive fast-twitch fibers were distinguished from the minority of slow-type fibers in the PLD by immunofluorescence using an antibody against slow-type myosin. Whereas mature PLD muscle fibers are focally innervated, at embryonic day 11 (E11) many of the fast-type fiber fragments from the PLD (44 ± 6% mean ± SEM, n = 7 embryos, mean fragment lengths 500-600 μm) displayed two or more large (longer than 2 μm) AChR-C. Double labelling with anti-neurofilament antibody suggested that most of these AChR-C (82 ± 2%) were associated with neuromuscular contacts. There was a progressive decline in the number of large AChR-C per 1000 μm of fiber from 3.2 ± 0.5 at E11 to 0.4 ± 0.1 at E18. By E18 fiber fragments with more than one large AChR-C were rare (1 ± 1%). Primary generation muscle cells identified at E11 and E16 by tritiated thymidine labelling showed a decline in the number of large AChR-C per 1000 μm proportional to that seen in the fiber population as a whole, suggesting that distributed synaptic AChR-C are eliminated from individual fibers as they mature. Thus it would seem that establishment of the mature pattern of innervation on avian fast-twitch fibers involves the elimination of distributed synaptic sites as well as the loss of multiple axon terminals at those sites which remain. Both processes occur during the period E11 to E18. Partial paralysis of embryos with d-tubocurarine starting at E6 prevented the loss of distributed AChR-C from fast-type PLD fibers between E11 and E14, suggesting that the activity of the motoneurones may play an important role in establishing the focal synaptic site AChR-C.

393.8 INVOLVEMENT OF CALCIUM CHANNELS IN THE NEUROTROPHIC REGULATION OF ACETYLCHOLINESTERASE IN MUSCLE CELLS. N. Rosenberg*, A. Yaron*, B. Attali*, F. Rieger, L. Garcia*, A.M. Tassin* and Z. Vogel. Depts. of Neurobiology and Biophysics, Weizmann Institute of Science, Rehovot, 76100, Israel, and Dev. Biol. Pathol. Neuromuscul., INSERM U153, 75005, Paris, France.

Muscle electrical activity as well as neurotrophic factors have been reported to play a role in acetylcholinesterase (AChE) accumulation in muscle. In addition, Ca^{2+} ions have been implicated in regulation of AChE in muscle cells in culture.

The high speed supernatant of extract prepared from one-month old rat brains stimulated the accumulation of AChE in C_2 cultured mouse muscle cells. We have purified the stimulatory activity by various HPLC procedures. A purification of more than 20-fold was obtained by gel filtration on TSK-G 3000SW column. This neurotrophic factor (NTF) preparation increased AChE accumulation in a dose dependent manner (1- to 4-fold). NTF is of a low molecular weight (<3000 daltons) and its $t_{1/2}$ at 100°C is 30 min. Incubation for 48 hr with NTF increased all AChE molecular forms in the muscle cultured cells. However, the increase of the 16S form was relatively more pronounced reaching 7-fold, compared with the lighter forms which increased by 2-fold. In addition, treatment with NTF changed the cellular distribution of AChE and increased the number of membrane patches containing high concentrations of enzyme. Increasing Ca^{2+} concentration in the culture medium synergistically increased the effect of NTF on AChE accumulation, while Ca^{2+} by itself had only a marginal effect. The voltage dependent Ca^{2+} channel blocker, nitrendipine, markedly inhibited the effects of NTF on the accumulation of AChE (by more than 60%). It also reduced the amount of AChE in the untreated cells although to a somewhat lesser extent (ca. 30%). Nitrendipine also sharply inhibited the clustering of AChE seen in NTF-treated cells. Moreover, preincubation of C_2 cells with the factor increased the $^{45}Ca^{2+}$ rate of influx into the cells by 50 to 80% compared with control cells.

In summary, our results suggest that NTF increases the uptake of Ca^{2+} into the cells, possibly through nitrendipine sensitive sites. This uptake of Ca^{2+} plays a role in the regulation of AChE in the cells.

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393.9 DELAYED EXPRESSION OF 200 KILODALTON NEUROFILAMENT PROTEIN AT THE NEUROMUSCULAR JUNCTION. S.P. Donahue*, A.W. English, and J. WOOD*. Dept. of Anatomy and Cell Biology. Emory University School of Medicine. Atlanta, GA 30322 (SPON: Barbara L. Brown).

The 200kD neurofilament protein (200kD NFP) is thought to stabilize axons in the adult CNS. We tested the hypothesis that the 200kD NFP stabilizes PNS axons by observing immunoreactivity to the 200kD NFP during neuromuscular synapse elimination, a postnatal process whereby polyinnervated muscle fibers lose innervation from all but one axon. If 200kD NFP selectively stabilizes axons, its appearance in a presynaptic axon might favor connections made by that axon. Axons lacking the 200kD NFP would be eliminated because of their inherent instability.

Fisher rats of postnatal ages zero to 19 days were deeply anesthetized and perfused through the heart with 4% paraformaldehyde and 0.1% glutaraldehyde. The triceps surae muscles were sectioned in a cryostat, reacted to demonstrate 200kD NFP immunoreactivity, and processed to localize acetylcholinesterase (AChE) at endplate sites. Animals five days and older show immunoreactivity to the phosphorylated 200kD NFP as far distally as preterminal axons. Animals younger than three days of age show immunoreactivity in axons, but not in presynaptic axons within AChE-stained endplates. Immunoreactivity appears in preterminal axons on the third postnatal day, after which time several immunoreactive axons can be observed at many endplates.

The number of axons observed to contact each fiber is comparable to that obtained by intracellular recording from muscle fibers (see also Bennett et al., J. Physiol 381:351-376, 1986), suggesting that the 200kD NFP appears in each axon that makes a functional synapse.

Some evidence suggests that the 200kD NFP becomes phosphorylated after it has been transported down the axon. Since our antibody recognizes a phosphorylated epitope, it is possible that a non-phosphorylated 200kD NFP is present during the perinatal period. To test this idea, we reacted sections from triceps surae muscles with an antibody to non-phosphorylated 200kD NFP. The results are similar to those described above, suggesting that post-translational modification of the 200kD NFP does not account for the delayed appearance we observed.

We conclude that selective appearance of the 200kD NFP does not determine which axons survive. We are unable to rule out the possibility that the subsequent selective loss of the 200kD NFP in some axons might trigger their demise. Manipulating synapse elimination by varying activity may determine if 200kD NFP expression is related to the process of synapse elimination or proceeds independently. Grant support: NS17731 and NS20545.

393.10 ULTRASTRUCTURAL CHARACTERIZATION OF SYNAPTIC BOUTONS IN THE CARDIAC GANGLIA OF POSTMETAMORPHIC AND ADULT *XENOPUS LAEVIS*. L. C. Streichert*[1,2], C. Magill[1] and P. B. Sargent[2]. (SPON: M. A. Baker). [1]Neurosciences Program, Stanford University, Stanford, CA 94305, and [2]Division of Biomedical Sciences, University of California, Riverside, CA 92521.

During postmetamorphic growth parasympathetic neurons in the frog cardiac ganglion undergo a dramatic increase in cell body size. Previous work from this lab has demonstrated that during growth there is a direct correlation between the surface area of a cell body and the number of synaptic boutons which terminate upon it. The overall density of boutons and the average size of individual boutons as seen in the light microscope remain constant. Therefore, the increase in synaptic contact which accompanies cell enlargement is regulated by increasing the *number* of synaptic boutons.

The preceding result suggests that synaptic boutons are the structural units of synaptic growth. If so, then the fine structure of boutons should appear similar in animals of different sizes and stages of postmetamorphic development. To examine this further, serial section electron micrographs of synaptic boutons from the cardiac ganglia of frogs which have just completed metamorphosis (stage 66) were analyzed and compared with those from adults.

Synaptic boutons appear as axonal swellings which contain synaptic vesicles. Active zones were defined by the presence of synaptic vesicles in proximity to electron-dense pre- and postsynaptic membrane specializations. At both stage 66 and in the adult the area of bouton-soma contact, the number of active zones per bouton, the area of active zone contact and the fraction of bouton area which is occupied by active zone were measured.

Synaptic boutons in adults usually have one active zone but may have as many as five. Larger boutons tend to have more active zones. The area of bouton-soma contact in adults varies over a 6-fold range, and the area of active zone contact varies over an 8-fold range. A similar degree of variability is observed for boutons at stage 66. No significant differences are observed for any of the parameters measured in stage 66 and adult animals, indicating that boutons at the two stages are morphologically similar. The only observed difference between synapses at the two stages is the number of boutons on the somatic surface. This result supports the hypothesis that boutons are the structural units of synaptic growth.

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393.11 FORMATION OF SYNAPSES BY SYMPATHETIC PREGANGLIONIC NEURONS. R.I. Hume and M.G. Honig. Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109.

The only synapse for which a detailed timetable of the development of synaptic function is known is the skeletal neuromuscular junction. One wonders whether the schedule of events might be different at other synapses. To address this issue we have examined the properties of developing synapses between sympathetic preganglionic neurons and their normal target neurons from sympathetic ganglia.

All experiments were performed on neurons in dissociated cell culture. Cells were labelled prior to co-culture with long-lasting fluorescent carbocyanine dyes so that we could determine the identity of each cell type (Honig and Hume, J. Cell Biol. 103:171). Our culture methods were similar to those previously described except that we found that the incidence of synaptic connections was higher when collagenase rather than trypsin was used to dissociate the cells of the spinal cord. Preganglionic neurons from stage 30-32 chick embryos were added to cultures of sympathetic ganglion neurons (from stage 33-35 embryos) that had been established 1-20 days previously.

The earliest we detected synaptic potentials was at 3 days of co-culture. The frequency with which we detected connected pairs of cells increased until about day 6 of co-culture. At 3 days synaptic function usually ceased after only a few stimuli, but by 4 days we could typically record hundreds of sequential synaptic potentials. The physiological properties of connected pairs studied between 4 and 10 days seemed quite similar. The EPSPs ranged in size from 2-30 mV and fluctuated between discrete levels (typically of 1-2 mV spacing), even when the calcium level was high (5 mM) and the stimulation rate was low (.2 Hz). At higher stimulus rates (.5-5 Hz), EPSP amplitude rapidly declined with repetitive stimulation, though the spacing between steps did not seem to change. These results suggest that release is quantal and that the number of release sites might be quite small. To test this possibility, we injected pairs of synaptically connected neurons with intracellular markers (Lucifer yellow or horseradish peroxidase). An individual ganglion cell received from 2 to 40 boutons from the preganglionic neuron that innervated it. These boutons were typically located on some, but not all, of the dendrites of the ganglion cell, and often on its cell body as well.

These results indicate that several properties of mature synapses develop relatively rapidly.

393.12 CHRONIC STIMULATION ALTERS SYNAPTIC CONNECTIVITY IN A COMPARTMENTAL TISSUE CULTURE SYSTEM. E.A. Neale, C. Yu*, L.M. Bowers*, S.C. Fitzgerald*, and P.G. Nelson. Lab. of Develop. Neurobiol., NICHD, NIH, Bethesda, MD 20892

Synapse elimination and stabilization are important phenomena during development of neuromuscular and neuro-neuronal synaptic connections. In an effort to understand the cellular mechanisms involved in these processes, we have undertaken a study of the effect of electrical stimulation on synaptic interactions occurring in multicompartment culture chambers (Campenot, R.B., PNAS, 74:4516, 1977).

Cells dissected from the ventral horn (VH) of the embryonic mouse spinal cord are plated on a layer of non-neuronal feeder cells in the central compartment of the chamber. A week later, dorsal root ganglion (DRG) cells are plated in the side compartments. Within several days, DRG neurites grow under a grease barrier into the central compartment and form synaptic contacts with the VH neurons.

Platinum coil electrodes attached to the culture dish lids are used for chronic stimulation across the chamber barriers. Intracellular recordings from DRG neurons in the side chambers are obtained in dishes connected in parallel with chronically stimulated dishes to ensure adequacy of stimulation. Stimulus parameters used in the chronic experiments are: five bipolar 100-200 μsec pulses, 1-2 msec separation, delivered at 10-20 Hz every 1-1.5 sec. Stimulus intensity is about 4X threshold.

Intracellular recordings are obtained from VH neurons in the central compartment to assess synaptic connectivity. The number of separate synaptic inputs to individual neurons is determined using graded stimulation across each barrier. (Occasional apparent antidromic activation of VH neurons is seen). VH neurons in the central compartment, but close to one of the barriers, have more inputs from DRG neurites crossing under that barrier from the adjacent side compartment than from DRG neurons in the opposite side compartment. After four days of chronic stimulation, the relative connectivity between chronically activated DRG neurons and VH neurons near the opposite barrier is increased. Preliminary data from control cultures indicate that the ratio of inputs from the adjacent as compared to the opposite side is 1.9 (n=41; SD=0.6). In chronically stimulated cultures, this ratio for VH neurons near the unstimulated barrier is 1.2 (n=29; SD=0.5). This difference is significant at $p<0.05$.

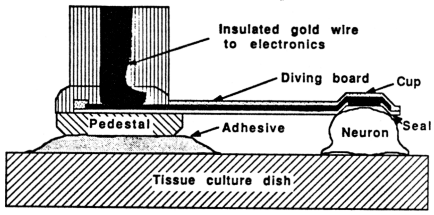
393.13 EFFECTS OF ALTERATIONS IN THE LEVEL OF SPONTANEOUS BIO-ELECTRIC ACTIVITY ON THE DEVELOPMENT OF FIRING PATTERNS IN CULTURES OF DISSOCIATED RAT CEREBRAL CORTEX. Ger J.A. Ramakers* and Michael A. Corner. Netherlands Institute for Brain Research, 1105AZ Amsterdam, The Netherlands.

Synaptogenesis in cultures of dissociated cerebral cortex of fetal rats has been found to be lowered when spontaneous bio-electric activity (SBA) is chronically suppressed with tetrodotoxin (TTX), and accelerated during treatment with the GABA-antagonist picrotoxin (PTX). Spontaneously active single neurons in normally grown cultures show a development from highly stereotyped burst activity, with overall high interval dependency and a low variability in the burst period (i.e. the interval between the onset of two successive bursts) at around 10 days in vitro (DIV), to firing patterns having a low interval dependence and high variability in the burst period by 21 DIV.

To see if TTX and PTX also affect the development of the firing patterns, cultures were grown in medium containing 0.1 uM TTX or 1 uM PTX. In control cultures (recorded at around 14, 21 and 42 DIV) the overall interval depence decreased with age. In TTX-grown cultures, when recorded in control medium, interval dependence remained as strong as at the early ages. Conversely, the PTX cultures (which were only recorded at 21 DIV) tended towards higher interval dependence than in the controls. At 21 DIV variability in the burst period was significantly lower in TTX cultures and higher in PTX cultures. These results suggest that the amount of SBA during development in vitro affects the rate of development of firing patterns, parallel to effects on synaptogenesis.

393.14 A CHRONIC *IN VITRO* MICRODEVICE-NEURON CONNECTION. W. G. Regehr*†, S. B. Kater‡, and J. Pine† (SPON: D. C. Van Essen). †Departments of Physics and Applied Physics, California Institute of Technology, Pasadena, CA 91125. ‡ Program in Neuronal Growth and Development, Colorado State University, Fort Collins, CO 80523.

A new method for long term recording from, and stimulation of cultured neurons has been developed. Silicon-based microelectrodes have been fabricated using integrated circuit technology. The figure below is a schematic of such an electrode with its silicon pedestal glued to the bottom of a tissue culture dish and in electrical contact with a neuron. Attached to this pedestal is an insulated gold wire to make contact to the electronics, and a long "diving board" structure that consists of a gold lead sandwiched between two insulating layers. This gold lead is exposed only at the bottom of a cup structure that makes a seal to the cell in much the same way as a conventional glass patch electrode.



These electrodes have been used to stimulate and record from identified *Helisoma* neurons 5 and 19. To understand device operation two electrode experiments were performed, with a diving board electrode sealed to the top of a cell, and an intracellular electrode in the same cell. With the routinely attained seals of the order of 1MΩ it is possible to record action potentials. It is also possible to stimulate a neuron with a diving board electrode and to record the resulting action potential with the same electrode. Preliminary results indicate that it will be possible to noninvasively stimulate and record from neurons for several days. Using such a long term *in vitro* connection it will be possible to answer questions about development and plasticity that are difficult or impossible to answer using conventional techniques.

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393.15 NONINVASIVE TECHNIQUES FOR MEASUREMENT AND LONG-TERM MONITORING OF SYNAPTIC CONNECTIVITY IN MICROCULTURES OF SYMPATHETIC NEURONS. C.-B. Chien*, W. D. Crank*, and J. Pine. Division of Biology, California Institute of Technology, Pasadena, CA 91125

We are trying to gain insight into the cellular mechanisms of learning and memory by studying simple neuronal cultures, containing two to ten neurons each, whose connectivity we can map completely. To this end, we have developed noninvasive techniques which allow us to stimulate any selected cell(s) in one of these microcultures, and record from all the cells simultaneously. These techniques will allow us to measure all the synaptic strengths of a culture and observe their development over time. Further, we will be able to observe the effects of specific patterns of chronic stimulation (applied for days) on this development.

We have chosen cultures of principal neurons from the rat superior cervical ganglion as a simple, well-characterized system. These neurons grow reliably in long-term microculture on a substrate of extracellular matrix produced by bovine endothelial corneal cells, and form excitatory cholinergic synapses.

We apply extracellular stimulation using electrodes built into the bottom of the culture dish.[1] These electrode dishes, made with standard integrated-circuit technology, have a hexagonal array of 61 electrodes spaced such that any cell on the array may be stimulated. The electrode tips are platinized for low impedance; the electrode leads are made of indium tin oxide, a transparent conductor, to avoid obscuring the cells during optical recording.

For noninvasive recording, we stain cells with the voltage-sensitive fluorescent dye RH423.[2,3] An array of 256 optical detectors records the fluorescence signals, allowing simultaneous recording from each cell in the microculture. This dye gives a 1% fluorescence change during an action potential, which is detectable without any signal averaging; subthreshold EPSPs are detectable with averaging. Preliminary data indicate that photodynamic damage caused by the dye is not significant under the conditions we will use. Recording from all the cells in a culture while stimulating each cell in succession gives the physiological strengths of all the synapses in the culture.

Initial measurements of synaptic connectivity using these techniques will be presented.

[1]Pine, J. and Gilbert, J., Abstract 190.9, 12th Annual Meeting, Soc. for Neuroscience, 1982. [2]Grinvald, A., Hildesheim, R., Farber, I. C., and Anglister, L., Biophys. J. 39, 301-308, 1982. [3]This dye was graciously supplied by A. Grinvald. We are grateful for his invaluable assistance in setting up our dye-recording system.

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394.1 IMMUNOCYTOCHEMICAL CHARACTERIZATION OF A DORSAL ROOT GANGLION CULTURE SYSTEM DERIVED FROM TWO-DAY OLD RATS. J.J. Lah* and R.W. Burry (SPON: J.S. King). Dept. of Anatomy, The Ohio State Univ., Columbus, OH 43210.

Study of early events in the formation of synapses is often complicated by the presence of previously formed synapses. Dorsal root ganglion (DRG) cells do not form synapses on each other in culture. A culture system of DRG neurons could therefore be used advantageously in the study of early molecular events involved in synapse formation.

Dorsal root ganglia from 2-day old rats were pooled and incubated with collagenase and DNAse at 37°C. After 1 hr., the ganglia were mechanically dissociated, and neurons were grown on a collagen substrate with medium containing NGF at 20 ng/ml. Cultures were treated with cytosine arabinoside from 12 hrs. to 6 days in culture. This procedure yields DRG neuronal cultures contaminated by very few non-neuronal cells. Morphometric analysis has shown that the culturing procedure selects for neurons with maximum cell diameters ranging between 8um-56um (mean=22um). Cell counts of cultures have shown that there is approximately 30% neuronal cell survival at 14 days in culture.

Using immunocytochemical techniques, we have begun more detailed characterization of the expression and localization of several antigens in this culture system. Monoclonal antibodies directed against MAP-2 and Tau protein (courtesy of Dr. Lester Binder) were employed for initial characterization of processes formed by DRG neurons in culture. At 7 days in culture, staining for MAP-2 was largely restricted to the cell body. In contrast, all processes appeared to stain positively for Tau protein. Cultures were also probed for the expression of synapse-specific antigens using antibodies directed against SVP-65 (courtesy of Dr. William Matthews) and Synapsin-1 (courtesy of Dr. Pietro de Camilli). When followed for 14 days in culture, SVP-65 staining could be detected through the entire period. The pattern of staining, however, showed variable intensity in individual cell bodies and processes. Synapsin-1 immunoreactivity could also be followed through 14 days in culture, and, like SVP-65, there was variability in the intensity of staining of cell bodies. However, most processes appeared to be positive for Synapsin-1.

From the data described, we can draw several conclusions: 1) Viable, long-term DRG neuronal cultures can be conveniently established from 2-day old rats. 2) These cultures contain neurons with a wide range of cell diameters. 3) Processes produced by these neurons in culture stain for Tau protein but not MAP-2, indicating that they are axons. 4) In the absence of synapse formation, cultured DRG neurons show positive immunoreactivity for the synapse-specific proteins Synapsin-1 and SVP-65; it is not yet clear whether or not this is related to formation of synapses prior to removal of DRG neurons for culturing.

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394.2 PROTEINS ISOLATED FROM POLYLYSINE-COATED BEADS INCUBATED WITH NEURONALLY ENRICHED CEREBELLAR CULTURES Richard W. Burry and Diane M. Hayes*, Department of Anatomy, and the Neuroscience Research Laboratory, The Ohio State University, Columbus, Ohio. 43210-1239

The mechanisms for control of synapse formation are not known, but proteins are probably involved as receptors for signals between cells and/or as the signals themselves (Burry, 1985, Brain Res. 344:109-119). In an attempt to identify developmental synaptic proteins, we have isolated proteins from fractions of neuronally enriched cell cultures. An enriched population of neurons was obtained from mixed glial and neuronal cultures after antimitotic drug treatment. The presynaptic elements were further purified by isolating polylysine-coated beads from the cell cultures after presynaptic elements had formed on the surface of the bead.

Cerebella from 3 day rats were mechanically dissociated, plated at 2.5×10^4 per cm^2, and treated at 2 days with cytosine arabinoside. At 11 days in culture, an average of 77,500 neurons per cm^2 (31% cells plated) and 650 non-neuronal cells per cm^2 (0.3% cells plated) were seen. Thus, at 11 days in vitro, neurons represent 99.3% of the cells in culture.

The proteins present in these neuronal cultures were examined with NEPHGE after incubation of the culture in ^{35}S methionine. With autoradiography over 60 different proteins were detected. Most of the proteins were under 120 kd and only a dozen were basic proteins. To determine if the cultures were enriched with some proteins, the pattern seen in the cultures was compared to a silver stain of NEPHGE gels from 7- to 10- day rat cerebellum. While many of the proteins seen in cultures were detected in animals, the cultures showed numerous different proteins not detected in the animal

To further investigate the synaptic proteins, polylysine coated-beads were isolated from enriched neuronal cultures. Presynaptic elements which form on the surface of the bead (Burry, 1982, Brain Res. 247:1-16) were isolated from the cultures by centrifugation through a PBS Percoll gradient. Microscopic analysis showed that neuronal aggregates were separated at the top of the gradient. The pellet contained no neuronal aggregates and greater than half of the beads. Scintilation counts of the beads showed that between 3% and 10% of the counts from the neuronal culture were isolated with the beads. As a control, cultures were incubated for a few minutes with beads and had less than 1% of the counts. NEPHGE of the isolated bead proteins showed good correlation with the proteins from the whole culture. Enrichment for several proteins and reductions in other proteins was seen.

It is concluded that the enriched cerebellar culture system is an excellent source of neuronal proteins for studies of development and synaptogenesis.

Research supported by a grant from USPHS, NIH NS-19961, and funds from the Department of Anatomy.

394.3 EXPRESSION OF A UNIQUE NERVE TERMINAL PROTEIN DURING SPINAL CORD DEVELOPMENT. L.M. Cabalka*, T.C. Ritchie, M.A. Thomas* and J.D. Coulter. Dept. of Anatomy, The University of Iowa, Iowa City, IA 52242.

The S-7B8 monoclonal antibody binds to a membrane protein associated with select nerve terminals in rat central nervous system. In spinal cord, laminae I and II display the highest density of nerve terminals containing S-7B8 antigen, with a moderate density extending through lamina V. Primary sensory afferents contribute the majority of S-7B8 immunoreactive terminals. No S-7B8 immunoreactivity is present in cell bodies or most fiber tracts, in the adult. However, immunocytochemical studies on developing rat spinal cord indicate that S-7B8 antibody strongly stains growing nerve fibers. Staining becomes concentrated in nerve terminals during and after synaptogenesis. Primary afferent fibers in the spinal cord first exhibit S-7B8 immunoreactivity between embryonic day 15 and 17. Staining first appears in deeper laminae of the dorsal horn by postnatal day 2 (P2) and expands dorsally between P4-P6. Staining also occurs along the superficial margin of the dorsal horn at this developmental stage. By P7, S-7B8 immunoreactivity extends throughout the superficial laminae. Thereafter, staining in laminae I and II increases and achieves the characteristic adult pattern in the third postnatal week. Axons in the developing corticospinal tract (CT) also exhibited S-7B8 immunoreactivity. By P2, staining appears in the brainstem, and at P7 the stained CT was clearly discernible in lumbar spinal cord. The staining density peaked at P15, then diminished. The development of the S-7B8 staining pattern in the spinal cord dorsal horn confirms previous studies of the synaptic development of the primary afferent and corticospinal systems.

The adult ventral horn contains only sparsely distributed S-7B8 immunoreactive nerve terminals, but transient staining was evident between P0 and P15, with peak density between P7 and P9. Loss of this staining in later development may indicate developmental regulation of S-7B8 antigen production or a pruning back of nerve endings containing the antigen. A dot-immunobinding assay was used to compare the amount of S-7B8 antigen in particulate fractions of developing and adult spinal cord. The specific activity of the S-7B8 antigen peaks during the second postnatal week, correlating well with the wide distribution of S-7B8 immunoreactivity in the spinal grey matter at this developmental stage.

In summary, the results suggest that the S-7B8 antibody is a useful probe for studying the development of synaptic connections in the spinal cord. Supported by NS23783.

394.4 EXPRESSION OF A SELECTIVELY DISTRIBUTED NERVE TERMINAL PROTEIN IN CEREBELLAR CORTEX. D.J. Wright, T.C. Ritchie, and J.D. Coulter. Dept. of Anatomy, University of Iowa, Iowa City, IA 52242.

The monoclonal antibody S-7B8 recognizes an antigen localized to a subset of nerve terminals in rat brain. The antigen is an integral membrane protein and may be associated with synaptic vesicles. This report describes the distribution and developmental expression of the S-7B8 antigen within the rat cerebellum. In the adult rat, positive immunocytochemical labeling with the S-7B8 antibody is concentrated within the cerebellar molecular layer, although there is some sparse punctate labeling of the neuropil within the granule cell layer. No cell bodies were found to stain. Colchicine application to the cerebellum prior to processing for immunocytochemical staining did, however, cause the build up of the antigen within certain neuronal cell bodies. After 24 hours exposure to colchicine, numerous medium sized neurons identified as Golgi cells were labeled within the most superficial part of the granule cell layer. After 48 hours of exposure to colchicine, most of the granule cells also appear to contain diffuse S-7B8 positive staining. Hence the antigen recognized by the S-7B8 monoclonal antibody is localized to neurons that utilize different neurotransmitters, since Golgi cells and granule cells are believed to use GABA and glutamate respectively.

The developmental expression of the nerve terminal protein recognized by the S-7B8 antibody has been studied in vivo and in vitro. At a postnatal (P) age of 10 days, S-7B8 positive labeling is first discernible within the deeper half of the developing cerebellar molecular layer. The width of the S-7B8 positive zone broadens to the most superficial part of the molecular layer by P21. The time of appearance of the antigen recognized by the S-7B8 antibody corresponds to the formation of synapses by the parallel fibers of the granule cells, suggesting an important function for this protein within the newly forming parallel fiber synapses. Cultured neurons of dissociated P7 rat cerebellum plated onto poly-l-lysine coated glass coverslips express the S-7B8 antigen after three days in vitro. The S-7B8 positive labeling first appears within neuronal cell bodies, and subsequently becomes localized to growing neurites. Within 7 days in vitro the staining is punctate, and is presumably localized to presynaptic nerve terminals.

In conclusion, within the cerebellum, the antigen recognized by the S-7B8 monoclonal antibody is expressed by neuronal populations of apparently unrelated neurotransmitter content and is expressed by neurons at the time of synapse formation.

Supported by NIH grant NS23783.

394.5 ENDOGENOUS OPIOIDS REGULATE NEURONAL DEVELOPMENT AND SYNAPSE FORMATION IN THE RAT CEREBELLUM: ULTRASTRUCTURAL OBSERVATIONS. K.F. Hauser, P.J. McLaughlin and I.S. Zagon. Dept. of Anatomy, The M.S. Hershey Medical Center, The Pennsylvania State Univ., Hershey, PA 17033.

Utilizing Golgi-impregnations at the light microscopic level, we have previously demonstrated that endogenous opioid systems (i.e., endogenous opioids and opioid receptors) regulate Purkinje cell dendrite growth and spine formation (Hauser et al., 1987, Brain Res., in press). In these studies, continuous blockade of endogenous opioid-opioid receptor interactions (e.g., daily s.c. injections of 50 mg/kg naltrexone (NTX)) was used to accelerate growth, whereas intermittent (4-6 hour) blockade (e.g., 1 mg/kg NTX per day), which results in increased opioid-opioid receptor interactions, was utilized to inhibit growth. In the present experiment, the above opioid antagonist paradigm was used to examine the ultrastructural consequences of manipulating endogenous opioid systems on cerebellar development during the preweaning period. Sprague-Dawley rats were treated with sterile water (controls), 1 mg/kg NTX, or 50 mg/kg NTX from birth until sacrifice at day 10. Cerebellar lobules 7 and 8 were processed for electron microscopy, and Purkinje and granule cells were examined in detail, including quantitative analysis of molecular layer synapses.

Purkinje cells in rats receiving continuous opioid receptor blockade had more mature cytoplasmic features and synaptic development. Moreover, granule cell development was correspondingly accelerated by complete receptor blockade. Counts of molecular layer synapses/100 um^2 demonstrated that there were up to 2 fold more synapses following continuous opioid receptor blockade compared to controls. In contrast, with intermittent blockade, the degree of Purkinje cell maturation, as well as synaptic density, were comparable to controls. These results indicate that endogenous opioid systems regulate synapse formation, as well as cytologic differentiation, by functioning as an inhibitory influence. When findings from previous Golgi studies are considered, a direct correlation between Purkinje cell maturation and the density of afferent synapses is apparent. Because Purkinje cell development is highly dependent on the amount and type of presynaptic input, this suggests that opioid-dependent alterations in afferent cell populations are likely to be a significant mechanism by which these neuropeptides regulate Purkinje cell maturation.

Supported by NIH grant NS-20500.

394.6 PURKINJE CELL DENDRITIC SPINE MORPHOLOGY IN LEAD TREATED KITTENS: AN ELECTRON MICROSCOPIC STUDY. George W. Patrick and Douglas L. Franke*, Department of Anatomy, Indiana University School of Medicine, Fort Wayne, Indiana 46805.

Lead poisoning is known to cause neurological problems in children. Among these neurological disorders are hyperactivity, slowed learning rates, mental retardation and loss of fine motor coordination. Studies in rodents have shown that lead exposure causes a decrease in the dendritic arbor and number of synapses on cerebellar Purkinje cells. In previous studies of lead treated kittens using Golgi-Cox staining, we found changes in dendritic spine numbers and branching patterns of Purkinje cells. This study was designed to describe the ultrastructural consequences of lead on Purkinje cell synaptic spines. Kittens were obtained at birth from the colony at the Fort Wayne Center. Beginning the day after birth and continued daily, the kittens were given 20 mg/kg body weight lead acetate solution by esophageal intubation, with one kitten maintained as a control receiving sodium acetate solution (20 mg/kg). Kittens were killed at weekly intervals to determine the time course and sensitive period of lead induced pathology. The animals were anesthesized with 70 mg/kg pentobarbital and perfused by a transcardiac cannula with 0.9% saline followed by cold 6% glutaraldehyde in phosphate buffer. The brain was removed and a piece of cerebellum posterior to the central fissure (simple lobule) was excised. This was postfixed in glutaraldehyde and then osmium, and embedded in epon. Thin sections were obtained and electromicrographs of dendritic spines were developed. Synaptic spine counts and areal measurements were taken with a digitizer and WiCat computer. Students T-test and ANOVA were used for statistical analysis. No statistical differences were seen in spine area or number of synapses at any age. Preliminary data analysis indicates that control animals may have more short stubby and long thin shaped spines, while lead animals have greater numbers of short thin, pedunculated and polymorphic shaped spines. Differences in spine shape have been shown to represent alterations in functional status of the synapse. These results suggest that despite the increased density of dendritic spines seen in Golgi-Cox stained neurons, the brain of lead-treated animals is capable of sufficient plasticity to compensate at the synaptic level. However, this compensation does not preclude effects on the temporal and spatial properties of incoming electrical signals, thereby disrupting fine motor coordination without affecting the gross functioning of the cerebellum.

394.7 ULTRASTRUCTURAL LOCALIZATION OF A GROWTH-RELATED ANTIGEN (5B4) AND ITS RELATIONSHIP TO IMMUNOCYTOCHEMICALLY IDENTIFIED NEURONS IN THE OLFACTORY BULB OF THE ADULT RAT. Thomas J. Mahalik, K.H. Pfenninger, and Thomas E. Finger. Dept. of Cellular and Structural Biology, University of Colorado Medical School, Denver, Colorado.

The ultrastructural distribution of the growth-regulated antigen, 5B4, was examined in the olfactory bulb of adult rat. Previous light microscopic studies (Ellis et al. J. Cell Biol. 101: 1977:85) have shown that the 5B4 antibody labels the growth cones of fetal neurons and a subpopulation of olfactory axons of the adult rat.

Rat olfactory bulbs were prepared for immunocytochemistry. 5B4 immunoreactivity was detected with either the immunoperoxidase or immunogold methods. To determine whether 5B4 immunoreactivity was expressed in identified olfactory neurons, an antiserum against tyrosine hydroxylase (TH) was used as a marker for dopaminergic periglomerular cells; in these experiments 5B4 and TH immunoreactivities were detected simultaneously by combining the immunogold and immunoperoxidase methods.

By light microscopy, 5B4 immunoreactivity was present within the olfactory nerve and periglomerular layers of the olfactory bulb. TH-like immunoreactivity was present in cell bodies adjacent to each of the olfactory glomeruli. By contrast, significant amounts of 5B4 immunoreactivity were present in only a subset of olfactory glomeruli.

Ultrastructurally, 5B4 was present in clusters of axons of the olfactory nerve layer. 5B4 immunoreactivity was present on cytoplasmic membrane faces in axons of the olfactory nerve layer, and in axon terminals in the periglomerular layer; within labeled terminals, reaction product was present on the outer surface of round vesicles. 5B4 immunoreactivity also was observed in discrete patches along the plasmalemma (cytoplasmic face) of periglomerular cells, and of dendrites of the periglomerular layer. In some cases 5B4 immunoreactivity was co-localized with tyrosine hydroxylase-like immunoreactivity. If the 5B4 antigen is a marker for neuronal growth as the earlier data suggest, then our new findings suggest that the turnover of the olfactory axons is associated with remodeling of post-synaptic elements in the olfactory bulb.

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394.8 INCREASES IN MESSENGER RNA FOR ACTIN AND TUBULIN WITHIN THE DENERVATED NEUROPIL OF THE DENTATE GYRUS DURING LESION-INDUCED SYNAPTOGENESIS. L.L. Phillips, D.M. Chikaraishi*, and O. Steward. Departments of Neuroscience and Neurosurgery, Univ. of Va. Sch. of Med., Charlottesville, VA 22908, and Neurosciences Program, Tufts Univ., Boston, MA 02111.

Following the denervation of the dentate gyrus there is an increase in the proportion of dendritic spines with underlying polyribosomes (Steward, O., J. Neurosci., 3:177, 1983) and an increase in protein synthesis within the dendritic laminae (Fass, B. and Steward, O., Neurosci. 9:653, 1983). These increases are maximal during the period of lesioned-induced synaptogenesis. We have been interested in identifying the protein(s) which are produced at these sites of local synthesis. Our initial biochemical analysis of dissected samples of the neuropil layer showed that polypeptides of molecular weights 43, 52 and 56 kD contain the highest levels of radioactivity following a ^{3}H-leucine pulse (Phillips, et al., Soc. Neurosci. Abstr. 18:1019, 1984). These molecular weights correspond approximately to actin and tubulin, suggesting that actin and tubulin might be amongst the proteins that are synthesized in the denervated neuropil. In the present study we utilize in situ hybridization to evaluate this question, employing high specific activity riboprobes for the messenger RNAs (mRNAs) of beta-actin and beta-tubulin in order to determine the cellular localization of the respective mRNAs.

Adult male albino rats were sacrificed by vascular perfusion of mixed aldehyde fixative at 6 days after unilateral destruction of the entorhinal cortex. Vibratome sections were hybridized with ^{3}H-labeled riboprobes (Melton, D.A., et al., Nuc. Acid Res. 12:7055, 1984) prepared from either chick beta-actin or chick beta-tubulin mRNA and processed for light microscopic autoradiography. Qualitative analysis of autoradiograms at 6 days after lesion showed a clear increase in mRNA for beta-actin within the denervated neuropil when compared to the intact dentate gyrus on the contralateral side. High grain density was visible over the outer two-thirds of the molecular layer which contains the denervated dendrites of the dentate granule neurons. There was also an increase in labeling over the same zone using the riboprobe for beta-tubulin mRNA.

The present results suggest that local protein synthesis within the denervated neuropil of the dentate gyrus involves, in part, an increase in the production of cytoskeletal proteins. These proteins could play a role in the activity of neurons or glial cells, or both during the degeneration/regeneration cycle induced by lesion. Supported by NIH grant NS 12333 to O.S.

394.9 USE OF IN SITU HYBRIDIZATION AND IMMUNOCYTOCHEMISTRY TO EXAMINE THE DISTRIBUTION OF ACTIN AND TUBULIN AND THEIR MESSENGER RNA IN CNS NEURONS. P.A. Trimmer, L.L. Phillips, and O. Steward. Depts. of Neuroscience and Neurosurgery, Univ. of Virginia. School of Medicine, Charlottesville, VA 22908.

During early development, neurons elaborate different processes (axons and dendrites) which are structurally and functionally distinct, and which differ in molecular composition. How differences in the molecular composition of axons and dendrites occur is currently unknown, but one possibility is that the synthesis of certain proteins may be localized in particular regions of the cell. Lawrence and Singer (Cell, 45:407-415, 1986) detected localized concentrations of messenger RNAs (mRNA) for actin and tubulin in the cytoplasm of fibroblasts. The focus of this study is to determine if there is a similar compartmentalization of mRNAs in neurons and if their distribution correlates with the expression of their respective proteins. We addressed this question by examining the distibution of actin and tubulin and their mRNAs in cultures of neurons from the dentate gyrus using immunocytochemistry in combination with in situ hybridization.

Coverslip cultures of isolated neurons were hybridized with 3H-labeled riboprobes for actin and tubulin mRNA prepared from chick beta-actin and beta-tubulin by D. M. Chikaraishi according to the method of Melton, D. A. et al. (Nucl. Acid Res. 12:7035-7056, 1984). Parallel coverslip cultures were hybridized with a cDNA probe complementary to ribosomal RNA to determine the overall distribution of protein synthetic machinery. The cultures were subsequently immunostained with antibodies to actin, beta-tubulin or cell specific markers such as GFAP and MAP2 using indirect immunofluorescence. The coverslips were then processed for autoradiography.

The mRNAs for actin and tubulin appeared to be distributed throughout the cytoplasm of the cell soma, with no evidence of accumulations at poles of the cell giving rise to either axons or dendrites. These data suggest that actin and tubulin synthesis may not be regionally localized in these neurons. Immunocytochemical staining for actin and beta-tubulin was also distributed throughout the cytoplasm of these neurons. In future studies we plan to evaluate whether molecules that are differentially distributed in neurons (i.e., MAP2 and Tau) are synthesized in distinct intracellular locations. Supported by NIH grant NS12333 to O.S. P.T. is supported by training grant NS 07199.

394.10 A NOVEL SUBTYPE OF AXOSPINOUS SYNAPSES DISTINGUISHED BY A SEGMENTED POSTSYNAPTIC DENSITY. Y. Geinisman, F. Morrell and L. de Toledo-Morrell. Dept. of Cell Biol. & Anat., Northwestern Univ. Med. Sch. and Depts. of Neurol. Sci. and Psychol., Rush Med. Coll., Chicago, IL 60611.

The so-called perforated axospinous synapses, which are characterized by a discontinuous postsynaptic density (PSD), have been proposed to represent structural intermediates in the processes of synapse turnover or division (Nieto-Sampedro, M. et al., Proc. Natl. Acad. Sci. USA, 79:5718, 1982; Carlin, P.K. & Siekevitz, P., Ibid., 80:3517, 1983; Dyson, S.E. & Jones, D.G., Dev. Brain Res., 13:125, 1984). These hypotheses have postulated the existence of axospinous synapses with the PSD consisting of separate segments: the segmented PSD develops through the stages of perforated and U-shaped PSDs as a step of splitting of a large synapse into smaller ones. However, axospinous synapses with segmented PSD had not been directly observed so far. We now present direct evidence for the existence of such synaptic contacts.

Axospinous synapses were examined in the molecular layer of the rat dentate gyrus. Serial section analysis of synapses, which exhibited a PSD discontinuity, was performed. Reconstruction of each discontinuous PSD was made in a plane perpendicular to that of serial sections. The results obtained indicate that some profiles of perforated synapses visualized in random sections of osmicated tissue are produced by sectioning of synapses with perforated or U-shaped PSDs. This observation confirms the conclusions of earlier serial section studies (Peters, A. & Kaiserman-Abramof, I.R., Z. Zellforsch., 100:487, 1969; Cohen, R. & Siekevitz, P., J. Cell Biol., 78:36, 1978; Nieto-Sampedro, M. et al., 1982; Calverley, R. K.S. & Jones, D.G., Cell Tiss. Res., 247:565, 1987). Additionally, it has been found in this study that another synaptic subtype, namely synapses with segmented PSD, contributes to the number of profiles of perforated synapses. Synaptic contacts with segmented PSD are distinguished by the presence of 2-5 discrete PSD segments at the interface between a presynaptic axon terminal and a postsynaptic dendritic spine.

The demonstration of axospinous synapses with segmented PSD is consistent with the hypotheses of synapse turnover and division. However, the hypothesized final splitting of synapses may not be necessary. Conceivably, the segmented PSD may evolve from perforated and U-shaped PSDs to form a specialized synaptic contact of an unusually high efficacy. Each PSD segment is a component of a separate synaptic complex comparable to that of a small axospinous synapse with a continuous PSD. A concerted activation of several synaptic complexes within a single synaptic junction may provide a mechanism for an amplification of synaptic transmission.

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394.11 SYNAPSES ON DENDRITIC SHAFTS EXHIBIT A PERFORATED POSTSYNAPTIC DENSITY. D.L. Scholz*, Y. Geinisman, F. Morrell and L. de Toledo-Morrell. Dept. of Cell Biol. & Anat., Northwestern Univ. Med. Sch. and Depts. of Neurol. Sci. and Psychol., Rush Med. Coll., Chicago, IL 60611.

A postsynaptic density (PSD), which contains a perforation(s), has been hypothesized to underlie an augmentation of synaptic efficacy or to be a structural intermediate in the processes of synapse turnover or division (Peters, A. & Kaiserman-Abramof, I.R., Z. Zellforsch., 100:487, 1969; Greenough, W.T. et al., Science, 202:1096, 1978; Nieto-Sampedro, M. et al., Proc. Natl. Acad. Sci. USA, 79:5718, 1982; Carlin, P.K. & Siekevitz, P., Ibid., 80:3517, 1983). If any of these hypotheses is correct, then the presence of PSD perforations reflects a fundamental property of synapses, and different synaptic types should incorporate a proportion of junctions characterized by a perforated PSD. However, only the category of synapses on dendritic spines had been demonstrated to include synaptic contacts with a perforated PSD (Peters, A. & Kaiserman-Abramoff, I.R., 1969; Cohen, R.C. & Siekevitz, P., J. Cell Biol., 78:36, 1978; Nieto-Sampedro, M. et al., 1982; Calverley, R.K.C. & Jones, D.G., Cell Tiss. Res., 247:565, 1987; Geinisman, Y. et al., Soc. Neurosci. Abstr., 1987). To elucidate whether synaptic types other than axospinous junctions also include synapses with a perforated PSD, we undertook a serial section study of synapses on dendritic shafts.

The tissue was processed for electron microscopy by means of perfusion fixation with aldehydes, osmication, Araldite embedding and section staining with uranyl acetate and lead citrate. Asymmetrical synapses on dendritic shafts were examined in electron micrographs of serial sections obtained from the molecular layer of the rat dentate gyrus. Some of these synapses were found to exhibit profiles of a discontinuous PSD. PSD reconstructions from serial sections showed that profiles of discontinuous PSDs observed in random sections of dendritic shaft synapses were generated by sectioning of PSD plates that contained 1-3 perforations.

The demonstration of dendritic shaft synapses distinguished by a perforated PSD complements the results of serial section studies cited above, which document the existence of axospinous synapses with a perforated PSD. Moreover, some axosomatic synapses may also have a perforated PSD, since PSD discontinuities have been noticed in their random sections (Peters, A. et al., The Fine Structure of the Nervous System, Saunders, Philadelphia, 1976: 147). These data, taken together, indicate that the presence of PSD perforations is a general phenomenon common to subpopulations of different synaptic types. Such a phenomenon may, therefore, represent a structural correlate of a basic synaptic function.

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394.12 DEVELOPMENT OF HIPPOCAMPAL SYNAPSES, SPINES AND LTP. K.M. Harris, F.E. Jensen, and B. Tsao. Dept. Neurosci., Children's Hospital, Boston, MA 02115

In an earlier study it was shown that the magnitude of hippocampal longterm potentiation (LTP) produced at postnatal day 15 (P15) in stratum radiatum of area CA1 is 3-4 times greater than the magnitude observed in hippocampal slices from adult rats (Harris KM. and Teyler TJ., 1984, J. Physiol. 346:27-48). We hypothesized that this peak and falloff in LTP during development could be mediated by a population of plastic synapses which are consolidated by maturation-related experience so that in the adult fewer of the total synapses are available for tetanus-induced potentiation.

The goal of the present study is to test whether changes in synapse or spine morphology could distinguish 'plastic' from 'consolidated' synapses at P15 and Adult ages. An accurate and relatively quick method for surveying the neuropil was developed. To determine the density of each synaptic type located in s. radiatum, a randomly-selected field (150-200 um^2) located 200-250 um from the pyramidal cell body layer is photographed and viewed through adjacent serial sections. Then to distinguish changes in synaptic density from changes in synaptic size, shape, or orientation with respect to the plane of sectioning, the total area of each synapse with a portion found on the middle section of the series is measured through serial sections using a computer-assisted reconstruction system. Synaptic densities were corrected for changes in the probability of observing synapses on the analyis section by subtracting the percent mean change in the total number of sections required to contain the synaptic area from the total area of the neuropil analyzed. Table I illustrates preliminary analyses from 2 fields at each of ages P15 and adult:

TABLE I: COMPARISON OF SYNAPSES AT P15 AND ADULT.

SYN LOCATION	SYN DENSITY/100um^2		SYN AREA (um^2, mean±sd)	
	P15	ADULT	P15	ADULT
Stubby Spine	6	2	0.16±0.10	0.08±0.03
Mushroom Spine	4	8	0.16±0.10	0.35±0.20
Thin Spine	4	27	0.07±0.05	0.06±0.04
Asymmetric Shaft	3	2	0.24±0.10	0.21±0.27
Symmetric Shaft	1.5	2	0.16±0.12	0.14±0.09

Dendritic spines were classified 'stubby' when the neck diameter $\geq$ neck length; 'mushroom' when the neck diameter <<<< head diameter; 'thin' when the neck diameter <<<< neck length and neck diameter $\leq$ head diameter. The decrease in stubby spine density was exactly matched by the increase in mushroom spine density between P15 and adult. Synapses on adult mushroom spines were on average 2 times larger than synapses on P15 stubby or mushroom spines. The density of thin spines increased dramatically between these two ages, though the size of synapses on thin spines did not change. Shaft synapse density and size did not change significantly.

Changes in individual thin spine synapses are not likely to be involved in tetanus-induced LTP as many more of them are present in the adult than at P15 when LTP is maximal. Similarly changes in shaft synapses do not parallel the development of LTP.

Stubby spines at P15 have many ultrastructural features of more mature mushroom spines, showing relatively large, often perforated synapses and abundent amounts of smooth endoplasmic reticulum. Perhaps the stubby become mushroom spines whose synapses are 'plastic', and age-related experience or tetanic stimulation induces an enlargement of the spine and area of synapses on the spine up to a maximum. In the adult, proportionately more of the synapses have reached that maximum ('consolidated' synapses) and therefore are unavailable for further enlargement from tetanus-induced LTP.

394.13 DENDRITIC PROLIFERATION AS A MECHANISM OF SPARING AND RECOVERY OF FUNCTION IN THE RAT. B. Kolb and R. Gibb*. Dept. of Psychology, Univ. of Lethbridge, Lethbridge, Canada, T1K 3M4.

We have demonstrated in previous work that the developing brain is especially vulnerable to the effects of cortical injury until cell migration in the cortex is completed, around 7-10 days of age in the rat. Thus, damage before 7 days produces more severe behavioral consequences than similar damage in adulthood, whereas damage around 10 days allows almost complete sparing of function. In adulthood, recovery of function following cortical lesions is quite limited but sometimes can be attenuated by serial lesions, enriched experience, or certain drug treatments. The goal of the current experiments was to examine the possibility that dendritic arborization might correlate with the presence or absence of behavioral sparing or recovery. Rats were given a) bilateral or unilateral lesions of the frontal cortex at 1, 10 or 90 days of age; b) two-stage lesions of the same tissue at 1 and 10 or 90 and 120 days, or c) hemidecortication at 1 or 90 days. Animals were raised in standard laboratory conditions or in "enriched environments" and given a battery of behavioral tests before the brains were stained with Golgi-Cox. Layer II/III pyramidal cells were drawn and the apical and basal dendrites quantified in several regions.

The results showed that the behavioral outcome was correlated with the extent of dendritic arborization. 1) Rats with 1-day frontal lesions showed no behavioral sparing and showed stunting of dendrites throughout the cortex. 2) Rats with 10-day lesions showed good sparing of function and an expansion of dendritic arborization, especially in the parietal, visual and temporal cortex. 3) Serial lesions in infancy allowed partial sparing of function along with stunted growth on the 1-day side and expanded growth on the 10-day side. 4) Hemidecortication produced increased arborization in the forepaw area, consistent with their partial recovery of motor function.

The results are interpreted as showing an anatomical basis of sparing and recovery of function in the rat cortex.

394.14 THE POSTNATAL DISTRIBUTIONAL CHANGES AND SYNAPTOGENESIS OF CORTICOSPINAL TRACT NEURONS IN THE CEREBRAL CORTEX OF THE RAT WITH HRP-LABELING MEHTOD. R. Ohtani*, T. Shirai* & H. Kato* (SPON:J.Yamada). Department of Anatomy and Physiology*, Yamagata University, School of Medicine, Zaoiida, Yamagata 990-23, Japan

We studied the establishment of the distributional pattern and the synaptic formation of corticospinal tract (CST) neurons in the postnatal rat.

By injecting HRP into cervical cords, CST neurons were labeled at various ages. CST neurons of neonatal rats were distributed in layer V as one continuous band from the medial region to the lateral one of the frontal and parietal areas. By the end of the second postnatal week, CST neurons became localized into two narrow bands. These two bands consisted of a dorsomedial band in the frontal and the anterior parietal cortices and a relatively smaller band in the lateral parietal cortex, and no labeled neurons appeared between these two bands. This distributional pattern of CST neurons was maintained to adult rats.

Subsequently, the HRP-labeled cells in the motor cortex were observed with the electron microscope to study the postnatal development of the synapses on the soma of the CST neuron. Synapses were found neither on the soma nor at the proximal region of dendrites of CST neurons on day 1, but appeared in both areas by day 7 and increased rapidly in number by day 14. The average number of synapses per 1mm CLS(circumferential length of the soma) was 0, 41±20, 93±35, 111±30, 115±35, on day 1, 7, 14, 28 and in adult rats, respectively. These studies indicate that the distributional reorganization of CST neurons and the synaptic formation on their somata and proximal dendrites were almost completed by the end of the second postnatal week.

Furthermore, the synaptic organization on distal dendrites and their spines of CST neurons were investigated using intracellular HRP-injection in the slice preparation of motor cortices of various ages. Together with these findings, we would show the synaptogesesis on the whole CST neuron.

This work was supported by the Grant-in-Aid for Encouragement of Young Scientist (60770054, 61770036) from the Ministry of Education, Science and Culture of Japan.

396 SYMPOSIUM: NEURONAL SEROTONIN RECEPTORS. J.M. Palacios, Sandoz Ltd. Basle (Chairperson); M. Hamon*, INSERM U.288 Paris; P. Hartig, Johns Hopkins Univ.; S. Maayani, Mount Sinai Med. Ctr.; G.K. Aghajanian, Yale Univ.; B.P. Richardson*, Sandoz Ltd., Basle

Selective new drugs permit the pharmacological classification of serotonin (5-hydroxytryptamine, 5HT) receptors into three categories: $5HT_1$, $5HT_2$ and $5HT_3$. Although $5HT_2$ receptors are homogenous, subtypes of $5HT_1$ and $5HT_3$ receptors clearly exist. All serotonin receptor subtypes occur in the CNS or PNS and this symposium will review the considerable progress made in their pharmacology, biochemistry, distribution and function.

Michel Hamon shows how radioligand studies have permitted identification of different $5HT_1$ receptor subtypes in rat brain membranes. Of these, both $5HT_{1A}$ and $5HT_{1B}$ receptors are located mainly postsynaptically, but with differing regional distributions. Photoaffinity labelling has demonstrated the $5HT_{1A}$ binding subunit to be a 63 kD protein functionally coupled to adenylate cyclase.

Paul Hartig demonstrates that the $5HT_{1C}$ receptor is present at high density in the choroid plexus and at low density in several other brain regions. It is located on the apical face of the choroid plexus epithelium, and is activated by serotonin in the CSF. Recently the $5HT_{1C}$ receptor has been cloned.

Saul Maayani explains how radioligand binding studies originally permitted the identification of $5HT_2$ sites in the CNS. Both biochemical (phosphatidylinositide hydrolysis) and behavioural correlates for these binding sites now exist. The density of $5HT_2$ binding sites, which is not uniform throughout animal and human brain, can be modulated in vivo by psychoactive drugs.

Brian Richardson shows $5HT_3$ receptors are widely distributed throughout the PNS, where they mediate exclusively excitatory actions of 5HT. Recently three subtypes of $5HT_3$ receptor have been identified using selective pharmacological tools. Evidence for the existence of $5HT_3$ receptors in the CNS will also be presented.

George Aghajanian presents electrophysiological studies showing that $5HT_{1A}$ ligands are full agonists in the dorsal raphé, mimicking 5HT inhibition of serotonergic neurones, whereas in the hippocampus they are only weak, partial agonists. In both regions, 5HT inhibition is mediated by a pertussis toxin-sensitive G-protein. Hallucinogenic indolamine and phenethylamines act as $5HT_2$ agonists.

José Palacios explains how binding and autoradiographic techniques have shown the human brain to contain $5HT_{1A}$, $5HT_{1C}$, $5HT_{1D}$ and $5HT_2$ sites but no $5HT_{1B}$ sites. $5HT_{1A}$ and $5HT_{1D}$ sites are enriched in limbic areas and the basal ganglia respectively, while $5HT_2$ sites occur predominantly in the cerebral cortex. Altered densities of $5HT_{1A}$ and $5HT_2$ sites occur in senile dementia.

397 WORKSHOP. NEW DIRECTIONS IN MAMMALIAN CNS *IN VITRO*: BEYOND THE SLICE. K. Walton, New York Univ. Med. Sch. (Chairperson); J. Feldman, Univ. Calif. Los Angeles ; P. Getting, Univ. of Iowa; L. Renaud, Montreal Gen. Hosp. and McGill Univ.; R. Llinás, New York Univ. Med. Sch.

The advantages of studying the electrophysiology of mammalian CNS *in vitro* have been elegantly demonstrated over the last decade where, in brain slices, many new ionic conductances have been characterized. However, in the slice many essential features of brain organization, such as the integrity of neuronal circuits, are lost. In order to combine the advantages of *in vitro* recording with complete circuitry, several mammalian CNS *en bloc* preparations have been introduced. This workshop will explore recent advances in the development and use of these preparations and consider the place of such preparations in the Neuroscience armamentarium.

Kerry Walton is using an SC-brainstem-cerebellum preparation isolated from neonatal rat to study the role of neuronal activity in the development of the motor system. She will discuss the contribution of motoneuron membrane properties and spinal circuit elements in the generation of rhythmic activity and the importance of such oscillations in SC development.

Jack Feldman and Jeff Smith are using an isolated neonatal rat SC-brainstem preparation (with/without ribcage) to study the motor control system for respiration. Jack's talk will focus on (1) the necessary role of inhibition in generating respiratory patterns and (2) the class of neuromessenger (possibly an excitatory amino acid or related peptide) transmitting respiratory drive from the brainstem to respiratory motoneurons.

Peter Getting and George Richardson have developed a perfused *in situ* adult guinea pig brain preparation. Their procedure uses non-pulsatile flow of perfluorocarbon artificial blood. Peter will discuss its usefulnesss in studies of neural network function and evaluate the effects of peripheral and central afferent systems on the neural control of respiration.

Leo Renaud has been studying neurosecretory neurons in the supraoptic nucleus in arterially perfused hypothalamic explants from adult rat. He will discuss the properties of local circuits transmitting cardiovascular input to neurosecretory cells and evaluate the membrane actions and hormone release capacities of neurotransmitters and modulators in the neurohypophyseal axis.

Rodolfo Llinás has developed an isolated adult guinea pig whole brain preparation. He will discuss recent experiments concerning the olivo-cerebellar system, pontocerebellar system and the interaction between mossy and climbing fiber inputs to Purkinje cells.

398.1 RELATIONSHIPS BETWEEN BRAIN VOLUME LOSS, INTELLIGENCE, AND POSTINJURY INTELLECTUAL DEFICITS. E. Irle*§, B. Wowra*§§, V. Sturm*§§ and St. Kunze* (Head)§§ (SPON: G. Raisman). Dept. of Psychology§ and Dept. of Neurosurgery§§, Univ. of Heidelberg, D-6900 Heidelberg,FRG

Forty-six patients sustaining surgery for tumors of the brain and 12 patients sustaining surgery of the spine were investigated. All subjects were inpatients of the neurosurgical division of the University Clinic of Heidelberg. The tumor patients were chosen on the basis of having a tumor restricted to cortical tissue of the frontal, temporal, or parietal lobes; the control subjects were matched for age and education to the tumor group. Pre- and postoperatively, all subjects were tested in a variety of cognitive and mnemonic tasks, e.g. a shortened version of the WAIS, Benton test, tests of endurance and psychomotor speed, and a paired associate learning task using lists with semantically related words. If the performance of a subject on one of these tests was impaired because of paresis, or neglect, or aphasia, these data were excluded from the statistical analysis. The tumor location and tumor volume were determined with quantitative computed tomographic analysis of both pre- and postoperative scans.

The volumes of the tumors varied between 1 and 87 cm^3. As the sizes of the tumors and the surrounding edemas were correlated ($r=.52$, $p<.01$) only the tumor volume was considered for further analysis. The tumors were not separated for different types as analyses of variance revealed no effects of groups for the tumor volume as well as for the general IQ measured preoperatively ($p's>.1$). Compared to their controls, the tumor patients performed significantly worse in the WAIS and the paired associate learning (t-tests, $p's<.05$). A preliminary correlation analysis revealed that the age of the tumor patients was not related to the preoperatively measured IQ ($r=-.06$, $p<.5$). However, the premorbid intelligence (estimated by age, gender, education and occupation) predicted the preoperatively measured IQ, and the performance in the paired associate learning (IQ: $r=.4$, $p<.01$; paired associate learning: $r=.32$, $p<.05$). Furthermore, the volume of the tumor was positively correlated with the preoperative IQ ($r=.2$, $p<.2$), that is, patients with larger tumors had higher IQ's. The same is true for the paired associate learning ($r=.17$, $p<.3$). However, the premorbid IQ not only correlated with the preoperative IQ, but also with the tumor volume, in that way, that larger IQ's were related to larger tumors ($r=.24$, $p<.1$). Thus, it may be suggested that subjects with higher intelligence tolerate a growing tumor for a longer time, until neurological or psychological deficits appear. More important, it may be true that the likeliness of functional compensation increases with the lesion extent and thus the necessity of the brain to fulfill plastic changes.

We thank S. Gauggel, J. Keller, S. Kubath, M. Peper, C. Ramrath and J. Werner for the testing of the patients.
Supported by grant Ir 15/3 of the Deutsche Forschungsgemeinschaft.

398.2 COGNITIVE ABNORMALITIES AND RATE OF PROGRESSION IN ALZHEIMER'S DISEASE. J.T. BECKER, F.J. HUFF, R.D. NEBES*, A. HOLLAND*, F. BOLLER, Alz. Dis. Res. Centr., Univ. Pittsburgh, Pittsburgh, PA, USA

The relationship between the pattern of cognitive deficits in Alzheimer's Disease (AD) and the progression of the dementia may reveal important clues to the pathophysiology of the disorder. Previous studies have suggested that the age at which clinical symptoms first appear may be an important predictor of both clinical presentation and disease progression. Younger individuals (i.e., <=65 yrs) may have more pronounced language deficits, and a more rapid decline of function.

The performance of 92 normal elderly controls (NC) and 87 AD patients was examined on tests of language and visuoconstructional abilities as part of a longitudinal study of dementia. A principal components analysis of the patient data revealed two components: one of visuoconstructional skill (VC) and the other of access to lexical/semantic knowledge (LS). Composite scores were created, and each subject's score was adjusted for age, education and sex. Fiften patients were identified with relatively "focal" patterns of impairment: 11 with LS defects and 4 with prominent VC impairments. There were no differences between the non-focal patients and those with LS or VC defects in terms of education, global measures of dementia, or duration of illness. However, the mean age at onset of illness among the LS patients (71.2 yrs) was greater than that of the non-focal (64.1 yrs) and VC patients (62.0 yrs).

One year later, seven of the patients had died, all of whom were in the "non-focal" group. Of the seven patients who had been institutionalized, all but two were in the non-focal group. The rate of decline in function, whether measured by change with repeated testing using the two composite variables or the Mini-Mental State Exam, did not differ as a function of patient group. Measures of expressive language, both written and oral, also failed to predict more rapid rates of disease progression.

These results confirm previous reports which identified VC and LS components of the cognitive deficit in AD, and specifically a similar component structure by Martin, et al. (1986). The predicted relationships between the pattern of presentation and disease progression, however, were not found. A longer follow-up interval may be necessary to differentiate among the groups of patients. Our finding that the LS impaired patients were the older ones in our group is also contrary to expectation. This result suggests that differences between young and old AD patients with regard to pattern of cognitive abnormalities may not be as consistent as was previously thought (supported by: AG03705, AG05133, MH30915).

398.3 THE ROLE OF THE FRONTAL CORTEX IN THE USE OF ADVANCE INFORMATION. B. Alivisatos* (SPON: B. Milner). Montreal Neurological Institute and Hospital, McGill University, Montreal, Quebec, Canada, H3A 2B4.

The present study explored the ability of patients with unilateral frontal or temporal-lobe excisions to use advance information in a choice reaction-time paradigm requiring the discrimination of alpha-numeric characters on the basis of their form of presentation, normal or mirror-image. The target stimuli were presented in various angular orientations, and the subject had to respond by pressing one key with one hand when the letters or numbers were normal and another key with the other hand when they were mirror-image. In the No-Information (NI) condition, a warning signal appeared for 1500ms, followed by the target, which remained on until the subject responded. In the Advance-Information (AI) condition, an informative cue was provided for 1500ms, followed for another 1500ms by the same warning signal as in the NI condition and finally being replaced by the target. In this AI condition, the cue conveyed the target's identity and orientation and always appeared in its normal form.

A total of 66 patients and 18 normal control subjects were tested assigned to the following groups: (1) left frontal (n=9); (2) right frontal (n=12); (3) left temporal (n=22); (4) right temporal (n=23). In accordance with findings for normal subjects (Cooper, L.A. and Shepard, R.N., Memory and Cognition, 1:246, 1973), in the NI condition, reaction time for all groups increased as a function of the target's angular departure from the standard upright orientation. In the AI condition, reaction time remained constant across changes of orientation for all subject groups, except the group with right frontal-lobe lesions. These results suggest that patients with right frontal-lobe removals did not use the cues to the same extent as normal control subjects but, instead, continued mentally rotating the target to the upright position before responding. The deficits found in the right frontal-lobe group cannot be attributed to a simple inability to make 'Same-Different' judgments, because these same patients performed normally in a control condition requiring the comparison of letters or numbers presented sequentially.

Previous findings in a visuospatial reaction-time study (Alivisatos, B. and Milner, B., Proc. Eastern Psychol. Assoc., 56:19, 1985) showed that patients with frontal-lobe removals were impaired in making use of directional cues, which indicated the spatial location where a target was about to appear. The present study supports and extends further the notion that the frontal cortex is involved in making use of cues to anticipate an event and prepare for a specific response.

398.4 A DOPAMINE DEFICIENCY SYNDROME OF DEMENTIA. N. Wolfe, D.I. Katz*, M.L. Albert*, M.C. Smith*, R. Durso*, L. Volicer, A. Almozlino*. Boston University School of Medicine, Boston, MA 02130

Preliminary findings from the first 12 patients entered in a study of dopaminergic function and dementia reveal a distinctive pattern of neuropsychological test results in a subset of demented patients with low cerebrospinal fluid homovanillic acid (CSF HVA). We hypothesized that such a dopaminergic deficiency syndrome may cut across traditional diagnostic categories, and thus included subjects with: Alzheimer's disease (n=4), Parkinson's disease (n=4) and Major Depression (n=4). Subjects were excluded from study if they had a Hachinski Ischemia Score > 4 (suggesting multi-infarct dementia), structural lesion on CT scan, evidence of central nervous system infection or chronic seizure disorder.

Cerebrospinal fluid HVA was measured using high pressure liquid chromatography with electrochemical detection. Neuropsychological testing emphasized patterns of cognitive decline which have been associated in the past with dopamine deficiency (slowness in rate of information processing, impairment in memory, and impaired manipulation of acquired knowledge). We identified two groups of demented subjects based on CSF HVA level. The mean HVA level in the low HVA group (11.7 ± 2.7 ng/ml)(n=3) was significantly lower than the mean HVA in the high HVA group (21.9 ± 7.1 ng/ml)(n=9) (Mann-Whitney $U=2$, $p<.016$, one-tailed). The low HVA group included 2 patients with Alzheimer's disease and 1 with Major Depression.

The low and high HVA groups also differed significantly in two specific neuropsychological measurements. First, speed of processing, assessed using a revised Paced Auditory Serial Addition Test (PASAT-r) was significantly slower in the low HVA group ($U=4$, $p<.045$ one-tailed). Second, it has been observed that patients with fronto-subcortical dysfunction perform better on cognitive tasks when provided with more structure. Consequently, we used the verbal fluency test (Controlled Oral Word Association Test, F.A.S.) to assess performance under greater structure (semantic categories: animals,fruits and vegetables) and lesser structure (phonemic categories: words beginning with F,A and S). Patients in the low HVA group showed significantly greater benefit from structure on the verbal fluency task, compared to those in the high HVA group ($U=1$, $p<.008$). The correlation between dopamine measurement (HVA level) and benefit from structure (FAS score) across all 12 patients was significant (Spearman $r=-.59$, $p<.025$, one-tailed).

These neuropsychologic differences: decreased speed of processing and greater benefit from structure on a verbal fluency task, can NOT be explained simply as a decrease in overall cognitive abilities, since the two groups did not differ significantly on the Mini Mental State Test (Folstein, M.F. et al., J Psychiatr Res., 12:189, 1975).

398.5 SINGLE-PULSE SENTENCE CONSTRUCTION WITHIN LAYERED, HEBBIAN, NEURAL NETWORKS. R. Martin. Department of Chemistry, Brooklyn College, Brooklyn, NY 11210.

The simulations to be reported illustrate how a "blank" network of nerve cells, linked together by connections of uniform strength, can develop the capacity to respond to questions like "What do lions do during the night?" and "Where do tigers live?" with logical and grammatically correct answers such as: "During the night lions hunt" and "Tigers live in India". Each of the sentences produced by the network is planned or constructed as a consequence of a single pulse of discharge from cells within a central "speech center". Apparently no additional time is required for determination of correct conceptual and semantic responses and selection of an appropriate grammatical structure for the answer. Connection patterns that develop within the net are able to select novel combinations of words when novel statements are required. These connection patterns also specify which word combinations are appropriate and which are not, so that under ordinary circumstances illogical statements like "Tigers hunt during India" will not be produced even if they conform to formal rules of grammar. The network learns quite rapidly if connection values are adjusted in large increments. As little as one "training example" involving a new word enables the word to be used correctly within all of the sentence structures previously learned by the network. And as few as two illustrations of a novel sentence structure are sufficient for the network to learn how to construct variations on this structure that include any appropriate combination of previously encountered words. As outlined previously (1-3) word selection proceeds within limits determined by many thousands of behavioral constraints which govern the use of concept hierarchies.

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398.6 EFFECTS OF A BENZODIAZEPINE RECEPTOR ANTAGONIST IN PATIENTS WITH PANIC DISORDER. S.W. Woods, D.S. Charney, J.M. Silver* and G.R. Heninger. Clinical Neuroscience Research Unit, Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508 and the Hoffmann-LaRoche Co., Nutley, NJ 07110.

To evaluate the hypothesis that abnormal regulation of benzodiazepine receptor function may relate to the pathophysiology of panic disorder, the benzodiazepine receptor antagonist flumazenil (RO 15-1788) was administered to panic disorder patients and healthy subjects.

METHODS: Eleven patients (age 37±7 yrs) drug-free for four weeks and five healthy subjects (age 24±4 yrs) participated after giving informed consent. All 11 patients received flumazenil 600 mg, ten received flumazenil 200 mg, and eight received matching placebo orally in random sequence on separate test days seven days apart. Too few healthy subjects were recruited to permit statistical analysis because of premature termination of the study due to unexplained EKG changes in two subjects. Subjective visual analog scale (100 mm) mood ratings, heart rate, blood pressure, and plasma cortisol and 3-methoxy-4-hydroxyphenylglycol (MHPG) measurements were obtained 30 minutes and immediately preceding and 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 minutes following drug administration.

RESULTS: On placebo days, ratings of anxiety decreased from baseline by 11±14 mm ($p<.10$) at 30 minutes and by 29 to 40 mm at each time point ($p<.05$) thereafter. On 200 mg days, anxiety ratings increased by 19±39 mm ($p<.05$ vs. placebo) at 30 minutes and then fell below baseline by 5 to 23 mm at later time points (all N.S. vs. placebo). On 600 mg days, anxiety ratings decreased from baseline by 3±18 mm at 30 minutes (N.S.) and to levels similar to those on placebo days at later time points. Neither dose of flumazenil significantly altered heart rate, blood pressure, or plasma cortisol or MHPG in comparison to placebo.

DISCUSSION: Flumazenil was not anxiolytic at these doses in panic disorder patients. The 200mg dose produced a significant anxiogenic effect. These results suggest that flumazenil at these doses does not antagonize an increased interaction of benzodiazepine receptors with an endogenous benzodiazepine receptor inverse agonist in panic disorder patients. The data do not exclude an intrinsic inverse agonist or partial agonist effect of flumazenil at these doses or the existence of a hypofunctioning endogenous benzodiazepine agonist system in panic disorder.

398.7 BRAIN ELECTRICAL ACTIVITY MAPPING IN PARANOID SCHIZOPHRENIA AND RELATED DISORDERS. J.D. Raese, K.D. Pool*, R.G. Paulman*, T. Finitzo*, C.R. Judd*, R.R. Gregory*, and J. Steinberg, Schizophrenia Research Center, Dallas VA Medical Ctr., and Dallas Neuroscience Associates, Dallas, TX 75216.

This study examined the similarities and differences of electrocortical activity as well as their topography in psychotic disorders. Brain electrical activity mapping, consisting of twenty five channel conventional and quantitative EEG, and auditory, visual and somatosensory evoked potentials were used to evaluate electrocortical function in twenty-two psychotic male patients between the ages of 21 and 47 years. Thirteen were diagnosed (by DSM III Criteria) as paranoid schizophrenics, four as nonparanoid schizophrenics and five as atypical psychosis. Patterns of electrocortical activity were examined in relationship to diagnostic category and neuropsychological measures obtained from the Luria Nebraska and/or Halstead-Reitan Neuropsychological Batteries. These data were compared to 95 age-matched control subjects who by history and examination were free of neurological and psychiatric illness. Statistical analyses were done as t-tests corrected for small sample size. A serial binomial exclusion was employed to correct for multiple measures-effects. The alpha level for T-determination was a two-tailed T measure set at $p<0.01$.

Changes in EEG spectra in all psychotic patients were mild. More robust and persistent changes were seen with sensory activation. Analysis of spectral data with rigorous attention to eye movement artifact did not confirm the increase in frontal delta activity previously described by Morihisa et al. (Arch. Gen. Psych., 40:719, 1983). We confirmed, however, the decrease in higher frequency beta activity in psychotic patients compared to controls. In paranoid schizophrenics the reduction in beta activity was seen over the left paramedian parietal territory extending over the occipital head regions. In nonparanoid patients this finding was more prominent and less focal.

Evoked potential mapping showed characteristic deficits common to all three diagnostic groups. The major finding consisted of a reduction in vertex potentials in response to auditory and visual stimuli. For the AER and VER this occurred both in the normally seen N100 and P200 waves. Paranoid schizophrenics additionally showed focal abnormalities particularly in the left hemisphere. Patients showing neuropsychological deficits in the right posterior quadrant differed in their somatosensory mappings over right temporal, posterior temporal and occipital regions. These results confirm that electrocortical activity in psychotic patients differs significantly from normal controls. Moreover, electrocortical changes appear to have neurobehavioral correlates.

398.8 SHIFTS IN SPATIAL ATTENTION AND EVENT-RELATED POTENTIALS (ERPs). M. R. Harter. Psychology, Univ. N. C. at Greensboro, Greensboro, N.C. 27412.

ERPs were recorded over the left and right hemisphere of twenty-eight 6-7 year old children. Two processes associated with a shift of spatial attention from the central to either the right or left visual field were investigated: those associated with a cue (an arrow) which shifts attention and which presumably causes contalateral hemispheric activation; and those associated with the differential processing of targets depending on whether or not they are in the relevant (cued by the arrow) visual field.

The arrow was presented for 1000 msec and pointed randomly either to the right or left. The direction of the arrow determined the relevant visual field. 600 msec after arrow onset, a small target was flashed 10 degrees randomly to the right or left visual field. The subjects were required to give a finger-lift reaction-time response to targets only if in the relevant (cued) visual field. ERPs (O1, O2, P3, P4, C3, C4, F3, and F4) and EOGs were measured for 1000 msec following the onset of the arrow.

A switch in attention influenced the amplitude of a slow negativity (termed switch in attention negativity or SAN) which started at approximately 300 msec following arrow onset. The direction of the shift influenced SAN differently depending on the electrode position: a) Frontal SAN was generally larger when attention was switched to the right than left visual field. This effect was symmetrical over the left and right hemispheres and peaked at about 450 msec after the shift. Reaction times were faster to right (437 msec) than left (464 msec) visual field stimuli which suggests that frontal SAN may reflect an excitatory process. The direction of an attention shift had little influence on central SAN. Occipital and parietal SAN were larger over the hemisphere ipsilateral to the direction of the attention shift--they were greater over the right hemisphere when attention was shifted to the right and greater over left hemisphere when attention was shifted to the left. This ipsilateral effect peaked at about 650 msec after a shift, just after the presentation of the target at 600 msec. Given the ipsilateral occipital hemisphere is the projection area for the irrelevant (ignored) visual field, occipital SAN most likely reflects some inhibitory process. This interpretation is supported by the effects of field relevance on ERPs to the targets.

The P1 and N1 ERP component to targets were greatest a) over the occipital and parietal regions, b) to targets in the relevant visual field, and c) over the hemisphere contralateral to the relevant visual field. The larger the SAN prior to the target, the smaller the P1 and N1 to the target.

398.9 PARALLEL COMPUTATIONAL MODEL OF VISUAL ERRORS IN ACQUIRED DYSLEXIA. B. Gordon and J.M. Sieracki*. Cognitive Neurology Div., Dept. of Neurology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Acquired dyslexia has typically been explained in terms of disruptions to or disconnections between processing components. Yet the specific operations performed by these components have been left underspecified at best. Recently, detailed models of processing in neural networks have been offered as examples of how these operations might be accomplished. Testing these models has been difficult because of the complexity of the reading process in normal individuals. We now describe a detailed computational model applied to the specific pattern of reading performance in a patient with a left hemisphere lesion, in whom the lesion and specialized testing allowed data to be obtained from relatively early stages of visual word recognition.

This patient makes frequent (7-37%, depending upon word frequency) visual errors in reading single words aloud (e.g., misreading MIDST as "MAST"). These errors can be shown to be visually-mediated rather than phonologically-mediated, to arise relatively early in the direct lexical access route (at or before the visually-based word store, the orthographic lexicon), and to be highly reproducible (Gordon, Goodman-Schulman, & Caramazza, submitted). Both normal reading and this patient's specific errors could be simulated by a two-stage computational model similar to several recently proposed ones (e.g., McClelland, 1986). In the model, a letter perceptual stage is connected in parallel to an orthographic lexical stage, with feedback. Letters in the stimulus activate many possible candidates in the lexical stage; the most strongly activated one is normally the correct one. This patient's cerebral damage is assumed to make the correct orthographic representation less available, permitting a lexical representation that would not normally be activated enough by the stimulus to be produced as a visual error (cf. Gordon et al., submitted).

While this simulation was necessarily post hoc, it does provide a plausible fit of much more detailed data than is typically considered in constructing accounts of acquired dyslexia. More importantly, independent estimates of some of the model's critical parameters should be possible in other patients, permitting a more rigorous, a priori, test. The visual word recognition process and its disruption may therefore provide a useful means for testing computational models of reading and for understanding the effects of cerebral damage on these higher-level functions.

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398.10 SUPPLEMENTARY MOTOR AND PREMOTOR RESPONSES TO ACTUAL AND IMAGINED HAND MOVEMENTS WITH POSITRON EMISSION TOMOGRAPHY. P.T. Fox, J.V. Pardo, S.E. Petersen*, M.E. Raichle. Washington Univ. Medical School, St. Louis MO 63110.

Imagined performance of a motor task has been reported to selectively activate brain regions performing "motor programming". Initial studies of imagined movement indicated exclusive but bilateral activation of the supplementary motor area (SMA) (Roland et al., J. Neurophysiol 43:118). Pursuant to these earlier observations, we measured brain blood flow in normal human volunteers with positron emission tomography during: 1) repetitive (1 Hz) opening and closing of the hand, 2) imagined performance of task 1, 3) vibratory finger stimulation, 4) eyes-closed rest. Responses were identified by a maximum-detection algorithm applied to subtraction images (task state minus control) after stereotactic inter-subject image averaging to enhance signal:noise.

SMA was activated equally by all 3 tasks (7%) increase), passive tactile input being as potent as overt or covert movement. SMA responses were unilateral, contralateral to the engaged hand. Posterior, inferior premotor cortex (PIP, in or near Brodman area 44), however, was more active during imagined (6%) than actual (4%) movement, was not activated by tactile stimulation, and was lateralized toward the dominant hemisphere. Both overt movement and tactile stimulation activated rolandic sensorimotor cortex and cerebellum; covert movement activated neither. We suggest that the PIP, being preferentially activated by motor planning, independent of sensory feedback, and lateralized to the dominant hemisphere, is upstream to SMA. SMA would then be construed as allowing lateralized application of the abstract motor plan (created in PIP) to primary motor cortex and cerebellum within the context of ongoing sensory feedback.

398.11 A COMPARISON OF AUDITORY AND VISUAL PROCESSING OF SINGLE WORDS USING AVERAGED IMAGES OF CEREBRAL BLOOD FLOW CHANGE. S.E. Petersen*, P.T. Fox, M.I. Posner, M.E. Raichle. Depts. of Neurology, Neurological Surgery and Radiology, and the McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, Missouri, USA.

Brain areas activated during the processing of single words (lexical processing) were identified as changes in local blood flow during a stepwise progression of tasks in 12 right-handed normals. Condition 1 was simply to fixate on a "+" synbol presented on a video monitor. Fixation was a component of all tasks. For Condition 2, single nouns were presented at 1 Hz either auditorily or visually. The 2nd level tasks were expected to produce passive sensory processing, and possibly earlier lexical processing. Vocal repetition of the presented words (Condition 3) added rate-controlled motor output. Higher level processing was added at the final level (Condition 4): a verb appropriate to the presented noun was said aloud (e.g. cake...eat). Both auditory and visual presentations were tested during a single session. Focal blood flow changes induced by the different level tasks were identified by subtractions of pairs of blood flow images.

"Passive" observation (2 minus 1) activated primary visual (for visual presentation) and primary auditory (for auditory presentation) cortical areas bilaterally. Lateral, extra-striate, occipital cortex was also bilaterally activated by visual nouns; left supramarginal gyrus was activated by auditory nouns. These extraprimary responses could be related to early lexical processing. Vocalization (3 minus 2) recruited several areas not active in Task 2, areas that are consistent with the word production demands of this comparison. These included: rolandic cortex (sensorimotor mouth, L = R), supplementary motor area, inferior frontal cortex (Broca's area) (L > R), and medial superior cerebellum. Verb generation (4 minus 3) gave strong activation of anterior cingulate gyrus, right inferior lateral cerebellum, and left inferior anterior frontal cortex (at or near area 45). Further studies, using a task during which subjects monitor lists for target words in a semantic category, activated a region of inferior anterior frontal cortex in a location similar to that in the verb generation task. This converging evidence implicates frontal area 45 in semantic processing.

The use of image averaging and stepwise task design has allowed us to identify brain areas related to different levels of cognitive activity. Further experiments are aimed at determining the specific functions related to these areas.

398.12 PREOPERATIVE ASSESSMENT OF CEREBRAL DOMINANCE FOR LANGUAGE USING POSITRON EMISSION TOMOGRAPHIC MEASURMENTS OF BRAIN BLOOD FLOW. J.V. Pardo, P.T. Fox, S. Goldring, M.E. Raichle. Washington University Medical School, St. Louis Mo, 63110

Preoperative assessment of cerebral dominance for language requires selective carotid catheterization and anesthesia of each cerebral hemisphere (Wada test), a highly invasive procedure. Regional brain activation lateralizing to the left cerebral hemisphere and right cerebellar hemisphere in right-handed normal volunteers has been reported by this laboratory (Soc. Neurosci Abst.12:1161) using PET measurements of brain blood flow. While this study reported averaged data, responses often were seen in individual images, suggesting PET as a noninvasive test for language laterality.

Based on our work in normals, 3 conditions and 4 response zones were tested in 9 candidates for surgical treatment of partial complex epilepsy. Tasks were: 1) repetition aloud of visually presented words (nouns), 2) generation aloud of verbs semantically related to visually presented nouns, 3) eyes-closed rest (control state). Response zones assessed were: 1) sensorimotor mouth areas (rolandic; repeat vs rest); 2) posterior, inferior frontal cortex (Broca's area; repeat vs rest); 3) anterior, inferior frontal cortex (generate vs repeat); 4) inferior, lateral cerebellum (generate vs. repeat). Areas of task-induced neuronal activation were identified as focal increases in the distribution of oxygen-15 labeled water, a blood flow tracer,and required only an intravenous catheter. Wada testing indicated left-hemishere dominance in 8 subjects and was indeterminate in 1 subject.

Anterior, inferior frontal responses were both robust and strongly lateralized to the left hemisphere in 7/9 patients. Sensorimotor (rolandic) responses were robust (7/9 pts), but bilateral with weak lateralization (L>R 4/9 pts). Posterior, inferior frontal responses were present in 7/9 pts, lateralized L>R in 4/9 pts, and bilateral in 2/9. Inferior cerebellum was seen only sporadically (3/9 pts), perhaps due to poor sampling of this area, but was strongly lateralized to the right. Only one case activated a region (Broca R>L) inconsistent with the Wada results.

PET measurement of brain blood flow during language performance has considerable potential as a preoperative technique for assessing cerebral dominance for language with minimal invasiveness.

399.1 SEGREGATION OF FUNCTIONALLY DISTINCT OPTIC AXONS IN THE OPTIC TRACT OF OLD WORLD MONKEYS. B.E. Reese* and A. Cowey*. (SPON: R.W. Guillery) University of Oxford, Departments of Human Anatomy and Experimental Psychology, Oxford OX1 3QX, U.K.

The optic tract of old world monkeys displays a partial segregation of its optic axons according to diameter: coarse axons are most abundant superficially, near the tract's pial surface, while at progressively deeper locations in the tract, only fine calibre axons are present (Reese & Guillery, J. Comp. Neurol. in press). We have interpreted this segregation by size as a segregation of functionally distinct ganglion cell axons, but an alternative interpretation, consistent with classical neuro-ophthalmologic descriptions of the optic tract, is also available, namely, that this size segregation reflects a centro-peripheral retinal gradient. In order to test between these two alternatives, implants of HRP have been surgically inserted into one optic tract at varying deep-to-superficial locations in five rhesus or cynomolgous monkeys. Following a 48-72 hour survival, monkeys were perfused with saline followed by 2% paraformaldehyde. Eyes were then removed, retinae were dissected out and reacted for HRP histochemistry, and wholemounts were prepared. The perfusion was continued with 1.25% paraformaldehyde + 2.5% glutaraldehyde. Brains were then cut frozen at 50µm, and sections were processed for HRP histochemistry.

Implants of HRP deep in the optic tract retrogradely labelled a population of retinal ganglion cells with small somas, the majority of which possessed the morphological appearance of the primate's P_β cell. Cellular labelling was densest centrally, near the fovea, but labelled cells extended to the retinal periphery. These implants produced anterograde labelling that was mainly confined to the parvocellular laminae of the lateral geniculate nucleus. Implants placed at more superficial positions labelled a population of retinal ganglion cells many of which had substantially larger somas and a dendritic morphology characteristic of the primate's P_α cell. Density of cellular labelling showed little variation across retinal eccentricity, but measurements of density near the fovea and at the retinal periphery indicated that even these superficial implants labelled more central than peripheral retinal ganglion cells. Anterograde labelling after such superficial implants was found primarily in the magnocellular laminae of the lateral geniculate nucleus.

The present results indicate that the deep-to-superficial axis of the monkey's optic tract does not simply contain a single representation of the foveo-peripheral radial dimension. Rather, this axis contains a partial segregation of axons arising from morphologically distinct retinal ganglion cell classes, each population having its own, independent, visual field representation.

399.2 POSSIBLE NEUROTRANSMITTERS IN THE MONKEY RETINOGENICULATE PATHWAYS. Ricardo Molinar-Rode and Pedro Pasik. Neurobiol, Grad. Prog., Depts. Neurol. & Anat., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

The retinogeniculate pathways in the monkey offer the possibility of analyzing the two major retinal ganglion cell subsystems which remain segregated within the magnocellular and parvocellular laminae of the dorsal lateral geniculate nucleus (LGNd). Current electrophysiologic, pharmacologic and biochemical findings suggest an acidic substance with glutamate-like properties as one neurotransmitter in these pathways. Glutamate (GLU), aspartate (ASP) and acidic dipeptides rich in these amino acids (AA), such as N-acetyl-aspartylglutamate (NAAG) have been considered putative candidates.

We investigated this hypothesis by measuring the concentration of these substances in the two segments of both LGNd, in 5 adolescent monkeys (M. fascicularis), 7 days after transection of the right optic tract, just behind the optic chiasm (histologically verified). Additional information was obtained from the distal portions of the optic tracts (OT). The animals were sacrificed with an overdose of barbiturates, and the brain removed in the cold within 10 sec of death. Blocks containing the structures distal to the lesion were frozen within additional 5 min. Cryostat sections, 256 µm thick, were mounted on glass, kept frozen and microdissected with a 22-gauge punch on a freezing stage. Approximately 40 discs were collected from each structure separately. AA and NAAG were extracted by homogenization in 0.4N perchloric acid, and analyzed by HPLC.

Of the 40 AA studied only ASP and GLU showed important decreases on the lesion side as compared to the intact side. In the LGNd, there were no significant differences between magnocellular and parvocellular laminae on either side so that values were combined for further analyses. ASP and GLU decreased 22% and 16%, respectively ($N=5$, $p < 0.05$). A greater reduction, 39%, was noted in the levels of NAAG ($N=4$, $p < 0.05$). Preliminary results in the OT showed even more marked drops, amounting to 71%, 67% and 80% in ASP, GLU and NAAG, respectively. The latter data reached significance in ASP, and only approached it in GLU and NAAG, probably due to the small sample ($N=3$).

The findings are consistent with the hypothesis that ASP, GLU, and NAAG are putative neurotransmitters in the retinogeniculate pathway. The lack of significant differences between the two segments of the LGNd, may indicate that the same neuroactive substances are used by both retinal ganglion cell subsystems. The more prominent decreases obtained in the OT probably reflect the more uniform population of elements present in this structure, since in the LGNd, ASP, GLU and NAAG may also be contained in geniculate neurons and/or afferents from sources other than the retina.

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399.3 **MORPHOLOGY OF CORTICOGENICULATE AXON ARBORS IN A PRIMATE.** E.A. Lachica*, J.B. Hutchins and V.A. Casagrande. Depts. Cell Biology and Psychology, Vanderbilt Univ., Nashville, TN 37232

While it is clear that a feedback circuit exists between visual cortex and the lateral geniculate nucleus (LGN), its function remains obscure. One way to address this question is to examine the arborization patterns of cortical axons that project to the LGN. Iontophoretic injections of horseradish peroxidase into the optic radiations near the LGN have allowed us to reconstruct putative corticogeniculate axons in *Galago crassicaudatus*, a prosimian primate. In galago, physiologically-distinct cell classes are segregated within magno- (Y-like), parvo- (X-like) and koniocellular (W-like) LGN layers. Hence, in the LGN we can examine (1) how corticogeniculate arbors interact with LGN laminae that receive input from different eyes, and (2) how these arbors interact with laminae that contain different physiological cell classes.

Our results suggest that there may be three types of axons that enter the LGN from the optic radiations. The first type gives off 2-3 collaterals that ramify within a single line of projection in several laminae and interlaminar zones; it then turns orthogonal to its original course and issues 2-3 more collaterals within a single layer over more than one projection column. The terminal arbors of these axons are composed of short side branches which possess strings of *boutons en passant* as well as *boutons terminaux*, and resemble the Type I corticogeniculate axons described in cats by Guillery ('66,'67) and Robson ('83).

Additionally, we find two other axonal arbor classes. For convenience, we refer to these as types II and III. (Since type III arbors were only seen in cases in which the main injection was made close to the LGN, we cannot be certain that they originate in cortex. However, in all cases, parent axons were traced into the optic radiations.) Type II axons have richly-branched terminal fields, restricted to individual LGN layers. They are oriented parallel to a line of projection and have *boutons en passant* in strings. Type III arbors also follow a course that is roughly parallel to a line of projection, giving off clusters in functionally matched pairs of layers. Like type II axon arbors, the rich terminal branches of type III arbors are of fine caliber and possess strings of *boutons en passant* as well as *boutons terminaux*. So far, we have identified terminals of type III axons within the magno- or koniocellular layers, but not in the parvocellular layers. If all three axon types are indeed of cortical origin, then our results suggest that the cortex can modulate LGN activity through at least three channels. Type I axons are in a position to influence several layers within one retinotopic region as well as across a broad retinotopic zone in one layer. In contrast, types II and III axons restrict their influence retinotopically and relate either to just one monocularly-innervated layer (type II) or two layers of the same functional type (type III). Supported by NIH EY01778 and the University Research Council (VAC).

399.4 MORPHOLOGY OF IDENTIFIED FERRET LGN NEURONS CHARACTERIZED IN VIVO AND IN VITRO. M. Esguerra*, A. W. Roe* and M. Sur. (SPON: J. Arezzo). Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

The ferret lateral geniculate nucleus (LGN) contains the W, X and Y functional cell classes as initially described in the cat. In the present study, we describe the morphology and laminar distribution of ferret LGN neurons identified physiologically as X or Y cells in vivo and as relay cells in vitro.

In vivo, we characterized cells as X or Y based on a battery of tests and injected them intracellularly with horseradish peroxidase (HRP). To date, we have recovered 6 Y cells and 2 X cells. For these cells, we have identified sublaminar locations, measured soma sizes and estimated dendritic arbor extents. The somata of ON center X and Y cells lie in the inner leaflets, and those of OFF center cells in the outer leaflets of laminae A and A1, reflecting the physiological division of these laminae into ON and OFF leaflets (Stryker and Zahs, J. Neurosci. 3:1943, 1983). Y cell somata are found in laminae A, A1, C, and interlaminar zones, and have somal areas that range from 163-580 um2. Their dendritic arbors arise from 5-9 primary dendrites and freely cross laminar and sublaminar borders, extending up to 800 um parallel and 600 um orthogonal to laminar borders. These sizes are similar to those of retinogeniculate Y axon terminal arbors (Roe et al., Soc. Neurosci. Abst. 12:9, 1986). One X cell (soma size, 237 um2) has 6 primary dendrites, with a dendritic arbor that is confined to a narrow region perpendicular to laminar borders and spans the entire inner leaflet of lamina A. Retinogeniculate X axons have arbors of similar size and shape. The other X cell (soma size, 574 um2) has 10 primary dendrites. Its soma is situated in the interlaminar zone between laminae A and A1 as are most of its dendrites; its dendritic extent, predominantly parallel to laminar borders, is at least 400 um.

We have also begun in vitro intracellular recording and injection of cells in the ferret LGN, as a prelude to studies of transmitter-induced conductances in identified cells. Rhodamine-latex microspheres are injected into areas 17 and 18 of visual cortex in adult ferrets to retrogradely label relay cell somata in the LGN (Katz, J. Neurosci. 7:1223, 1987). Rhodamine-labeled cells are identified in slices of thalamus 300 um thick and injected with Lucifer Yellow to reveal their dendritic morphology. Initial observations with this preparation suggest that area 18 receives projections from large LGN cells with morphology similar to that of HRP-filled Y-cells, while a heterogeneous population of cells projects to area 17.

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399.5 INNERVATION OF THE CAT'S LATERAL GENICULATE NUCLEUS BY INDIVIDUAL CELLS OF THE PERIGENICULATE NUCLEUS.

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The cat's perigeniculate nucleus (PGN) consists of a thin layer of GABAergic cells lying just dorsal to the lateral geniculate nucleus (LGN); the PGN may be part of the thalamic reticular nucleus. PGN cells are innervated by the LGN, by the visual cortex, and by several ascending brainstem pathways. PGN cells in turn provide a dense, apparently inhibitory innervation to the LGN that strongly modulates geniculate cell activity. To reveal the morphology of the pathway from the PGN to the LGN, we intracellularly labeled individual PGN cells with HRP after recording from them electrophysiologically.

Visual responsiveness of each of our labeled sample of PGN cells was strongly dominated by one or the other eye; PGN cells driven equally by both eyes have not yet been labeled and recovered. Each labeled PGN cell has 5 or 6 primary dendrites that branch and produce beaded secondary and tertiary dendrites. The dendritic arbor extends horizontally for roughly 1mm. Each axon generally originates from a proximal dendrite, and it branches several times within 500um of the soma. One branch usually arborizes within the dendritic arbor, and two sets of branches descend into the LGN A-laminae to form terminal arbors that are mediolaterally separated by 150-300um. Of these, the more lateral arbor, which contains many more boutons, is 500-600um wide and is confined either to lamina A if the contralateral eye dominated PGN cell responses or to lamina A1 if the ipsilateral eye dominated. The more medial arbor, which contains relatively few boutons, innervates laminae A and A1; the innervation is via boutons en passant or several small clusters of boutons (20-150um wide) on short collateral branches. Our prior electron microscopic analysis (Cucchiaro et al., Neurosci. Abstr. 11:231, 1985) suggests a different pattern of contacts between the medial and lateral arbors of these PGN axons. We have as yet observed no projection of PGN axons to the C-laminae or medial interlaminar nucleus. Finally, some PGN cells project an axon branch caudo-medially towards a presently unknown destination, possibly in the midbrain. We are currently labeling a limited number of PGN cells with discrete extracellular injections of the anterograde tracer Phaseolus vulgaris leucoagglutinin to confirm and extend these observations.

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399.6 CONVERGENCE OF CORTICAL AND RETINAL W-CELL INPUT TO CELLS OF CAT SUPERIOR COLLICULUS.

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The superficial layers of the cat's superior colliculus receive input from retinal Y- and W-cells and from the visual cortex. Many collicular neurons ("W-direct cells") appear to receive direct retinal input exclusively from W-cells. Corticotectal afferents densely innervate the upper half of the superficial gray where W-direct cells predominate, but it is not certain that cortical input reaches W-direct cells. For example, some collicular cells exhibit a polysynaptic Y-cell influence mediated by corticotectal input, but this "Y-indirect" influence is not seen in W-direct cells (Hoffmann, J. Neurophys. 36: 1973).

To determine whether W-direct cells receive cortical input, responses of collicular neurons to intracortical stimulation of area 17 were tested in barbiturate-anesthetized cats. W-direct cells were identified on the basis of the slow conduction velocity of their retinal afferents (<13 m/s), as determined from differences in their minimal latencies of activation from the optic disk, chiasm and tract. W-direct cells were common in the superficial layers, making up 94% (44/47) of cells whose afferent conduction velocities could be estimated with confidence. Nearly three-fourths of these W-direct cells (32/44; 73%) could be driven from area 17, a proportion comparable to that among superficial-layer cells overall (106/133; 80%). The fraction of W-direct cells receiving excitatory cortical influence is probably even higher, since retinotopic alignment of stimulus and recording sites was only approximate in this study. Latencies of cortical activation of W-direct cells ($\bar{X}$ = 6.5 $\pm$ 3.1 ms; range: 2.6 - 16.5; n = 32) were similar to those of superficial-layer cells overall ($\bar{X}$ = 5.3 $\pm$ 2.7 ms; range: 0.5 - 16.5; n = 112).

Convergence of W-cell and corticotectal input to collicular neurons is perhaps not surprising, in view of the overlapping terminal distributions of these projections to the superficial gray. Further, most W-direct cells are binocular and direction-selective (Hoffmann, 1973), properties thought to depend largely on corticotectal influence. It is unclear why W-direct cells fail to exhibit the polysynaptic "Y-indirect" influence seen in other collicular cells with cortical input. One possibility is that functionally distinct sets of corticotectal neurons influence the two collicular cell types. In support of this view, preliminary results suggest that cells with Y-indirect input exhibit shorter latencies of activation from the cortex and lie deeper in the superficial layers than do W-direct cells.

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399.7 TOPOGRAPHICAL ORGANIZATION OF THE CONNECTIONS FROM THE DIENCEPHALON TO THE SUPERIOR COLLICULUS IN THE CAT.

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In the last years several authors have proposed that the superior colliculus (SC) plays an important role in the regulation of eye and head orientation movements, being involved in the transfer of locational and behavioral information to the motor system. On the basis of retrograde HRP studies Tortelly and Reinoso-Suárez (Neurosci. Lett., 18: 257, 1980) described projections from several diencephalic formations to the SC, which could serve as a morphological substrate for such a role. With the aim of confirming and understanding more thoroughly these connections we have analyzed the topographical organization of the diencephalo-collicular projections by using the HRP and HRP-WGA retrograde and anterograde tracing techniques. In 29 adult cats, three groups of HRP injections (Sigma VI) were placed in the SC: massive injections, injections above the stratum opticum (SO) and small injections in the strati grisei intermedium (SGI) and profundum (SGP). Fourteen additional animals received HRP-WGA injections (Sigma VI) in different parts of the diencephalon in order to examine the anterograde labeling in the SC. The SC receives billateral projections (mainly from the ipsilateral side) from the dorsal (DHA), lateral, posterior and anterior hypothalamic areas, zona incerta (ZI), the fields of Forel, reticular thalamic nucleus (RT), ventral lateral geniculate nucleus (GLV), ventromedial hypothalamic nucleus and other hypothalamic structures. Almost all these projections end in the deep collicular layers of the SC, although those arising in GLV, which also extend above the SO. Every portion of the SGI and SGP of the SC receives projections from large areas of the diencephalon. However we have verified a certain segregation in the distribution of the connections to the SC from different diencephalic formations. The medial part of the SGI and SGP is the one receiving most abundant and consistent projections from the hypothalamus and the lateral zone of the SC receives connections principally from the lateral and caudal hypothalamic formations. The central (mediolaterally) part of the SC receives few hypothalamic projections, which mainly originate in the lateral part of the anterior hypothalamic area and ventral hypothalamus. The DHA projects widely to the deep collicular layers. The major output from the ZI reaches the rostral and lateral part of the deep collicular layers and the RT projects to the whole extension of the SC, although we have observed a rostrocaudal and mediolateral segregation in these projections. Finally, we have found that the GLV also projects to the entire extension of the SC with a reversed mediolateral topography.

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399.8 CORTICOTECTAL CELLS IN CAT ARE GLUTAMATE IMMUNOREACTIVE: A DOUBLE LABEL STUDY USING WGA RETROGRADE TRANSPORT AND TRANSMITTER IMMUNOCYTOCHEMISTRY.

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The corticotectal pathway in cat arises primarily from layer V pyramidal cells and is thought to be excitatory. Evidence for which neurotransmitter mediates this excitation is contradictory. Removal of visual cortex reduces glutamate levels in the superior colliculus (SC), but few or no corticotectal cells are retrogradely labeled by ^{3}H-D-aspartate, a substance thought to be transported by glutamate neurons. We have used an antibody raised directly against a glutamate conjugate to determine if corticotectal cells in cat contain this excitatory amino acid neurotransmitter.

Either ^{3}H-n-acetylated wheat germ agglutinin (WGA) or HRP conjugated WGA were injected into SC in order to retrogradely label corticotectal cells. ^{3}H-WGA was localized by autoradiography, WGA-HRP by cobalt intensified diaminobenzidine histochemistry. Cells in visual cortex which were labeled by the glutamate antibody were stained using the avidin-biotin (ABC) technique. Preabsorption of the antibody with 4 mM L-gamma-glutamic acid dramatically reduced specific antibody staining. By contrast, preabsorption with 4 mM GABA had no effect on specific staining. The antibody therefore appears specific to neurons containing glutamate but not GABA.

Anti-glutamate positive cells were found throughout visual cortex. Pyramidal cells in layers II,III, and V were especially heavily labeled. The apical and basilar dendrites of these cells were also well-labeled. Many retrogradely labeled corticotectal cells were also found in visual cortex. These cells were found only in layer V. After large injections involving both the superficial and deep layers of SC, retrogradely labeled corticotectal cells were found in areas 17, 18, 19, and several regions of lateral suprasylvian cortex (LS). Smaller injections resulted in labeling of corticotectal cells in more restricted regions.

Approximately 92% of the retrogradely labeled corticotectal cells were double labeled by the glutamate antibody. Thus, all but a small fraction of neurons projecting to SC contain glutamate. Double-labeled corticotectal cells were all pyramidal neurons, most with prominently labeled apical dendrites. These cells varied in size and were widely stratified within layer V.

Our double-label technique demonstrates that the vast majority of all cortical neurons projecting to the cat superior colliculus contain glutamate, regardless of the visual cortical area in which the cells are located. Glutamate is therefore a likely neurotransmitter candidate of the corticotectal system in cat. This conclusion is also supported by evidence that 1) laminae of SC which are known to receive cortical input are densely stained by the glutamate antibody; and 2) visual cortical lesions reduce glutamate levels in SC.

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399.9 RETINAL AND CORTICAL PROJECTIONS TO THE NUCLEUS OF THE OPTIC TRACT AND DORSAL TERMINAL NUCLEUS IN MACAQUE MONKEYS. K.-P. Hoffmann, R. Erickson* and C. Distler*. Allg. Zoologie und Neurobiologie, Ruhr Universitaet Bochum, Postfach 102148, 4630 Bochum, FRG

The nucleus of the optic tract (NOT) in the pretectum and the dorsal terminal nucleus (DTN) of the accessory optic tract in vertebrates are essential for slow horizontal eye movements stabilizing the image on the retina (Optokinetic Reflex OKR). Comparative neuroanatomical studies of mammals have shown that with increasing 'corticalization' of the visual system the midbrain receives an increasing proportion of its input from cortical areas as opposed to the well established direct retinal projection.

To extend this comparative study towards the primate visual system we investigated the relative strength of retinal and cortical projections to the NOT and DTN in the macaque monkey. Electrical stimulation was applied to the optic tract (OT), striate cortex (V1), and different cortical areas in the superior temporal sulcus (STS) while recording extracellularly from single neurons in the NOT and DTN of anaesthetized paralyzed animals. NOT-DTN cells were identified by their antidromic spike following electrical stimulation of their axons projecting to the inferior olive and by their high degree of movement and direction selectivity. All NOT-DTN cells on the left side of the brain prefer leftward movement of visual stimuli in the visual field and vice versa. The receptive fields always include the fovea and mostly extend far into both the contralateral and ipsilateral hemifields. NOT-DTN neurons respond to stimulus velocities from less than 0.1°/s up to several hundred degrees per second. The mean peak sensitivity of the whole population occurs at 20-30°/s.

Only about half of the NOT-DTN cells could be activated by OT-stimulation. The low conduction velocity of the retinal axons to the NOT and DTN (4-9m/s) implies that they originate from so called 'rarely encountered' ganglion cells with special receptive field properties (Schiller,P.H., Malpeli, J.G., (1977), J. Neurophysiol. 40, 428-445). Electrical stimulation in V1 as well as STS activated all cells recorded in the NOT-DTN with average latencies of 3.2 ±0.7 and 2.1 ±0.5ms, respectively. Anatomical studies confirm our physiological results by showing direct projections from both V1 and STS to NOT and DTN.

A comparison of retinal and cortical input to the NOT and DTN in different mammals shows that the strongest preponderance of cortical influence exists in monkeys. This could explain why in monkeys all NOT-DTN neurons can be activated from either eye and why monocularly tested horizontal OKR is completely symmetrical. In humans the cortical input to NOT and DTN may be so important that in patients with cortical blindness the retinal input alone cannot maintain the OKR.

399.10 TREE SHREW PULVINAR NUCLEUS: DIFFERENTIAL PROJECTIONS TO ACHE-RICH AND -POOR ZONES FROM THE SUPERIOR COLLICULUS BASED ON CELL SIZES, DEPTH AND MORPHOLOGY. R.G. Carey. Div. of Neurobiol., Barrow Neurol. Inst., St. Joseph's Hosp. & Med. Ctr., Phoenix, AZ 85013.

We recently showed that in the tree shrew two distinct pathways exist from the superior colliculus (SC) to the pulvinar nucleus (Pul) (Carey et. al., 1985). The first is bilateral and projects diffusely to widespread regions of the AChE-rich portions of the Pul, while the second is strictly ipsilateral and projects topographically to specific discrete regions of the AChE-poor Pul. The present investigation examined the distribution of HRP-labeled cells in the SC following small electrophoretic tracer injections (WGA/HRP or cholera toxin/HRP) into restricted portions of the pulvinar nucleus in anesthetized tree shrews. Each of these tracers, when reacted properly, result in Golgi-like visualization of the labeled cells for use in the morphological analyses.

Following injections restricted to AChE-rich Pul, labeled cells were located bilaterally within the deep part of the lower stratum griseum superficiale (SGS) and the most superficial portion of the stratum opticum (SO) with a mean depth of 375um (S.D.=55um). Labeled cells ipsilateral to the injection consistently were found throughout widespread regions of the SC and often covered the entire rostral-caudal extent of the colliculus. These cells were typically medium to large horizontally or vertically oriented fusiform, multipolar or triangular cells with cross-sectional areas ranging from 100 to 500um^2 ($\bar{X}$=215um, S.D.=70um), but were skewed heavily towards the larger cells. The various classes appeared to occur with no particular order, except that the cells were generally larger at the deeper depths. Contralateral cells, while basically similar, were less variable in depth and size.

Following injections of the AChE-poor Pul, a different pattern of labeled cells emerged. These labeled cells were strictly ipsilateral and occurred only within limited areas of the SC; their position varied with the placement of the injection in the Pul. Further, they principally were located in the mid to deep part of the lower SGS with fewer cells occurring in SO, but with a number of cells occurring in the lower part of the upper SGS with a mean depth = 335um (S.D=60um). These labeled cells characteristically were small to medium spherical and flat fusiform cells, with a cross-sectional area of their cell bodies ranging from 30-250um^2 ($\bar{X}$=109um, S.D.= 38um) and reflecting a fairly normal distribution slightly skewed towards smaller cells.

Thus, in the tree shrew two distinct projection pathways exist between SC and Pul that are distinguished not only by termination pattern within the Pul, but by cellular characteristics as well. These paths may represent separate "Y" and "W" channels to Pul and eventually to extrastriate cortex, analogous to that occuring in the retinogeniculate pathways.

399.11 SPATIO-TEMPORAL RESPONSE PROPERTIES OF NEURONS WITHIN THE FERRET VISUAL CLAUSTRUM. K.M. Horn and R.G. Carey. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Quantitative examination of visual response characteristics of over 100 neurons within the claustrum of paralyzed, anesthetized ferrets (Mustela putorius furo) has revealed three major spatio-temporal response properties. (1) Even though the receptive fields of claustral cells are relatively long (mean=24°), only a few cells (<20%) demonstrate length summation. In fact, the majority of the claustral neurons (>60%) exhibit peak responses to stimuli that are less than 50% of the receptive field length. These neurons can be classified as a form of special complex cell since their responses are either 'tuned' to a particular slit length and exhibit either response decrements to longer slits or show no further summation to longer length slits. A major subgroup of these cells prefers stimuli that are less than 5% of the receptive field length. (2) Claustral cells prefer moving stimuli and concomitantly are less responsive to stationary stimuli. Over 50% of the claustral neurons prefer fast moving stimuli (>100°/sec) and can be categorized as velocity-high pass cells since they respond poorly to slow moving slits and have responses of over 70% of maximum when tested at 1000°/sec. Velocity-broad band (22%) and velocity-tuned (20%) cells constitute the remaining claustral neurons. The only decrements in response activity in velocity-broad neurons are found at either slow (<25°/sec) or very fast (>500°/sec) velocities, while velocity-tuned cells exhibit response decrements when the velocity of the stimulus is either less than or greater than a narrow range of velocities (usually less than 75°/sec in width). No cells in the claustrum have been sampled that respond exclusively to slow moving stimuli (<25°/sec). (3) Directional preferences to moving stimuli are shown in many cells (>30%). The majority of these cells continue to exhibit directionality when tested at nonpreferred or fast velocities. The remaining neurons exhibit either a directional bias (30%), pan-directionality (20%), or bi-directionality (16%). Finally, we have noted interactions between the spatio-temporal characteristics of stimulus length, velocity and direction.

The dorsal claustrum has extensive reciprocal connections with visual cortices (striate, adjacent extrastriate, and lateral suprasylvian) and thalamus (lateral posterior and intralaminar nuclei). These anatomical connections in conjunction with the spatio-temporal properties of the majority of ferret claustral cells provide preliminary evidence that these cells are involved in either providing 'attentional tonus/gating' or fast saccade processing information to multiple visual cortices.

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400.1 Double-labelling Retrograde Transport in the Mouse Thymus and adjacent tissue. K. Bulloch and L. Tollefson. Neuroimmune Physiology Laboratory, Helicon Foundation, San Diego, CA 92109.

In view of the close anatomical positions of the esophagus, neck musculature, and intercostal regions to the thymus gland in the mouse, and the reported labelling of these areas of the spinal cord and by the vagal complex of the brain stem, it is possible that early retrograde transport studies might have produced ambiguous results. Therefore we have undertaken the re-examination of the innervation patterns of the thymus gland and the aforementioned tissues using a double-labelled retrograde transport technique.

To determine the best procedure for peripheral double-labelled retrograde transport, several different substances and protocols were developed and tested. Retrograde transport from peripheral tissue to the central nervous system using Rhodamine beads (Luma-Fluor) proved to be ineffective as a transporting agent but did serve as an excellent marker in later experiments for varifying the specificity of the injection sites. There were some drawbacks with the use of Rhodamine in the periphery. These beads initiate a massive migration of phagocytic cells to the injection site which may be responsible for the failure of this agent to be taken up by autonomic nerves. The most effective combination of double-labelled retrograde transport markers were found to be Horseradish peroxidase(HRP) (Sigma Type VI) and Fluoro-gold (Fluorochrome, Inc.). Fluoro-gold proved to be even a more sensitive label than HRP and thus could be injected in very small quantities.

To determine the pattern of innervation of the various aforementioned tissues and organs, HRP and Fluoro-gold were simultaneously injected into mice anesthesized with Ketamine and Rompun. Neck muscles, pectoral muscles or the areas of the esophagus adjacent to the thymus received multiple injections of a 30% HRP (1-3 ul) whereas the thymus received injections of 0.5-1.0 ul of 2.5% Fluoro-gold. Many different variations of the aforementioned protocol were used in these experiments. The results clearly show Fluoro-gold injections into the thymus when compared to HRP injections into the other tissue produce very few double-labelled neurons in the brain stem and the spinal cord. The presence of double-labelled neurons may reflect diffusion of one or both of the substances onto the adjacent areas in question or onto other tissue (heart, lung, thoracic duct, etc...) that receives vagal innervation. Supported by ONR grant #N00014-85k-0528.

400.2 SIGMA RECEPTORS IN HUMAN PERIPHERAL BLOOD LEUKOCYTES (HPBL) AND RAT SPLEEN: IDENTIFICATION, CHARACTERIZATION AND AUTORADIOGRAPHIC LOCALIZATION. S.A. Wolfe, Jr.*, C. Kulsakdinun* and E.B. De Souza. (SPON: K. Dismukes) Neuroscience Branch, NIDA Addiction Research Center, Baltimore, MD 21224.

Phencyclidine (PCP) and sigma opioids have been shown to produce psychotomimetic effects in humans and associated behavioral effects in animals through actions on specific receptors in brain. In addition, PCP has been reported to cause immunosuppression in vitro. The aim of the present study was to identify, characterize and localize the sites of PCP action (sigma and/or PCP receptors) in immune tissues. Sigma receptors were labeled in homogenates of rat cerebellum, rat spleen and HPBL, and in slide-mounted sections of rat brain and spleen with ^{3}H-haloperidol in the presence of 25 nM spiperone. PCP receptors were labeled with ^{3}H-(1-[1-(2-thienylcyclohexyl)]piperidine) (^{3}H-TCP). Nonspecific binding was defined as ^{3}H-haloperidol or ^{3}H-TCP bound in the presence of 30 μM (-)butaclamol or 20 μM PCP, respectively. ^{3}H-Haloperidol binding in rat spleen, rat cerebellum and HPBL was saturable and of high affinity, with comparable K_D values (mean $\pm$ S.E.M.; n = 3) of 1.07 $\pm$ 0.16, 0.74 $\pm$ 0.12, and 0.63 $\pm$ 0.09 nM, respectively. In competition studies, the pharmacological profiles were virtually identical in all three tissues; the rank order of potency was haloperidol >> (-)butaclamol = pentazocine > [3-hydroxyphenyl]-N-(1-propyl)piperidine (3-PPP) > (+)SKF 10,047 = (+)butaclamol > ethylketocyclazocine = PCP = (-)SKF 10,047 = levallorphan > TCP. The highest density of ^{3}H-haloperidol-labeled sigma receptors (B_{max}, fmol/mg protein,) was found in spleen (1198 $\pm$ 105, n = 4), with comparable but significantly lower densities present in HPBL (884 $\pm$ 143, n = 3) and rat cerebellum (753 $\pm$ 26, n = 4). Autoradiographic studies carried out in slide-mounted sections of rat spleen demonstrated that ^{3}H-haloperidol-labeled sigma receptors were localized primarily in the white pulp areas, which contain a high density of lymphocytes. In contrast, there was a notable absence of ^{3}H-TCP-labeled PCP receptors in rat spleen and HPBL using either homogenate binding or autoradiographic methods. In summary, these results demonstrating high concentration of sigma receptors and the virtual absence of PCP receptors in immune tissues suggest that PCP exerts its immunomodulatory influence via sigma receptors. Furthermore, the data suggest that endogenous "sigma" ligands may play a role in immune regulation, either directly or by lymphokine or monokine induction. Studies of the effects of PCP and sigma agonists on immune function are presently underway in our laboratory. The sigma receptor in HPBL may also be a useful peripheral marker for assessing the role of sigma receptors in human brain.

400.3 EFFECTS OF MORPHINE ON THE PHENOTYPIC EXPRESSION OF T-LYMPHOCYTE CELL SURFACE MARKERS IN THE MOUSE. A.S. Kimes, W.J. Smith*, C.P. Jaffe* and E.D. London. Neuropharm. Lab., NIDA Addiction Research Center, Baltimore, MD 21224.

Intravenous drug abusers are at a high risk for infection by the acquired immune deficiency syndrome (AIDS) virus. Therefore, it was of interest to study the effect of opioids, the drug class most often abused by self-injection, on immune cells which are attacked by the AIDS virus, HIV. Peripheral blood lymphocytes (PBL) were obtained from blood of pathogen-free male C57/BL6J mice. While under halothane anesthesia, each mouse received a subcutaneous placebo (P) pellet, a low dose morphine (M) pellet (6.7 mg M sulfate), or a high dose M pellet (75 mg M sulfate) (n = 8-10 per group). Three days later, the mice were anesthetized with halothane again, and blood was obtained by cardiac puncture. Numbers of white blood cells (WBC), lymphocytes, total T-cells, T-helpers and T-cytotoxic/suppressors were measured in each sample using flow cytometry and monoclonal antibodies to cell surface antigens specific for T-cells, T-helpers and T-cytotoxic/suppressors (Thy-1, L3T4, and Lyt2, respectively). Spleen/body weight ratios also were calculated for each mouse.

All of the aforementioned measures were significantly lower in M-treated mice, showing a dose-response relation. In vitro incubation of PBL with naloxone (100 nM) partially reversed the M effect on Thy-1 expression but had no effect on Thy-1 expression in PBL from P-treated mice. The M effect (6.7 mg) on spleen-body weight ratios, number of lymphocytes and WBC count was blocked by naltrexone (40 mg/kg, s.c., twice daily).

Studies on PBL of parenteral M and heroin drug abusers have failed to demonstrate lower levels of T-cell markers compared to normal controls. It seemed reasonable that this failure might be due to antigenic stimulation found in drug abusers because of their needle sharing practices. In order to test the effect of antigenic stimulation on detectability of an opioid effect on immune markers, we gave mice injections of sheep red blood cells. Spleen/body weight ratios of P- and M-pelleted mice which received these injections were higher than those of vehicle-treated controls, demonstrating that M-treated mice could respond to an antigenic stimulus, and suggesting that such a response might obscure a reduction of T-cell numbers and a suppression of T-cell markers in human addicts. We detected no differences in ^{3}H-thymidine incorporation into splenocytes in the presence of mitogenic agents (concanavalin A, phytohemagglutinin, and pokeweed) comparing M- (75 mg) and P-pelleted mice.

We conclude that M may affect the phenotypic expression of cell surface markers and possibly number of T-cells. Inasmuch as mitogen assays reflect the immunological competency of T-cells, the present findings do not suggest a functional impairment.

400.4 CENTRAL OPIOID SYSTEMS ARE DIFFERENTIALLY AFFECTED BY PRODUCTS OF THE IMMUNE RESPONSE. P.M. Dougherty* and N. Dafny (SPON: F. Yatsu). Dept. of Neurobiology and Anatomy, The Univ. of Texas Med. Sch. at Houston, P. O. Box 20708, Houston, TX 77225.

The present study compares the effects induced by muramyl dipeptide (MDP), the smallest biologically active fragment of bacterial endotoxin (lipopolysaccharide), and interferon-alpha (IFN), the most rapidly produced defense against viral infection, upon various central opioid behavioral and electrophysiological activities. Forty-eight male Sprague Dawley rats were used to study the dose-response characteristics of MDP and IFN upon naloxone-precipitated withdrawal in morphine-dependent animals following both intracerebroventricular (i.c.v.) and systemic (i.p.) administration in the first half of this study. In the second half, the relationship of the similarities as well as differences in the actions of these two immune-response products upon the baseline and post-narcotic electrophysiological sensory-evoked responses of four brain regions essential for the in vivo manifestation of various opioid activities is investigated. Electrical activity from 24 male Sprague-Dawley rats previously implanted with permanent semi-microelectrodes in the hypothalamus, septum, mesencephalon and cortex was recorded prior to and following either MDP or IFN, and later following morphine and naloxone. The results obtained demonstrate that: 1) MDP attenuates withdrawal severity in a typical linear dose-related fashion, while IFN attenuates withdrawal severity in a U-shaped pattern 2) The cortex, mesencephalon and septum were modified differently following MDP versus IFN treatment; however, the responses obtained from the hypothalamus were the same for both agents. 3) In addition, MDP and IFN have differing effects upon the actions of morphine and naloxone in the cortex, septum and mesencephalon; but, again, these agents exhibit the same effects upon morphine activity in the hypothalamus. These results indicate that the shared effects of these two agents upon the behavioral expression of opiate withdrawal may be due to a common activity upon the physiologic sequelae of opioids in the hypothalamus. In addition, since each peptide also has unique dose-response characteristics as well as unique effects upon the electrophysiologic activities of other subcortical and cortical structures, these results support the conclusion that central opioid systems may provide a target for the perception and differentiation of sensory immunologic information by the brain.

400.5 CORTICOTROPIN RELEASING FACTOR STIMULATES PROLIFERATION OF RAT LYMPHOCYTES IN VITRO. J. P. McGillis, A. Park*, M. Dallman*, and D. G. Payan*. Dept. of Medicine and Physiology, and Howard Hughes Medical Institute, Universisty of California, San Francisco, San Francisco, CA 94143

An immunoregulatory role for neuropeptides is supported by evidence from functional studies, by the presence of specific neuropeptide receptors on lymphocytes, and by the ability of immunological tissues to produce neuropeptides. Production of immunoreactive (ir) ACTH and ß endorphin peptides, and POMC mRNA by lymphocytes suggests that immunological tissues utilize these peptides in a manner similar to the neuroendocrine system. This analogy is consistant with the observation that CRF can induce the production of irACTH and ß endorphin in lymphocytes in vitro.

In these studies a functional effect of CRF on lymphocytes was identified by demonstrating that CRF stimulates lymphocyte proliferation. Rat splenocyte suspensions were prepared from male rats and were cultered at 10^6 cells/ml in 96 well microtiter plates. The cells were treated with CRF (n=6) at doses ranging from 10^{-12} to 10^{-6} M. After 48 hrs 1 µCi of ^{3}H-thymidine (Tdr) was added and the cells were harvested 8 hrs later. Proliferation was quantified by determining the amount of ^{3}H-Tdr incorporated into cellular DNA. Both ovine and rat CRF caused a dose dependent cellular proliferation which peaked at 10^{-8} M CRF. A significant response was usually seen at doses as low as 10^{-10} M and the ED_{50} was about 2 x 10^{-9} M. The maximal response to 10^{-8} M CRF varied between 250 and 500 % of unstimulated controls. This effect of CRF appears to be pharmacologically distinct in that the antagonist αhCRF (9-41) acts as an equipotent agonist in inducing lymphocyte proliferation. This suggests that the 8 N-terminal amino acids of CRF are not required for lymphocyte stimulation. Further studies are being done with other fragments of CRF to localize the biological activity.

The response of lymphocytes from different lymphoid tissues and different subsets of lymphocytes were also studied. Lymphocytes were isolated from mesenteric lymph nodes (MLN), Peyer's Patches (PP), Inguinal lymph nodes (ILN), spleen, and thymus and were stimulated with CRF. The greatest response to CRF induced lymphocyte proliferation was seen in gut associated lymphoid tissue, MLN and PP (> 350 % unstim. control) followed by ILN and spleen (200-250 %). Thymocytes did not proliferate following CRF treatment. When enriched populations of T-cells, B-cells, and macrophages were treated with CRF, only B-cells responded by proliferating. The ability of CRF to stimulate B-cell proliferation supports the hypothesis that CRF has an immunoregulatory role in addition to its role in integrating autonomic and neuroendocrine stress responses.

400.6 NOREPINEPHRINE INHIBITION OF G-IFN INDUCED Ia EXPRESSION ON CULTURED ASTROCYTES SEEMS TO WORK THROUGH A BETA-2 RECEPTOR MECHANISM THAT INVOLVES ELEVATIONS IN INTRACELLULAR cAMP. E.M. Frohman, B. Vayuvegula, S. Gupta, and S. van den Noort. Departments of Neurology and Immunology, University of California at Irvine, Irvine, CA 92717

Recent studies show that astrocytes serve as mediators of intracerebral immune responses. Astrocytes do not normally express the MHC class II antigens (Ia in mouse and DR in man) that are necessary for the initiation of such responses, but instead must be induced to do so. A potent inducer of Ia expression is gamma-interferon (G-IFN). Despite the presence of this lymphokine in the CNS, there is a paucity of Ia (DR) expression in normal brain. Due to this apparent paradox, we sought to determine whether there are intracerebral modulators that prevent G-IFN induced Ia expression on astrocytes. For this purpose, enriched astrocyte cultures were made from brains of newborn Balb-C mice. When cultures were treated with 2 units/ml of G-IFN, 37%, 50.2% and 51.5% of cells were Ia+ at 24, 48, and 72 hours when analyzed by flow cytometry. When cultures were co-treated with 2 units/ml of G-IFN and varying concentrations of NE (10^{-7} to 10^{-3}M) the percent inhibition of Ia expression ranged from 18.4% to 86.2% at 24 hours; 0 to 89% at 48 hours; and 0 to 94.2% at 72 hours. To determine the mechanism by which such an inhibitory effect occurs, we co-treated cultures with 2 units/ml of G-IFN and varying doses of isoproterenol (10^{-8} to 10^{-4}M), a beta-2 adrenergic agonist. Results showed that this drug in a dose dependent fashion, inhibited G-IFN induced Ia expression on cultured astrocytes. Further, we have demonstrated that propranolol, a beta-adrenergic antagonist, but not atenolol (a beta-1 antagonist) or alpha antagonists, attenuated the NE inhibitory effect on G-IFN induced Ia expression. Since prostaglandin E, an inhbitory modulator of Ia expression, causes an increase in intracellular cAMP, NE may act through a similar mechanism. To test this hypothesis, we treated cultures with 2 units/ml of G-IFN and varying doses of dipyridimole (10^{-7} to 10^{-4}M), a phosphodiesterase inhibitor, and observed a significant inhibition of Ia expression (11.4% to 83.0% respectively). These findings suggest that NE inhibition of Ia expression acts via beta-2 adrenergic receptor transduction pathways, which are known to involve the activation of adenylate cyclase (PKA) and a rise in cAMP. This contrasts with Ia inducing signals such as G-IFN, that are believed to work through activation of protein kinase C (PKC).

400.7 Autonomic Nervous System Receptors as a Marker for T-Cell Differentiation and Function. T. Radojcic (1), M. Gersten (2), S.J. Divis (2), D. Darko (3), H. Moltusky (3), D. Smith (3), M. Cohn (2), and K. Bulloch (1). (T. Melnechuk, sponsor). Neuroimmune Physiology Laboratory, Helicon Foundation, San Diego, Cal. 92109 (1), Salk Institute, San Diego, Cal. 92138 (2), Univ. of Cal. San Diego, 92093 (3).

Recently, studies have indicated complex interaction between the nervous system and the immune system. Furthermore, it is becoming increasingly evident that the nervous system may play a significant role in the maturation of functional lymphocyte subtypes. In this study, murine-derived cloned cell lines in log phase of growth, representative of the ontogeny of the immunocompetent T-cell, have been investigated with respect to the development of beta adrenergic receptor distribution.

Normal thymocytes, a cloned thymoma-derived cell line BW 5147, and a splenic-derived, activated cloned T-cell line T-12 (non-tumoregenic) were screened for the presence of beta adrenergic receptors. Iodopindolol was used as the ligand probe at concentrations ranging from 10(-12) M to 10(-9) M. Sixty minute incubations at 37C were carried out in RPMI at 2 X 10(5) cells per assay point (in quadruplicate). Non-specific binding was determined by incubation in the presence of 1 X 10(-6) M propranolol.

The results of these experiments indicate that there is an increase in beta adrenergic receptor number in the mature activated T-cell line, as compared to the immature thymocyte cell line BW 5147, and to the normal thymocytes. These findings are consistent with a previous study that showed mature peripheral lymphocytes express a significantly greater number of beta adrenergic receptors than do normal thymocytes (Van De Griend, R.J. et al., Clin. Exp. Immunol. 53:53, 1983). Binding in the BW 5147 cell line is comparable to levels observed in normal thymocytes.

Further differences may become evident in receptor type, number, and distribution on the various effector function subsets of T-cells. It is also apparent that the use of cloned "normal" and tumoregenic T-cell lines may provide a useful specific tool in a more precise analysis of neuroimmune interaction. Supported by grant #N00014-85k-0528 from the Office of Naval Research and a grant from the Joan B. Kroc Foundation of Psychoneuroimmunology.

400.8 THE EFFECT OF NUMBER OF MICE HOUSED/CAGE ON IMMUNOLOGIC COMPETENCY. B.S. Rabin*, T. Caggiula, M. Lyte*, E. Hamill* (Spons: H. Barry, III). Depts. of Pathology and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA. 15261

This study has identified a change in immunologic functional activity at the level of the T-helper cell in male C3H mice housed 1 or 5 per cage. This resulted in elevated numbers of spleen lymphocytes producing antibody to sheep erythrocytes in individually housed mice, but did not affect the T-cell independent antibody response to PVP. Nonspecific responsiveness to the T-cell mitogens Con A and PHA, as well as IL-2 release, was also higher in the individually housed mice, but the mitogenic response to the B-cell mitogen LPS was not changed. No differences in IL-1 production between the differentially housed animals were found. The data suggest that functional changes have primarily occurred in the T-helper cell population of the male C3H mice. The difference of immune reactivity to S-RBC of the differentially housed was not present at 1 and 4 days of housing but was detected on days 8 and 10. By day 21 the difference in the antibody response to S-RBC was no longer present, but Con-A reactivity was still higher in the mice housed 1/cage. Castration of young male mice abolished the immune difference associated with housing. Four additional strains of mice housed individually or in a group (5/cage) were immunized with sheep erythrocytes (SRBC) to determine if the major histocompatibility complex (MHC) was associated with altered immune reactivity based on housing. Individually housed mice from two strains which shared neither the H_2D or H_2K loci, produced more antibody forming spleen lymphocytes to SRBC than group housed mice. Corticosterone levels were not related to the level of the immune response. Thus, genetic factors related to the MHC do not influence alteration of the immune response and male sex hormones may contribute to the immunologic differences which occur with differential housing conditions.

400.9 ALTERED IMMUNE FUNCTION IN YOUNG ADULT BASAL FOREBRAIN DAMAGED AND NORMAL AGED RHESUS MONKEYS. L.J. Kraus, M.B. Moss and D.L. Rosene. Departments of Neurology and Anatomy, Boston University School of Medicine, Boston, MA. 02118

A wide variety of recent research has demonstrated the important role of neural and endocrine influences in regulation of the immune system. Damage to specific brain areas has been shown to result in various changes in immune function. We previously reported preliminary findings of a decrease in one aspect of cell mediated immune function, natural killer cell activity (NKCA), in young adult rhesus monkeys with bilateral lesions of the basal forebrain. We report here our continuation of these studies to include a larger number of monkeys and several additional measures of immune function. We have now examined 10 young adult animals (5-10 yrs.) with lesions of the basal forebrain. Bilateral lesions of the basal forebrain, involving principally the substantia innominata and nucleus basalis (SI-NB), were produced by intracerebral injection of the neurotoxin, ibotenic acid. We have also examined 4 monkeys with caudate lesions and one with hippocampal lesions, 11 sham operated controls and 5 nonoperated controls in this age range. Further, 6 monkeys with SI-NB lesions and one animal with caudate lesions were assessed both before and after creation of the lesions. Finally, we have examined 17 normals in other age groups including 8 animals 25+ yrs. old. The immune measures studied include NKCA, response to T and B cell mitogens, production of interferons (IF), and percent of specific lymphocyte subsets determined by reactivity with monoclonal antibodies. As part of a multidisciplinary study of the basal forebrain and aging in the rhesus monkey, these measures were compared with chemical and behavioral data obtained from many of the same animals. Changes in the cholinergic system were evaluated with histochemical studies of the cholinergic marker, acetylcholinesterase (AChE) and were quantified with a scanning and integrating microdensitometer. NKCA, IF production, and response to T cell mitogens were all depressed in monkeys with SI-NB lesions. All immune measures tested in monkeys with caudate or hippocampal lesions were within normal range. NKCA and IF production were depressed in some, but not all, aged normal monkeys and mitogen response was depressed in this group. Monoclonal antibody reactivity was not altered in any test group. In aged monkeys and monkeys with SI-NB lesions where behavioral data were available, degree of behavioral deficit was correlated with immune impairment. Further, immune impairment appeared to correlate with AChE depletion in animals for which these data were available. These data suggest that cholinergic loss due either to specific brain lesions or degenerative processes associated with normal aging may greatly influence immune function and thus host resistance to autoaggression and disease.

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ACTION POTENTIALS AND ION CHANNELS XIII

401.1 PHYSIOLOGICAL INTERACTION BETWEEN CALCIUM AND CYCLIC AMP IN AN APLYSIA BURSTING NEURON. Richard H. Kramer* and Irwin B. Levitan Graduate Dept. of Biochemistry, Brandeis Univ., Waltham MA 02254

Ca^{2+} and cyclic AMP are well established intracellular messengers in neurons. Studies of physiological interactions between these messengers, however, are still in their infancy. The Aplysia bursting neuron R15 is ideal for studying such interactions because the individual roles that Ca^{2+} and cyclic AMP play are well understood. Intracellular Ca^{2+}, which increases during bursts of action potentials, regulates several ionic currents that participate in generating the bursting pattern of electrical activity. Cyclic AMP mediates the effects of serotonin (5-HT) and egg-laying hormone (ELH; see Levitan, Kramer and Levitan, this volume) on several ionic currents in cell R15. These include an increase in an inwardly rectifying K^+ current (I_R) and a voltage-gated Ca^{2+} current (I_{Ca}).

Last year we reported that Ca^{2+} influx during bursts of spikes or under voltage-clamp leads to the inactivation of I_R. We have investigated two possible mechanisms of this inactivation of I_R: 1) Ca^{2+} could bind to I_R channels and directly regulate their activity, and 2) Ca^{2+} could alter the cyclic AMP cascade and thus regulate I_R indirectly. Several types of evidence suggest that the second possibility is more likely. Greatly elevating the level of cyclic AMP, by application of high concentrations of 5-HT, forskolin, or PCPT-cyclic AMP, all block the effect of Ca^{2+} on I_R. This happens in spite of the fact that these agents increase I_{Ca} and also increase the number of I_R channels available to be inactivated by Ca^{2+}. Loading neuron R15 with Ca^{2+}, by applying periodic depolarizations, reduces the magnitude and speeds the decay of the I_R increase evoked by locally applied "puffs" of 5-HT or ELH. Thus, Ca^{2+} can decrease the magnitude and duration of cyclic AMP action, possibly by accelerating the removal of cyclic AMP by activating a phosphodiesterase (PDE), or by accelerating the dephosphorylation of proteins that regulate I_R by activating a phosphatase. We favor the hypothesis that Ca^{2+} causes the inactivation of I_R by activating a Ca/calmodulin-dependent PDE because 1) we have measured such enzyme activity in extracts of single R15 somata, 2) R15 neurons which have been hyperpolarized to prevent Ca^{2+} influx have more cyclic AMP than control (bursting) R15 neurons, and 3) the Ca^{2+}-dependent inactivation of I_R is reduced by adding the PDE inhibitor IBMX. We propose that Ca^{2+}-dependent modulation of cyclic AMP metabolism has at least two consequences in neuron R15. First, it causes a nearly complete inactivation of I_R in the normal bursting neuron. Second, it decreases the sensitivity of I_R, and perhaps other ionic currents, to neurotransmitters that elevate cyclic AMP.

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401.2 MODULATION OF CHARYBDOTOXIN-SENSITIVE CALCIUM-DEPENDENT POTASSIUM CHANNELS BY PROTEIN PHOSPHORYLATION. Peter H. Reinhart* and Irwin B. Levitan (SPON: Daniel Dagan) Graduate Dept. of Biochemistry, Brandeis University, Waltham MA 02254.

The regulation of calcium-dependent potassium channels from rat brain has been investigated using single channel recording techniques. When plasma membrane fractions from rat brain are reconstituted into planar lipid bilayers, at least three types of calcium-dependent potassium channels with different single channel conductances (approximately 240 pS, 140 pS and 75 pS in symmetrical 150 mM KCl), and distinct gating kinetics, can be observed. These channels do not exhibit any rectification between -40 mV and +40 mV, nor do they show any inactivation at hyperpolarized potentials. The open probability of the 240 pS channel can be increased dramatically by the addition of the catalytic subunit of the cAMP-dependent protein kinase; this increase is due largely to an increase in the channel mean open time. In contrast, the gating activity of a 240 pS channel from muscle t-tubules, which has similar properties to the brain channel, is not modulated by the catalytic subunit. The open probability of the 75 pS channel from rat brain is also increased by phosphorylation, but in this case resulting from a decrease in the channel mean closed time. Preliminary experiments suggest that the activity of the 140 pS channel may be decreased by the catalytic subunit. All three types of rat brain channels are inhibited by nanomolar concentrations of the polypeptide scorpion toxin charybdotoxin (CTX). Thus there may be a family of calcium-dependent potassium channels which differ in their single channel conductances, but have certain common regulatory and toxin binding sites.

Channel	Voltage-Sensitive	Calcium-dependent	CTX-sensitive	Modulated
1. Muscle 240 pS	Yes	Yes	Yes	No
2. Brain 240 pS	Yes	Yes	Yes	Yes ↑
3. Brain 140 pS	Yes	Yes	Yes	? ↓
4. Brain 75 pS	Yes	Yes	Yes	Yes ↑

Supported by NIH Grant NS17910 to IBL.

401.3 MODULATION OF MEMBRANE CURRENTS AND EXCITABILITY BY SEROTONIN AND cAMP IN PLEURAL SENSORY NEURONS OF APLYSIA. D.A. Baxter and J.H. Byrne, Department of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

In the pleural sensory neurons of Aplysia, serotonin (5-HT) modulates not only the novel 5-HT sensitive K^+ current (Ik,s), but also the delayed K^+ current (Ik,v) (Baxter & Byrne, 1986). In order to determine whether modulation of both of these currents by 5-HT is mediated by cAMP, two-electrode voltage- and current-clamp techniques were used to compare the effects of application of 5-HT, 8-bromo-cAMP and 8-4-chlorophenylthio-cAMP on membrane currents, spike duration and excitability in isolated clusters of sensory neuron cell bodies.

Computer isolation of membrane currents modulated by 5-HT clearly revealed two affected currents (Baxter & Byrne, 1986). One current had properties consistent with Ik,s. It was relatively voltage-independent, noninactivating, not blocked by 4-AP and relatively insensitive to TEA. The second current had properties consistent with Ik,v. It was highly voltage-independent and was blocked by 4-AP and TEA. Computer isolation of the membrane currents modulated by the cAMP analogues revealed only one prominent current, Ik,s. The cAMP analogues occluded further modulation of Ik,s by subsequent application of 5-HT, but did not occlude modulation of Ik,v by 5-HT. Thus, application of the cAMP analogues mimicked the action of 5-HT on Ik,s, but did not mimic the action of 5-HT on Ik,v.

During current-clamp, sensory neurons were held at -45 mV, and spikes were elicited by short (3 ms, 5 nA) or long (1 s, 0.5 to 3 nA) depolarizing current pulses. The brief pulses produced single spikes that had an average duration of 7 ms. The long pulses usually produced brief bursts of no more than 5 spikes. Application of 5-HT broadened the spikes to an average duration of 23 ms and doubled the number of spikes produced during long pulses (also see Klein et al, 1986). Application of cAMP analogues produced similar increases in the number of spikes during long pulses, but only modestly broadened the spikes to an average duration of 9 ms. The cAMP analogues occluded further increases in spike number during subsequent 5-HT application, but did not occlude 5-HT induced spike broadening.

These results suggest that in pleural sensory neurons only one of the two currents modulated by 5-HT is sensitive to elevated intracellular levels of cAMP. This current (Ik,s) appears to be critical for membrane excitability, with modest effects on spike duration. In contrast, modulation of Ik,v by 5-HT dramatically broadened the spike. This action of 5-HT may require an as yet unidentified second messenger system. Supported by AFOSR grant 84-0213.

401.4 MYOMODULIN, A NOVEL NEUROPEPTIDE, MODULATES ACTION POTENTIALS IN PLEURAL SENSORY NEURONS OF APLYSIA. L.J. Cleary, D.A. Baxter and J.H. Byrne. Department of Neurobiology and Anatomy, The University of Texas Medical School, Houston, TX 77225.

Myomodulin is a neuropeptide that has recently been purified from buccal muscle of Aplysia (Weiss et al, this volume). This peptide contributes to arousal of feeding behavior by potentiating muscle contractions evoked by motor neurons. Because other transmitters that modulate feeding behavior also modulate defensive behaviors, we were interested in determining whether myomodulin alters the properties of neurons mediating the tail withdrawal reflex. We therefore examined the effects of myomodulin on the action potential produced by tail sensory neurons in the pleural ganglion.

Clusters of pleural sensory neurons were isolated, and myomodulin (12.5-165 uM final concentration) was applied to the bath. In artificial sea water (ASW), there was no apparent change in the duration of action potentials produced by brief depolarizing current pulses. Myomodulin could, however, narrow action potentials that were first broadened by serotonin (5-HT) or tetraethylammonium (TEA). 5-HT (50 uM) increased the average duration of action potentials from 7 to 20 msec. When myomodulin was subsequently applied, the average duration was reduced to 15 msec. Similarly, 10 mM TEA broadened the action potential from an average of 6 to 12 msec, and myomodulin narrowed it back to 10 msec. Myomodulin also decreased membrane excitability, but this effect was not apparent in ASW alone. When 50 uM 5-HT or 10 mM TEA was added to the bath, the number of spikes produced in response to long (1 sec) depolarizing current pulses was increased. Application of myomodulin reduced the number of spikes to control levels. These effects are similar to those produced by another modulatory peptide, FMRFamide.

The effect of myomodulin on synaptic potentials was examined by eliciting action potentials in sensory neurons and monitoring the monosynaptic EPSPs in tail motor neurons. On average, application of myomodulin does not affect the amplitude of the EPSP. However, preliminary results indicate that it reduces the facilitation produced by subsequent application of 5-HT.

While we do not yet know if myodulin is, like FMRFamide, present in the pleural and pedal ganglia, the close similarity between the effects of these two peptides is intriguing. We suggest that they modulate sensory neuron activity by a "silent depression". The effects of myomodulin or FMRFamide on the pleural sensory neurons seem most prominent when membrane conductances are first modulated by transmitters such as 5-HT. This suggests that the neural circuits utilizing these peptides have a function antagonistic to that of facilitatory circuits.

401.5 INVOLVEMENT OF G PROTEINS IN THE INHIBITORY ACTION OF FMRFAMIDE THROUGH LIPOXYGENASE METABOLITES OF ARACHIDONIC ACID IN APLYSIA SENSORY NEURONS. A. Volterra, J.D. Sweatt* and S.A. Siegelbaum. HHMI, Ctr. for Neurobiol. & Behav., Dept. of Pharmacol., Columbia Univ., NY, NY 10032.

The molluscan neuropeptide FMRFamide hyperpolarizes Aplysia sensory neurons as a result of an increase in K conductance due to an increase in the open probability of the resting S K channels (Belardetti et al., Nature 325:153, 1987). This effect of FMRFamide is mediated by the lipoxygenase metabolites of arachidonic acid (Piomelli et al., submitted). Here, we investigate the possible role of GTP-dependent processes in this cascade by studying the effects of intracellular injection of GTP-γ-S, an irreversible activator of G proteins, in Aplysia sensory neurons.

Membrane potentials were recorded from sensory neurons of Aplysia abdominal ganglia using microelectrodes filled with 0.5 M KCl (resistance of 20-50 megohms) with or without addition of 25 mM GTP-γ-S. In the absence of GTP-γ-S, brief pressure application of FMRFamide (from a wide-mouthed pipette containing 10 μM peptide) onto the sensory neurons produced a transient hyperpolarization of 4.56 ± 2.55 mV (mean + S.D., n = 7) associated with an increase in membrane conductance that lasted for 1-2 min. After impalement of a sensory neuron with a GTP-γ-S containing microelectrode, a similar application of FMRFamide produced a hyperpolarization of 5.75 ± 2.21 mV (n = 12) that was now largely irreversible. Upon subsequent iontophoresis of GTP-γ-S into the cell (using 0.5 sec hyperpolarizing current pulses of 0.1 -0.3 nA, 1 Hz), the cell membrane potential further hyperpolarized irreversibly by 5.83 ± 2.76 mV over 5-15 min with a large increase in resting conductance. These effects of GTP-γ-S were blocked by about 85% in presence of 50 μM NDGA, a lipoxygenase inhibitor (n = 8). Current injections from a microelectrode lacking nucleotide had no effect.

Reapplication of FMRFamide after injection of GTP-γ-S produced little or no further hyperpolarization, suggesting that the FMRFamide cascade was already fully activated. The normal depolarizing response of the sensory neuron to serotonin was also blocked at this time. Serotonin normally activates adenylate cyclase through a G protein and leads to S channel closure via cAMP-dependent phosphorylation. Thus, the net effect of G protein activation via GTP-γ-S appears to be the opening of S channels (leading to the increase in membrane conductance and hyperpolarization). This result suggests that the activation of the FMRFamide cascade antagonizes the excitatory action of 5-HT, in agreement with Belardetti et al.

G proteins thus appear to play an important role in mediating the inhibitory action of FMRFamide in Aplysia sensory neurons (see also Brezina and Eckert, Soc. Neurosci. Abstr. 12:1341, 1986). Since blockade of the lipoxygenase pathway of the arachidonic acid cascade with NDGA inhibits the response to GTP-γ-S, a likely role for a G protein lies in the receptor-mediated release of arachidonic acid metabolites.

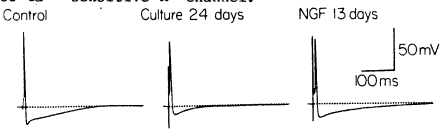
401.6 SUPPRESSION OF IA AND BACKGROUND K+ CURRENT IN HERMISSENDA NEURONS BY cAMP, 5-HT, THE NEUROPEPTIDE SCPb AND INJECTION OF EXOGENOUS Ca++/CALMODULIN DEPENDENT KINASES. Juan Acosta-Urquidi. Friday Harbor Labs, Univ. Washington, Friday Harbor, WA 98250.

Voltage-clamp studies of a set of identified Hermissenda neurons revealed two species of IA, based on different kinetics and voltage range of activation-inactivation. Pedal cells (LP1-3) with short duration spikes (3-6 ms at half-amplitude), little broadening and accomodation to sustained depolarization, have a "fast" IA (IAf). IAf peaks in<10ms, has tauoff ca. 30-50 ms, activates ca. -40 mV and is half-maximally inactivated at ca. -65 mV, at 18°C. Left pleural cells (LP1tc, LP1c and LP11) have slow spikes (10-20 ms) with a shoulder on the falling phase which show pronounced broadening and rapid accomodation to depolarization. These cells have a "slow" IA (IAs), which peaks ca. 20-30 ms, has tauoff ca. 200-300 ms, and activation-inactivation curve shifted more positive (activation at ca. -35 to -20 mV and half-maximal inactivation at ca. -40 mV). Both IAf&s are blocked by 5mM 4-AP. IAs has kinetics of decay that closely match IK(v) decay, but IK(v) is resistant to 4-AP and is blocked by 100mM TEA which does not suppress IAs.

The following compounds all reduced peak IAf&s amplitude and the steady-state (s-s) component of outward current: 8-BrcAMP (25 uM), Forskolin (25 uM), Ro-20-1724 (25 uM), SCPb (10 uM) and 5-HT (10 uM). The s-s component corresponds to IR, a "background" residual K+ current sensitive to 5-HT and cAMP (Cell. Molec. Neurobiol. 5: 407-412; Soc. Neurosci. Abstr. 11:788,1985) that resembles the S K+ current. IR is linear and voltage-insensitive in the range -30 to -160 mV. At Em more positive than -30 mV, IR shows moderate voltage-dependence. IR is Ba++ and TEA-resistant, is slightly reduced by Cd++, 4-AP and EGTA injection, and is blocked by internal Cs+. IR is probably carried mostly by K+ ions since it reverses at ca. -65 mV and shifts more positive in high external K+ in agreement with the Nernst equation. Iontophoretic injection of exogenous Ca++/calmodulin kinases: Phosphorylase kinase (Phk) and type II CaM kinase also suppressed IAf&s (Soc.Neurosci. Abstr.11: 788,1985) and IR. These data suggest that activation of cAMP and Ca++/CaM second messenger pathways, interact or converge at some stage to effect phosphorylation of a common substrate which modulates the same two distinct K+ channels.

401.7 NERVE GROWTH FACTOR RESTORES ACTION POTENTIAL AFTERHYPERPOLARIZATION BUT NOT SPIKE WIDTH IN EXPLANT CULTURES OF BULLFROG SYMPATHETIC GANGLIA. P. Traynor*, W.F. Dryden and P.A. Smith. Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

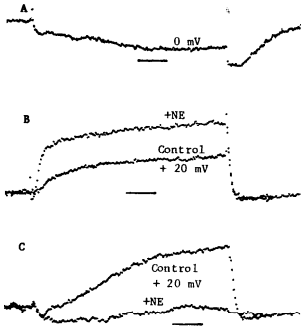
Transection of the axon (axotomy) of a peripheral vertebrate neurone promotes biochemical, morphological and electrophysiological changes in the cell body. These changes may be related to the neurone's ability to regenerate an axon. Axotomy of B-cells in bullfrog sympathetic ganglia results in an increase in action potential (a.p.) duration (spike width) and a reduction in the amplitude and duration of the afterhyperpolarization (a.h.p.) which follows the a.p. (Kelly et al., Neurosci. Letts., 67: 163, 1986). One explanation for this effect may be that axotomy prevents the retrograde axonal transport of a trophic substance from the periphery. In the case of a sympathetic neurone, such a trophic substance could be nerve growth factor (NGF, Levi-Montalcini, Prog. Brain. Res. 45: 235, 1976). To test whether loss of NGF was responsible for axotomy-induced electrophysiological changes, we studied the electrophysiological properties of (axotomized) bullfrog sympathetic neurones growing in explant culture (Groul et al., Brain Res. 233: 81, 1981) and examined the effect of NGF. Standard current clamp microelectrode techniques were used to measure electrophysiological characteristics of cultured neurones. A.p.s were generated by injection of brief, depolarizing current pulses. As the neurones regenerated in culture, there was a significant increase in spike width and a decrease in the duration and amplitude of the a.h.p. These changes were similar to those previously seen in axotomized neurones (Kelly et al., Neurosci. Letts., 67: 163, 1986). When 2.5 S NGF (50 ng/ml) was included in the culture medium, the duration and amplitude of the a.h.p. were restored towards control values, whereas the spike width was further increased. These results support the hypothesis that part of the electrophysiological response to axotomy (attenuation of a.h.p.) results from loss of a retrograde supply of NGF. Furthermore, since different Ca^{2+} sensitive K^+ channels contribute to a.p. repolarization and to a.h.p. generation (MacDermott and Weight, Nature 300: 185, 1982); Pennefather et al., Proc. Natl. Acad. Sci. USA 82: 3040, 1985), it is possible that NGF has different effects on each type of Ca^{2+} sensitive K^+ channel.



Supported by AHFMR and the MRC of Canada.

401.8 Ca^{2+} AND K^+ CURRENTS IN UTERINE SMOOTH MUSCLE. DIFFERENTIAL EFFECT OF NOREPINEPHRINE ON TWO K^+ CURRENTS. L. Toro*, E. Stefani and S. Erulkar. Dept. Physiology. Centro de Investigación y de Estudios Avanzados del IPN. Apdo. Postal 14-740. México, D.F. 07000. Dept. Pharmacology and Mahoney Inst. Neuroscience. Univ. Pennsylvania. Philadelphia, PA. 19104.

Single isolated cells from the uterus of Wistar rats (200 g) were patch clamped, using the whole cell configuration. Cells were used up to 5 days after culture. Their dimensions varied from 50 to 200 μm in length, and from 7 to 13 μm width. When the cells were stimulated from a holding potential, V_H, of -90 mV to more positive potentials than -50 mV, inward and outward currents were elicited. K^+ currents were recorded in normal Krebs and with an intracellular solution containing (mM): 150 AspO-K, 1 $MgCl_2$, 2.5 $CaCl_2$, 10 HEPES-K, 5 EGTA-K_2 and 5 ATP-Na_2. Two types of voltage dependent K^+ currents were observed: an early one, I_{K-f} with a time to peak, t_p of about 3 ms (at +20 mV) and a slow K^+ current, I_{K-s}, with a t_p=35 ms (at +20 mV). Norepinephrine (NE), 1 μM, had a distinct effect on these currents. I_{K-f} was enhanced about 4 times, while I_{K-s} was greatly diminished. Ca^{2+} currents, I_{Ca}, were recorded with an external solution containing (mM): 120 TEA$(CH_3SO_3)_2$, 5 HEPES-TEA, 1 3,4-diaminopyridine, 65 sucrose; and a pipette solution where K^+ was substituted by Cs^+ and 20 mM TEA. Two calcium currents were observed: fast I_{Ca} had a t_p=2 ms (at 0 mV) and an activation potential, V_a=-70 mV; slow I_{Ca} had a t_p=20 ms (at 0 mV) and a V_a=-20 mV. These results show that channels at the plasma membrane of myometrial cells can be modulated by NE in a different way. NE is capable to suppress a K^+ current, as other neurotransmitters, like substance P and acetylcholine. A: Ca^{2+} currents. Bar=40 pA, 5 ms. B: Enhancement I_{K-f}. Bar=100 pA, 5 ms. C: Suppression I_{K-s}. Bar=100 pA, 5 ms.



Supported by grants NS1221 1, 5ROI-AR 35085-03 (NIH, USA) and ICCBXNA-020848 (CONACyT, MEXICO).

401.9 ESTROGEN REGULATION OF UTERINE mRNA THAT INDUCES POTASSIUM CHANNELS IN XENOPUS OOCYTES. M.B. Boyle, N.J. MacLusky, F. Naftolin, and L.K. Kaczmarek, Depts. of Pharmacology, Physiology, and Obstetrics and Gynecology, Yale Univ. Sch. Med., New Haven, CT 06510.

The electrical excitability of the smooth muscle of the mammalian uterus varies dramatically during adult life, depending upon the hormonal status of the animal. Administration of steroid hormones to ovariectomized animals can be used to mimic, at least in part, the changes in excitability occurring during pregnancy. In particular, estrogen treatment leads to a dramatic increase in the excitability of this smooth muscle.

We used the Xenopus oocyte translation system to express mRNA coding for potassium channels from the uterus. Previous work has shown that an unusual potassium current is expressed in oocytes injected with RNA from the uteri of ovariectomized rats treated for a few days with estrogen. This current takes many seconds to activate during depolarizations.

We have now compared the ability of mRNA prepared from mid-gestational and term pregnant, as well as from estrogen- and non-estrogen-treated uteri, to induce this current. Xenopus oocytes were injected with polyA+ RNA (200 ng), incubated for at least 3 days, and then voltage-clamped using 2 microelectrodes. The expression of the slowly activating potassium current was quantitated by measuring the amplitude of the slowly decaying outward tail currents measured at -40 mV following a pulse of several seconds to +70 mV in Na- and Ca-free saline. Control noninjected oocytes do not show slow tail currents resembling those in uterine-RNA-injected oocytes. The outward tail currents in oocytes expressing the slowly activating current from estrogen-treated uterine RNA ranged from 40 to 660 nA. No expression of the slowly activating current has been detected in any oocytes (n=25 oocytes, 5 frogs, 2 RNA samples) injected with polyA+ RNA from uteri of rats not treated with estrogen, although the current was expressed in oocytes from each of the same frogs injected with RNA from the estrogen-treated uteri. Oocytes injected with RNA from term (21-day, before parturition) uterus expressed the slowly activating current (n=10 out of 18 oocytes from 4 frogs, 2 RNA samples), although only at about 5% to 20% of the levels found in oocytes injected with RNA from estrogen-treated uteri. No expression of the slowly activating current has been seen in oocytes injected with RNA from uteri at 15 days of pregnancy (n=9 oocytes from 3 frogs, 1 RNA sample).

These findings support the hypothesis that estrogen regulates the concentration of the messenger RNA coding for this ion channel in the rat uterus. The current is expressed in oocytes injected with RNA, either from estrogen-treated uteri or from term pregnant uteri, which are largely under the influence of estrogen. In contrast, no expression of the slowly activating K current has been seen in oocytes injected with RNA from uteri of ovariectomized rats not given estrogen or from uteri at mid-pregnancy, which are mainly under the influence of progesterone. These findings suggest that regulation of the concentration of mRNA species coding for ion channels may play a physiological role in the changes in electrical excitability occurring during pregnancy.

401.10 ON THE IONIC MECHANISMS OF α_2-ADRENOCEPTOR MEDIATED PRESYNAPTIC INHIBITION IN GUINEA-PIG SUBMUCOUS PLEXUS NEURONES. A.Surprenant, Vollum Institute, Oregon Health Sciences Univ. Portland, OR. 97201.

Cholinergic neurones that have their cell bodies in the submucous plexus send processes which make synaptic connections with other cells in the plexus; the amount of acetylcholine (ACh) released at these synapses can be measured by recording the amplitude of the fast excitatory synaptic potential (epsp). Noradrenaline (NA) reduces the epsp, suggesting a presynaptic inhibition of ACh release. NA also increases an inwardly rectifying K^+ conductance (gK) by activating postsynaptic α_2 receptors; such an action on the cholinergic nerve fibres might underlie the presynaptic inhibition. The present study was undertaken to characterize the receptor involved in presynaptic inhibition of ACh release and to determine whether other postsynaptic actions of NA (i.e. calcium conductance decrease) might be due to α_2-adrenoceptor activation. All methods were as described in detail previously (Surprenant & Williams, J.Physiol., 1987).

NA, UK 14304 and clonidine caused a dose-dependent depression of the epsp; EC_{50} values (200 nM, 30 nM and 10 nM respectively) for this presynaptic inhibition were the same as EC_{50} values for agonist-induced hyperpolarizations or outward currents recorded from these neurones. Antagonist dissociation equilibrium constants (K_d) for idazoxan (25 nM) and phentolamine (100 nM) in preventing agonist-induced inhibition of the epsp were also the same as those obtained for the α_2-mediated gK increase. Experiments in which brief (0.5 - 5 ms) pressure (or ionophoretic) pulses of NA were applied showed that the time course of inhibition of the epsp mirrored that of the gK increase with a minimum latency of onset being 75 - 100 ms. These results indicate that NA-induced presynaptic inhibition of ACh release in sumbucous neurones is due to α_2-adrenoceptor activation and that it is not distinguishable from that causing gK increase.

Membrane currents in response to step depolarizations from -50 to -20 mV were recorded in the presence of TTX, TEA replacing Na^+ or with CsCl-filled intracellular electrodes plus external CsCl replacing KCl. Currents recorded under these conditions were an inward calcium current and a calcium-activated outward current. These manipulations abolished NA-induced outward current at -50 mV. NA, UK14304 and clonidine decreased the inward current; agonist EC_{50} values were 10 to 50-fold higher than for agonist-induced gK increase/presynaptic inhibition but idazoxan K_d determinations showed that this action was also mediated by α_2-adrenoceptors.

401.11 VOLTAGE AND TRANSMITTER GATED CHANNELS IN PURKINJE CELLS FROM ORGANOTYPIC CULTURED SLICES. I.Llano *, B.H. Gähwiler and A. Marty * (Spon:J. Bruner). Ecole Normale Supérieure, Paris, France and Sandoz Laboratory, Basel, Switzerland.

We have applied patch-clamp recording techniques to the study of voltage and transmitter activated channels from Purkinje cells in organotypic cultured slices of newborn rat cerebellum (Gahwiler, B.H., J. Neurosc. Meth. , 4:329:342). GΩ-seals were reliably formed without previous enzymatic treatment. In whole-cell recording mode (WCR) cells dialysed with K saline had resting potentials of -60mV and displayed spontaneous synaptic activity as well as action potentials similar to those recorded in in vitro slices. Under voltage-clamp, depolarizing pulses elicited large TTX-sensitive inactivating inward currents with fast on-kinetics, as well as large outward relaxations. Cs dialysis revealed TTX-insensitive inward currents with slower time course and incomplete inactivation. Whole cell voltage-clamp recordings showed evidence of lack of spatial control.

In contrast to the large inward currents observed in WCR, most outside-out patches (OOP) from cell somata had no inward currents. In some cases, a small inward current could be discerned in the absence of TTX. The channels giving rise to this current opened with short latency after pulse onset, had brief open times and γ of 14 pS. However, OOP displayed large voltage-dependent outward currents with an activation threshold of -30 mV (at -10 mV, peak current: 100-300 pA, time to peak: 3-4 ms). For depolarizations greater than -10 mV, the outward relaxations decayed to approx. 50% of their peak value during 40 ms. At least two types of K channels were identified from single channel recordings, with γ of 25 and 90 pS.

The responses to excitatory amino acids and GABA were studied in both WCR and OOP. Under symetrical Cl and cationic gradients, the application (via a fast perfusion system) of GABA (2 μM), led to large current responses (-0.5 to -1.8 nA at -60 mV) which were blocked by bicuculline (10 μM). The glutamate agonists quisqualate (2 μM) and kainate (10 μM) elicited currents of similar size. N-methyl-D-aspartate (100 μM) was ineffective, even in Mg-free saline and in the presence of glycine (10 μM).

In OOP from somatic membrane, application of GABA (0.5-2 μM) produced large currents (-100 to -300 pA at -60 mV). In contrast, quisqualate and kainate at concentrations which elicited large currents in WCR, led to none or very small (less than 5 pA) responses in OOP, suggesting that the current observed in WCR comes from dendritic membrane.

These results indicate that, in Purkinje cells, voltage-gated and transmitter-activated channels are spatially distributed in a highly differentiated manner.

401.12 REGIONAL DISTRIBUTION OF VOLTAGE-DEPENDENT SINGLE CHANNEL CONDUCTANCES IN CULTURED HIPPOCAMPAL NEURONS FROM THE RAT. Leona M. Masukawa and Anker Hansen*, Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

A previous study demonstrated that pyramidal neurons of the hippocampus possess distinct types of electrical responses that are different in cell bodies when compared to dendrites (Benardo, Masukawa and Prince, J. Neurosc., 2:1614, 1982). These specific ionic conductances function to control the activity of the different regions of the neuron. To study further the distribution of ionic conductances in different regions of hippocampal neurons, we have used the patch clamp technique. Cell attached and inside-out patches were examined for the presence of specific voltage-dependent single channel currents. Gigaohm seals were formed on cell bodies and dendrites of cultured hippocampal neurons dissociated from 1 day old rats. Cells were examined 2-15 days after plating.

Na single channel events which rapidly inactivated were observed only on cell bodies. They were activated by a step depolarization of 30 mV after a hyperpolarization of 60 mV in the cell attached configuration. The single channel events remained open for a period of 2-3 msec and then were inactivated. The conductance was approximately 20 pS and the channel was never observed when the pipette solution contained TTX or was Na free. The ensemble average of single channel events to a step depolarization produced a macroscopic current that had a similar time course to that of the inward Na current of the fast action potential.

Several types of K currents were observed. The most well defined of these was the outward delayed rectifier. This current was observed in both cell body and dendritic patches, and had similar characteristics in both areas. The single channel conductance was 10 pS and the channel opened at 20 mV depolarization. It did not inactivate during the 120 msec depolarization step. From ensemble averages of many traces the macroscopic current showed that there was a 5 msec delay before the current reached its peak amplitude and consequently resembled in time course a whole cell delayed rectifier current.

Both Ca and Na single channels that did not inactivate during a 120 ms depolarizing step were seen in both cell body and dendritic membrane patches. Their conductances were between 15 to 25 pS.

During the time period of 2-15 days in culture, dendritic patches did not show rapidly inactivating inward currents that would be consistent with Na and Ca action potential currents. It is possible after longer times in culture, that these channels will be incorporated into the dendritic membrane. At the time period studied here, the dendritic processes are in a state of development and rapid turnover of membrane as arborizations are extended. At the time of insertion of action potential channels into dendritic membrane the necessary K channels will be in place to regulate the inward currents. Supported by NIH grant NS 23077 (LMM) and NSF grant BNS 8519610 (LMM).

401.13 LOOSE-PATCH MAPPING OF ION CHANNEL DISTRIBUTIONS IN CULTURED LEECH NEURONS. R.J. Bookman*, H. Reuter*, J.G. Nicholls and W.B. Adams. Dept. of Pharmacology, Biocenter, CH-4056 Basel, Switzerland.

The excitable properties of axons, nerve terminals, and growth cones can often be infered only from measurements made at the cell body. We have used the loose-patch technique to measure directly qualitative and quantitative regional differences in voltage-dependent ion channels on single identified leech neurons. Retzius cells were removed from the ganglion with a 100-500μm length of stump (5-20μm in diam.), plated on a ConA substrate and studied after 1-8 days in culture. Low resistance (300-1000kohm) pipettes (filled with SES = 115NaCl, 5KCl, 5$CaCl_2$, 10HEPES, pH7.4) were sealed repetitively onto different regions of the cell surface to map the local currents. Seal resistances were 1-10Mohm. The density of Na channels was greatest at the tip of the stump where the neurites emerged and decreased sharply towards the cell body. Most somatic locations showed no inward current. Outward K currents showed a similar pattern of decline away from the stump tip although the decrease in density was not as steep. Pressure sensory (P) neurons differed in that clear Na currents were recorded from the cell body. Recording in a Ba-TEA solution, currents through Ca channels have been measured using Ba tail currents. These experiments set the stage for a mapping of currents in pairs of co-cultured cells that have formed synaptic connections.

Supported by grants from the Swiss Nationalfond and the U.S. Navy.

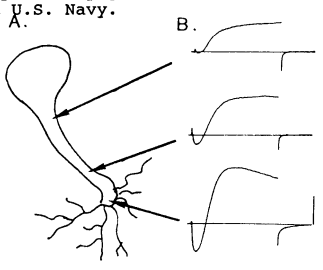


Fig. 1. A. Drawing of a Retzius cell in culture. B. Currents recorded from three locations on the cell. The pipette contained SES solution. The patch was depolarized by a 10ms pulse of -100mV applied to the inside of the pipette. Scale = 5nA.

402.1 SOMATOSTATIN (SS) AUGMENTS THE M-CURRENT IN HIPPOCAMPAL CA1 NEURONS. S.D. Moore*, S.G. Madamba*, M. Joëls*, and G.R. Siggins. Division of Preclinical Neuroscience, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Recent immunocytochemical evidence indicates the presence of abundant SS-containing somata and nerve fibers in the hippocampal formation. Previous studies in our laboratory suggest that SS acts as an inhibitory transmitter in the hippocampus and also interacts with other neurotransmitters, including acetylcholine (ACh). To investigate the mechanisms of these interactions, we applied current- and single electrode voltage-clamp methods to CA1 pyramidal neurons in vitro. Rat hippocampal slices were prepared for electrophysiology in the conventional manner, completely submerged and continuously superfused with artifical CSF. Intracellular recordings were made in pyramidal neurons using 3M KCl-filled micropipettes (60-80 Mohm). In some voltage recordings, SS-induced hyperpolarizations were verified. After obtaining a stable current-clamp recording (at least 10-30 min), TTX (0.5-1.0 μM) was added to the perfusate. In discontinuous voltage-clamp mode (Axoclamp amplifier; switching frequency: 3-5 KHz) the M-current was recorded as a slow inward relaxation during small hyperpolarizing command pulses (5-25 mV; 0.7-1 sec) from a depolarized holding potential (-40 to -45 mV). The peak M-current was measured as the difference between the instantaneous and steady state currents, the former determined by exponentially extrapolating the current relaxation back to command onset. The M-current was characterized on the basis of size and kinetics, voltage and K^+ sensitivity, inactivation by muscarine or barium ions, and insensitivity to extracellular CsCl (Halliwell and Adams, Brain Res. 250: 71, 1982).

In 6 of 9 cells, superfusion of SS-14 or SS-28 (0.5-1.0 μM) increased the amplitude of the M-current ($\bar{x}$=160%, range = 15-480%). These concentrations of SS also produced a steady outward holding current but had no apparent effect on other K^+ mediated currents such as the A-,Q- or AHP- currents. Muscarine (25-40 μM) or carbachol (50 μM) consistently blocked the M-current even in the presence of SS. To our knowledge this is the first demonstration of a putative transmitter-mediated facilitation of the M-current and thus of a unique reciprocal regulation of such a conductance by two different transmitter candidates, SS and ACh. Furthermore, the M-current augmentation might explain the SS-induced potentiation of cholinergic excitations seen in vivo (Mancillas et al, PNAS (USA) 83: 7518, 1986): in the presence of SS more M-channels are open for ACh to act upon. Supported by NIAAA (AA-06420, AA-07456), NIADDKD (AM-26741), and the C & C Huygens-Stipendium from the Dutch Z.W.O. (H88-145).

402.2 SOMATOSTATIN DEPRESSES NEURONAL EXCITABILITY IN THE SOLITARY TRACT COMPLEX (STC) VIA HYPERPOLARIZATION AND AUGMENTATION OF THE M-CURRENT. G.R. Siggins[1], J. Champagnat*, T. Jacquin* and M. Denavit-Saubie. (SPON: L. Koda). Lab. Physiol. Nerv., CNRS, Gif-sur-Yvette, France and [1]Div. Preclin. Neurosci., Research Institute Scripps Clinic, La Jolla, CA 92037.

The function of somatostatin- (SS) containing fibers in the extrahypothalamic nervous system is controversial. The complex of nuclei (STC) comprising the nucleus tractus solitarius and the n. dorsal motor vagus contains a profuse network of fibers and cell bodies immunoreactive for SS (Kalia et al, J.Comp.Neurol. 222: 409, 1984). Several fragments of the pro-somatostatin derived prohormone, including SS28, a precursor to SS14, and the N terminal fragment, SS28(1-12), also appear to co-exist in this STC network (L. Koda, unpublished). Therefore we used a slice preparation of the rat brain stem, prepared as described by Champagnat et al (Brain Res. 280: 155 and 325: 49), and current- and single electrode voltage-clamp recording to test the effects of pro-somatostatin-derived peptides on neurons of the STC. The slices were completely submerged and continuously superfused with artifical CSF. In extracellular and intracellular recordings superfusion of either SS14 or SS28, but not SS28(1-12) or SS28(1-10), inhibited spontaneous spike and subthreshold activity in 69% of 26 neurons tested. In intracellular recordings of 29 neurons, SS28 and SS14 hyperpolarized about 65% of the neurons, in association with a slight but reproducible decrease (8-25%) in input resistance. These responses were slow in onset and recovery. Response thresholds were about 0.2 μM with both SS14 and SS28. An increase in K^+ conductance may be the basis of these hyperpolarizations as they were augmented in depolarized cells and persisted in cells in which spontaneous inhibitory postsynaptic potentials became depolarizing after Cl^- injection. Under voltage-clamp, SS28 and SS14 induced a steady outward current of about 50-100 pA and augmented the voltage dependent, non-inactivating outward conductance (I_M) shown in previous studies on the STC (Champagnat et al., Pfluger's Arch 406: 372, 1986) to be blocked by activation of muscarinic cholinergic receptors. These results suggest: 1) that both SS14 and SS28 should be considered as candidates for novel inhibitory transmitters in the STC; 2) that SS-containing elements in the STC exert an inhibitory role through the activation of postsynaptic permeability to potassium ions, and 3) a novel reciprocal control of the same ion channel (M) by two different neurotransmitter candidates, SS and ACh. Supported by grants from the USPHS (AM-26741 and AA-06420), Fondation de l'Industrie Pharmaceutique pour la Recherche, and the Fondation pour la Recherche Medicale.

402.3 ACTIONS OF SUBSTANCE P AND CARBACHOL ON NIE-115 NEUROBLASTOMA CELLS M.A.Schumann*, J.Whitbread*, J.Prives, and I.Spector. Dept. of Anatomy, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

The actions of substance P (SP) carbachol (CCh), cholecystokinin octapeptide (nonsulfated) (CCK), bradykinin and vasoactive intestinal polypeptide (VIP) on differentiated cells of the mouse neuroblastoma clone NIE-115 were examined with intracellular microelectrodes. The N1E-115 cells used were grown in the presence of 2% dimethylsulfoxide (DMSO) to induce morphological and electrophysiological differentiation. Among the compounds tested, only SP and CCh showed a consistent effect on the N1E-115 cells. Bath application of SP (0.02 to 0.05mM) induced a prolonged hyperpolarization with an amplitude averaging 5.9±1.6 mV (30 out of 35 cells tested). The hyperpolarization was associated with an increase in cell membrane input resistance (17.8±8.1%) in 77% of the responsive cells and lasted for 21.1±11.1 sec. Application of carbachol (CCh) (0.1 to 0.25mM) also caused a prolonged hyperpolarization with an associated increase in membrane input resistance. The decreased membrane conductance elicited by both SP and CCh suggests closure of ion channels that may contribute to membrane depolarization at the resting state. The direction of the response (hyperpolarization) further suggests that SP and CCh do not affect potassium currents. These actions are thus very different from the recently described effects of bradykinin on potassium channels in the NG108-15 neuroblastoma-glioma hybrid cell line (Higashida & Brown, Nature, 323:333, 1986).

To determine if the SP-induced membrane conductance change is controlled by the intracellular messengers produced by the hydrolysis of the membrane inositol lipid, PLP_2,we monitored the effects of SP on the incorporation of ^{3}H-myo-inositol into inositol phosphates in DMSO-differentiated N1E-115 cultures. We found that in the presence of SP there is an increase in labeled inositol phosphates (IP_1,IP_2, and IP_3) suggesting that at least one of second messengers (IP_3) may have a role in the membrane conductance changes induced by SP. This work was supported by NIH grant NS22028

402.4 WHOLE-CELL AND CURRENT-CLAMP RECORDINGS REVEAL DIRECT AND INDIRECT EFFECTS OF RECEPTOR-SELECTIVE NEUROKININ ANALOGUES ON THE ACTIVITY OF SPINAL CORD NEURONES IN CULTURE. M. Wienrich*, J. DePeyer*, K. Reuss*, J. Harting*, G. Häusler* and A. Haase. E. Merck, Pharmaceutical Research, Frankfurter Str. 250, D-6100 Darmstadt, FRG.

The presence of three neurokinins, Substance P (SP) and Neurokinins A (NKA) and B (NKB) has been shown in the spinal terminals of primary afferents and in interneurones of the mammalian spinal cord. They have been proposed as neuromediators/neuromodulators of nociception at the spinal level. Moreover, in peripheral organ systems, three distinct neurokinin receptors have been identified (NK-1, NK-2 and NK-3). Since none of the endogenous agonists is selective at these sites, neurokinin analogues with high preference for a given receptor have been developed. The analogues Pro^9-C6 (pGlu^6, Pro^9SP6-11) and DiMe-C7 (pGlu^5, $MePhe^8$, $MeGly^9$ SP5-11) have been shown to be highly selective for the NK-1 and NK-3 receptor, respectively. We have investigated both direct and indirect effects of these neurokinin analogues on membrane potential, electrical activity, and membrane currents of rat spinal cord neurones in culture.

Both Pro^9-C6 and DiMe-C7 induced membrane depolarizations accompanied by an increase in spike activity in some neurones. The depolarizing action of both peptides was maintained after blockade of synaptic transmission by tetrodotoxin (TTX). No change in membrane resistance was observed. Whole-cell recording revealed an inward current being the cause for the observed membrane depolarizations. This could have been due to an activation and inactivation of two distinct channels by neurokinins.

In other neurones, the neurokinin-induced increase in spike activity occurred without membrane depolarization. Whole-cell recording showed that this increase was essentially due to enhanced synaptic discharge. In the presence of TTX these responses were abolished.

These results provide evidence for direct as well as indirect effects of neurokinins on spinal cord neurones in culture.

402.5 A NOVEL FORM OF LONG-TERM POTENTIATION OF EXCITATORY AMINO ACID TRANSMISSION IN LATERAL SEPTUM IN VIVO, ELICITED BY VASOPRESSIN AND BRAIN STIMULATION. I.J.A. Urban, P. van den Hooff and A. Ontskul. (SPONS. ENA). Rudolf Magnus Institute for Pharmacology, University of Utrecht, The Netherlands.

Electrical stimulation of fimbria-fornix (fi-fx) fibers elicits in lateral septum (LS) of rats large negative field potentials (NFPs), and induces in LS neurons in vitro excitatory postsynaptic potentials (EPSPs). Both, the NFPs and EPSPs are presumably mediated by excitatory amino acid (EAA) released from the fi-fx fibers. LS is innervated a.o. by a brain VP system that originates from neurosecretory neurons in the bed nucleus of stria terminalis (BNST) and projects to the LS via the medial forebrain bundle and the medial septum (MS).

Exogenous VP, added in 10^{-12} M concentration to the medium superfusing LS neurons in vitro, increased for many minutes the EPSPs elicited by fi-fx stimulation in the neurons. A VP-induced increase in the magnitude of EPSPs was also seen when inhibitory postsynaptic potentials that closely follow the EPSPs, were suppressed with picrotoxin. In series of experiments in vivo we used the NFPs as a measure of EAA transmission on LS neurons and examined the effect of the BNST and MS stimulation on the transmission. A three min. long stimulation with 30 sec long trains of stimuli at 8 Hz alternated with 30 sec rest periods, markedly increased the NFPS in 8 of 15 Wistar rats thus stimulated in the MS, and in 7 out of 14 Wistar rats stimulated in the BNST. The increase in NFPs elicited by the stimulation attained on average 18% of the NFPs amplitude measured prior to the stimulation, and lasted for more than 3 hrs following the stimulation. In the remaining rats, the NFPs did not changes or decreased following the stimulation. None of the 12 Brattleboro rats homozygous for a genetic deffect in synthesis of the brain and pituitary VP manifested by diabetes insipidus (HODI), showed an increase in NFPs following the MS stimulation. The litte mates heterozygous for the deffect, showed a mild increase in the NFPs, and homozygous normal Brattleboro rats exhibited a similar increase in the NFPs as did the Wistar rats. It is for the first time that a novel, long term potentiation of EAA transmission, elicited by electrical brain stimulation releasing presumably VP, is in vivo demonstrated.

402.6 **THE EFFECT OF THYROTROPIN-RELEASING HORMONE (TRH) ON SPINAL CORD NEURONS.** **M.M. Behbehani, R.Y.K. Pun and E.D. Means. Dept. of Physiology and Biophysics, and Dept. of Neurology, University of Cincinnati College of Medicine. Cincinnati, OH 45267-0576.**

There is considerable evidence that in addition to its involvement in hypothalamic function, TRH has a significant effect on other CNS neurons. Of particular interest is the observation that neurons in the rostral ventral medulla that have a direct projection to the spinal cord contain TRH and, in many instances TRH is co-localized with serotonin and with substance P. Since this pathway plays an important role in pain inhibition, it is possible that TRH plays a role in the transmission and modulation of pain. Currently the effect of TRH on the spinal cord and its mechanism of action is not clear. In experiments that are described, the effect of TRH on spinal cord neurons in vivo and in tissue culture was examined.

Male Sprague-Dawley rats were used for in vivo experiments. Animals were anesthetized with chloral hydrate and were not paralyzed. Single unit recordings were made from dorsal horn nociceptive and ventral horn neurons using a mutibarrel electrode containing recording,glutamic acid and TRH electrodes. TRH was applied using micropressure injection procedures. In tissue culture experiments, dorsal and ventral horn neurons were obtained from 12 to 14 day old mouse embryos. After dissociation, they were cultured separatel. Intracellular recordings were made from cultured cells two to eight weeks after plating using whole cell patch clamp techniques.

Application of TRH caused a significant increase in the firing rate of both ventral and dorsal horn neurons recorded in vivo. Usually application of TRH caused firing of silent neurons. In the majority of the neurons, the effect of TRH lasted for more than two minutes. There was no correlation between the response to TRH and response to peripheral stimulation.

The effect of TRH on dissociated spinal cord neurons was similar to its effect in the in vivo experiments. Application of TRH caused a short lasting hyperpolarization. This was then followed by a slight depolarization and a significant increase in the firing rate of the cell that lasted for a period as long as 20 minutes. The most striking finding in these experiments was the effect of TRH on synaptic potential. In TTX treated culture plates, application of 10 to 50 uM TRH produced a significant increase in synaptic activity as manifested by an increase in the frequency of post synaptic potentials.

In voltage clamp experiments, the outward current produced by step depolarization was affected by TRH. In 20% of the cells TRH caused an increase, and in 40% of the cells it caused a decrease, in the outward current. The increase in the outward current was frequently seen when ATP was included in the solution used to fill the patch electrode. The effect of TRH could be blocked by cesium,cobalt and barium, suggesting that it may be due to changes in potassium and/or a calcium activated potassium current.

It is concluded that TRH has an excitatory effect that is mediated through reduction of potassium currents. In addition, TRH has a considerable presynaptic effect that leads to significant increase in transmitter release.
Supported by PHS Grant NS 20643 and MDA.

402.7 HIPPOCAMPAL MOSSY FIBER DENERVATION INDUCES A SUPERSENSITIVITY TO CHOLECYSTOKININ OF CA_3 PYRAMIDAL NEURONS IN THE GUINEA PIG BUT NOT IN THE RAT. G. Debonnel and C. de Montigny. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Canada H3C 3J7.

Immunohistochemical studies have revealed the presence of cholecystokinin (CCK) in the guinea pig hippocampal mossy fiber projections (Gall, Brain Res., 306: 73, 1984), whereas CCK appears to be absent in these fibers in the rat (Greenwood et al., J. comp. Neurol., 203: 335, 1981). However, the rat mossy fiber system shows a strong opiate-like immunoreactivity which would consist mainly in methionine-enkephalin (mENK) and dynorphin (McGinty et al., P.N.A.S., 80: 589, 1983). The present electrophysiological studies were undertaken to determine the effect of mossy fiber denervation on the responsiveness of target pyramidal neurons to mENK, CCK and non-peptidic excitatory agents in these two species.

Colchicine, a selective neurotoxin for dentate gyrus granule cells (Goldschmidt et al., P.N.A.S., 77: 3047, 1980), was injected directly into the right dentate gyrus (20 µg in 5 µl in the rat and 1 µg in 3 µl in the guinea pig) under chloral hydrate anesthesia (400 mg/kg i.p.). Two to three weeks later, electrophysiological experiments were carried out under urethane anesthesia (1.25 g/kg i.p.). Five-barrelled glass micropipettes were used for extracellular unitary recording and microiontophoretic applications. The central barrel was filled with a 2 M NaCl solution and the side barrels with four of the following solutions: CCK 10 µM in 200 mM NaCl, pH: 5; mENK 0.5 mM in 200 mM NaCl, pH: 4; acetylcholine (ACh) 20 mM in 200 mM NaCl, pH: 4; quisqualate (QUIS) 1.5 or 3 mM in 400 mM NaCl, pH: 8; ibotenate (IBO) 100mM in 200 mM NaCl, pH: 8.

In both species, histological sections, prepared with a simplified Timm stain procedure for heavy metals (Sloviter, Brain Res. Bull., 8: 771, 1982), revealed a virtually complete destruction, by colchicine, of the dentate gyrus and its mossy fiber projection.

In the guinea pig, the mossy fiber denervation induced a 20-fold increase in the responsiveness of CA_3 pyramidal neurons to CCK, as compared to the intact side, but did not modify their responsiveness to mENK, QUIS, IBO and ACh.

In the rat, the mossy fiber denervation did not modify the responsiveness of CA_3 pyramidal neurons to CCK, mENK, QUIS, IBO and ACh.

These results provide novel electrophysiological evidence for the physiological role of CCK in the hippocampal mossy fiber projection of the guinea pig. The absence of supersensitivity to CCK in the rat is consistent with the lack of CCK-like immunoreactivity in the mossy fiber projection of this species. The failure of mossy fiber denervation to induce a supersensitivity to mENK in the rat might be due to the fact that such a lesion produces only a 50% decrease of the mENK content in the rat dorsal hippocampus (Grimes et al., Neurosci. Abst., 12: 116.13, 1986), the remaining mENK being presumably sufficient to maintain normosensitive postsynaptic opiate receptors.

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402.8 MECHANISM OF ACTION OF PERIPHERALLY ADMINISTERED CHOLECYSTOKININ OCTAPEPTIDE ON BRAINSTEM NEURONS IN THE RAT. H. Raybould and G.J. Dockray, Physiological Laboratory, University of Liverpool, U.K.

Peripheral administration of cholecystokinin octapeptide (CCK8) has been shown to influence the discharge of brainstem neurons responding to gastric distension; injection of CCK8 close-arterial to the stomach suggested a site of action within the splanchnic bed. We have investigated the pathway and the mechanism by which CCK-8 given peripherally may influence the discharge of brainstem neurons receiving an input from the stomach. Extracellular recordings of sponaneous activity were made from neurons in the nucleus of the solitary tract and the dorsal motor nucleus in urethane anaesthetized rats. Catheters were placed in the stomach through the oesophagus for gastric distension by instillation of saline and through the duodenum to measure intraluminal pressure in the body of the stomach. Gastric distension (2 ml) and CCK-8 (100 pmol) injected close-arterial to the stomach evoked excitatory (GE, n=29) or inhibitory (GI, n =29) responses. The responses were abolished by vagal section (6 GE and 2 GI), but maintained in animals from which the coeliac/superior mesenteric ganglion had been removed four weeks previously (4 GE and 5 GI). The responses originated at least in part from the gastric corpus as they remained intact in animals from which the gastric antrum was removed two weeks previously. The effects of CCK8 are unlikely to be secondary to changes in smooth muscle tone as CCK8 decreases pressure in the gastric corpus while distension increases it. Intravenous injection of VIP and noradrenaline (NA) also decreased intragastric pressure, but their action on neurons in the dorsal vagal complex were distinct from those of CCK8. Thus, while the response to gastric distension and CCK-8 were concordant, only 43% and 33% of GE, and 75% and 18% of GI neurons gave concordant responses to distension and NA and VIP respectively. Also, neurons that did not respond to gastric distension and CCK-8 were affected by VIP and NA. The results are consistent with the idea that CCK8 acts directly on vagal mechanoreceptor endings within the stomach wall. It is well known that peripheral administration of CCK8 influences feeding behaviours; the effects described here may represent the pathway by which circulating CCK8 influences CNS function.
Supported by the MRC.

402.9 CHOLECYSTOKININ-ENHANCED [3H]DOPAMINE EFFLUX FROM SLICES OF RAT NUCLEUS ACCUMBENS/OLFACTORY TUBERCLE: EVIDENCE FOR A DIRECT ACTION ON MESOLIMBIC DOPAMINE-CONTAINING NERVE TERMINALS. T.W. Vickroy and B.R. Bianchi*. Abbott Laboratories, Neuroscience Research Division, Abbott Park, IL 60064.

Cholecystokinin octapeptide (CCK-8) is the major form of CCK-related peptides that are present in brain and is believed to be the predominant biologically active form of this neuropeptide family. Studies in vivo with CCK-8 have demonstrated that direct injection of this peptide into discrete brain regions where CCK and tyrosine hydroxylase immunoreactivities are co-localized (primarily mesolimbic structures) causes a profound potentiation of dopamine (DA)-evoked behaviors (Crawley et al., J. Neurosci. 5:1972, 1985). However, few in vitro neurochemical correlates of this CCK-DA interaction have been reported. In this report, we describe a selective neurochemical action (enhanced [^{3}H]DA overflow) for CCK-8 in slices from the nucleus accumbens/olfactory tubercle region from rats.

For [3H]DA efflux experiments, all tissues were obtained from male albino Sprague-Dawley rats. Tissue blocks were removed from the nucleus accumbens/olfactory tubercle region or striatum and miniprisms (0.35 x 0.35 x 2.0 mm) were prepared with a mechanical tissue chopper. Tissue slices were prelabelled with [3H]DA (100nM, 30 min) and subsequently transferred to parallel chambers for superfusion with a warm, oxygenated physiological salt solution. Following an initial exposure to superfusion medium containing 35mM KCl (S1-drug free), CCK-8 or other drugs were added to the buffer and a second pulse of high KCl medium was applied (S2). Addition of CCK-8 (0.1-1000nM) to the superfusion buffer produces a concentration-dependent increase in the S(2)/S(1) ratio from 0.71 (control) to 0.85 (maximal response to CCK-8). The EC(50) for CCK-8 is approximately 3nM and the response achieves a maximum at 100nM peptide. Additional observations include: (1) desulfated CCK-8 (1-1000nM) and CCK-4 (10-1000nM) do not elicit this effect nor do these peptides block the action of 100nM CCK-8; (2) the magnitude of the CCK-8 effect is unaltered in the presence of the muscarinic antagonist atropine (1uM), the dihydropyridine calcium channel antagonist nifedipine (1uM) or tetrodotoxin (1uM); (3) under identical assay conditions, CCK-8 does not significantly alter [3H]DA efflux from rat striatal slices; and (4) CCK-8 enhanced [3H]DA efflux is not additive with the stimulatory effects of (-)sulpiride, a D2 selective antagonist, or phorbol 12,13-dibutyrate, an activator of protein kinase C. Taken together, these results imply that CCK-8 and certain related peptides selectively modulate the release of recently accumulated DA from mesolimbic neurons and that these changes may be related to the modulatory effects of CCK peptides upon DA-sensitive behaviors in animals.

402.10 KAPPA RECEPTOR MODULATION OF THE NIGRO-COLLICULAR GABA PATHWAY IN THE RAT. P.L. Wood, H.S. Kim, and C. Cosi Neuroscience Research, Research Dept., Pharmaceutical Division, CIBA-GEIGY Corp., Summit, N.J. 07901.

The nigrostriatal dopaminergic pathway in the rat is is modulated by mu and delta opioid receptors but not kappa receptors, as evidenced by work with synthetic opiates. In addition, the endogenous kappa ligand, dynorphin(1-13), does not alter striatal dopamine metabolism. However, there is a prominent dynorphinergic pathway with cell bodies in the striatum and nerve endings in the pars reticulata of the substantia nigra. The negative data with regard to kappa opiate effects on nigrostriatal dopamine metabolism, therefore, raise the question of what is the role of this dynorphinergic innervation of the substantia nigra? We now report a specific modulation of the pars reticulata GABAergic neurons which innervate the rat superior colliculus, by the kappa agonist U50488H.

In these studies, rats were treated with U50488H, morphine or saline and subsequently infused with [$^{13}C_6$]glucose for 9 min. The flux of this label through the CNS glucose, glutamate and GABA pools was monitored by gas chromatography-mass spectrometry and the turnover rates for glutamate and GABA were determined simultaneously in the same tissue extract. Using this method, morphine was found to significantly elevate GABA turnover in the superior colliculus in a naloxone-reversible manner. In contrast, the kappa agonist, U50488H, decreased GABA turnover and the effect was naloxone-reversible.

These data are consistent with a kappa receptor modulation of pars reticulata output neurons by the striatonigral dynorphinergic pathway.

402.11 ENDOGENOUS PEPTIDE RECEPTORS IN XENOPUS OOCYTES. E.M. Landau, T.M. Moriarty*, B. Gillo*, and S. Sealfon*. (SPON: J.R. Thornborough). Dept. of Psychiatry and Fishberg Center in Neurobiology, Mt. Sinai School of Medicine and Bronx V.A. Hospital, New York, N.Y.

Oocytes of the African frog Xenopus laevis have been shown by electrophysiological methods to possess receptors for a number of transmitter substances including acetylcholine, adenosine and norepinephrine. We report here that Xenopus oocytes possess receptors for the neuropeptides corticotropin releasing factor (CRF), arginine vasopressin (AVP), and cholecystokinin (CCK).

Oocytes were taken from mature female frogs. Oocytes with the follicular cell layer intact (follicles) and oocytes with the follicular cell layer stripped away by treatment with collagenase (denuded) were studied. Single cells were voltage clamped in a superfusion apparatus in standard fashion.

Application of 2 uM CRF to follicles induced an outward hyperpolarizing current. Voltage ramp studies revealed this current to be carried by K^+ ions. Pretreatment of follicles with 0.2 uM forskolin or 1 mM isobutylmethylxanthine (IBMX) potentiated the outward current indicating that the hyperpolarization is mediated by cAMP. Application of CRF to denuded oocytes (which cannot generate a cAMP dependent K^+ current) did not induce a current.

Application of 2 uM AVP to follicles also induced an outward hyperpolarizing current. Voltage ramp studies and denuded oocyte studies revealed this to be a K^+ current. Pretreatment of follicles with forskolin or IBMX showed that the AVP induced current was mediated by cAMP.

Application of 2 uM CCK to follicles induced an inward depolarizing current. Voltage ramp studies showed that this current is carried by Cl^- ions. Application of CCK to denuded oocytes gave the same inward current. This current is similar in shape, time course, and reversal potential to the Cl^- current produced by intracellular injection of IP_3 and that induced by transmitters known to act through the breakdown of polyphosphoinositides. This suggests that CCK acts through the phosphatidylinositol pathway.

Approximately 60% of our population of frogs were responsive to these peptides. We conclude that the native Xenopus oocyte possesses peptide receptors in addition to those receptors previously described.

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403.1 SELECTIVE DEAFFERENTATION OF THE HIPPOCAMPUS PROTECTS CA1 NEURONS AGAINST ISCHEMIC INJURY.

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Hippocampal CA1 neurons are selectively vulnerable to ischemic injury, dying between 48 and 72 hours following a period of global ischemia. To test the hypothesis that an excitatory input may determine this vulnerability, afferents to CA1 were cut and then the animals were exposed to ischemia.

Adult male rats were anesthetized and the right dorsal hippocampus was exposed by cortical and white matter aspiration. Septal afferents in the Fimbria/Fornix, Schaffer Collaterals and entorhinal afferents in the Perforant Path were cut. Separate groups were subjected to cortical aspiration only (sham); all 3 cuts (Tricut); Fimbria/Fornix only; Schaffer Collaterals only and Perforant Path only. All lesions were made on the right side, the left side serving as a control. Following 10-16 days rats were subjected to 30 minutes of complete forebrain ischemia using the four vessel occlusion model. 72 hours later animals were perfused, serial sections of dorsal hippocampus were then examined and irreversible injury to CA1 neurons was graded (0=normal;1<10%;2=10-50%;3>50% damaged). Differences in the degree of damage were analysed using Mann-Whitney U (MWU) and Kruskal-Wallis (K-W) tests.

	Mean grade of CA 1 neuronal damage (±SD)	
Type of lesion (no. of animals)	Intact left side	Deaff . right side
SHAM (5)	3.0 ±0	3.0 ±0
TRICUT (11)	2.73 ±0.3	0.5 ±0.6*
FIMBRIA/FORNIX (12)	2.97 ±0.1	1.85 ±0.8†
SCHAFFER COLLAT. (4)	2.89 ±0.1	2.86 ±0.1
PERFORANT PATH (4)	3.0 ±0	2.46 ±0.5

* p<0.0001vs.left side(MWU) and p<0.001 vs . right side of shams(K-W).
† p<0.002 vs. left side (MWU) and p<0.01 vs. right side of shams(K-W).

These data indicate that deafferentation of the hippocampus will protect CA1 pyramidal neurons against transient ischemia. They also indicate that the the septal (Fimbria/Fornix) input is more important than either the Schaffer Collateral or Perforant Path input. Specific neurotransmitters or neuromodulators carried with the Fimbria/Fornix pathway may be involved in the process of delayed but selective neuronal vulnerability.

403.2 DEXTROMETHORPHAN REDUCES NEOCORTICAL ISCHEMIC NEURONAL DAMAGE AND EDEMA IN AN *IN VIVO* MODEL OF TRANSIENT FOCAL CEREBRAL ISCHEMIA.

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The dextrorotatory morphinan dextromethorphan (DM) is an N-methyl-D-aspartate (NMDA) antagonist which has recently been shown to attenuate neuronal injury in cortical cell culture (Neurology 37,S1:250). DM was tested in an *in vivo* acute reperfusion model of cerebral ischemia to assess neuronal protective effect.

Twenty male New Zealand white rabbits underwent 1hr transient transorbital two or three vessel cerebral arterial occlusion. Fourteen animals had the left internal carotid artery (ICA) and distal A1 (dA1) segment of the anterior cerebral artery (ACA) clipped; six animals had the left ICA, dA1 and proximal middle cerebral artery clipped. Animals having two vessel or three vessel occlusion were separately randomized into DM or control normal saline (NS) groups. One hour prior to occlusion animals were blindly given either a 20mg/kg bolus followed by 10mg/kg/hr of 0.4% DM in NS or an equivalent volume of NS alone. Bilateral median nerve somatosensory evoked potentials (SEP) were obtained throughout the experiment. Brains were formalin-fixed and subsequently analyzed for changes in water content with magnetic resonance imaging (MRI). Axial and coronal T2-weighted images (TR2000, TE20 and 80) were obtained on a high field 1.5 Tesla GE Signa scanner using multiple spin-echo sequences. Ischemic neuronal damage (IND) was assessed at four coronal levels as a percentage of structural area with conventional hematoxylin and eosin (HE) histology.

There was a significant decrease in the percentage of left neocortical (CX) IND at all four levels examined in the DM treated group. However in the striatum only the tail of left caudate (CD) and most posterior left putamen (PU) showed a significant decrease in % IND with DM treatment.

	CX1	CX2	CX3	CX4	CD1&2	PU1&2	CDtail	PUpost
DM	24.1	8.6	6.7	3.5	42.9	38.5	12.3	21.4
NS	61.7	53.6	41.1	29	64.8	63.6	63.9	65.0
p	<.01	<.01	<.01	<.02	ns	ns	<.01	<.05

MRI lesions were localized to cortex and subcortical white matter with no evident basal ganglia involvement. MRI images were blindly scored according to area and intensity of lesion. The DM treated animals showed a significantly lower MRI score than the NS group: DM 13.3, NS 88 (n=19, p=.002). The abnormal cortical signal corresponded most closely to spongiform change in the neuropil (probable edema).

Amplitude ratios were calculated from the SEP primary cortical potential as a percentage of preocclusion values. Both left and right primary cortical potential amplitude ratios were greater in DM than in NS treated animals throughout reperfusion.

In summary, DM markedly decreases neocortical IND and edema. The disparity between neocortical and striatal DM effect as assessed by histology may reflect 1) differential NMDA receptor densities in each region, 2) the relative contribution of NMDA receptor mediated neurotoxicity to ischemic neuronal degeneration in each region, and/or 3) relatively more severe striatal ischemia in this model. Further investigation into the mechanism of action and possible therapeutic applications of this clinically available compound in cerebral ischemia should be undertaken.

403.3 DEXTROMETHORPHAN PROTECTS AGAINST HYPOXIC-ISCHEMIC BRAIN INJURY IN THE LEVINE RAT MODEL.

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Dextromethorphan (DM), an over-the-counter oral antitussive agent, and its metabolite dextrorphan (DX), are NMDA antagonists that have been recently shown to have a protective effect against anoxic neuronal injury in a cortical cell culture model (Goldberg et al., Neurology 37:250, 1987). Although most NMDA blockers have not been tested for their safety in man, DM and DX are readily available at present and appear to be relatively non-toxic. We therefore studied the effects of DM in an in vivo animal model of hypoxic-ischemic brain injury.

Eight day old Sprague-Dawley rat pups were divided into two groups using a split-litter design, anesthetized by cooling, and injected i.p. with either 35 mg/kg DM or with an equal volume of saline. The right common carotid artery was then isolated and ligated. One hour following the completion of surgery, animals were placed in a 92% N_2, 8% O_2 environment at 37°C for two hours, allowed to recover in room air, then returned to their dams. A group of control animals without carotid ties were treated identically to the saline-injected experimental animals except that the exposed carotid was never ligated. No obvious behavioral seizures were observed during or immediately following the hypoxia, although hypoxic animals appeared tremulous and unresponsive. After seven days, rats were anesthetized, perfused and sections stained with celestine blue and acid fuchsin for assessment of abnormalities.

Sections from unligated controls, ligated controls, and DM animals were assessed blind and rated for degree of hypoxic/ischemic damage. Types of abnormality included 1) presence of acidophilic or pyknotic neocortical neurons, 2) acidophilic and pyknotic hippocampal CA3/CA4 pyramidal cells with cells loss, 3) laminar cortical necrosis, 4) cortical microinfarction, and 5) frank cortical macroinfarction. Abnormalities 2-5 were ipsilateral to the occluded carotid and abnormality 1 was bilateral but more prominent on the occluded side. Cortical macroinfarctions were not seen in DM animals and laminar necrosis was much more prominent in untreated subjects. Both groups showed scattered dark cells and degrees of hippocampal damage. These preliminary results suggest that DM has protective effects against brain injury in this model and might be useful in treatment or prophylaxis of hypoxic/ischemic damage in man.

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403.4 PET STUDY OF THE EARLY CHANGES IN CEREBRAL BLOOD FLOW, OXYGEN AND GLUCOSE METABOLISM IN A PRIMATE MODEL OF STROKE.

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We have adapted a primate model of stroke for repeated measurements with PET for determination of regional cerebral blood flow (CBF), metabolic rate for oxygen ($CMRO_2$), and metabolic rate for glucose (CMRglu) during the first week following cerebral infarction. The motivation for this model is the need to obtain a controlled lesion in a species closely related to man. By using PET techniques a single animal can be studied repeatedly during the post stroke phase to assess the time course of the response to infarction/ischemia.

The cerebral infarction was carried out in Papio Anubis baboons (15-25 kg) by a transorbital coagulation occlusion of the M_1 segment of the middle cerebral artery while the animal was under anesthesia with alphaxalone and alphadolone. The animals were extubated 6-12 hours after the occlusion. During the study period the animals were progressively more independent with no major complications other than hemiparesis. PET studies were carried out 2 days before and 1-4hrs, 48 hrs, and 7 days after the lesion. Animals received similar doses of the anesthetic during each of the PET studies. EEG and Arterial blood gases were monitored during each study.

CBF, $CMRO_2$,and OEF were measured using the ^{15}O labelled CO_2 and O_2 steady-state method. CMRglu was obtained from a kinetic determination of k1-k4 derived from a 90-180 min dynamic PET scan of ^{18}F-deoxyglucose (FDG). CMRglu was calculated using these constants and the arterial histories of plasma FDG and glucose. An index of relative metabolism was calculated from the molar ratio of $CMRO_2$ to CMRglu. This ratio was correlated to CBF, ($r=0.8$, $p<0.01$) for pooled data obtained from regions of interest from each PET scan day and from the involved and uninvolved regions. The accuracy of these preliminary data might be improved by consideration of a regional blood volume correction and the effects of ischemia on the lumped constant.

A well defined lesion encompassing approximately 25% of the brain could be visualized on the initial post-occlusion PET image. This model combined with PET could offer an opportunity for repeated study of physiologic variables and could result in an understanding of the time course and the factors involved in the cerebral response to ischemia and stroke.

403.5 ATTENUATION OF THE MPTP-INDUCED DOPAMINE DEPLETION IN PRIMATE CAUDATE NUCLEUS BY FETAL NEURON TRANSPLANTS. J.D. Elsworth, D.E. Redmond, Jr., A.Y. Deutch, T.J. Collier, J.R. Sladek, Jr., R.H. Roth. Yale Univ. Sch. of Med., New Haven, CT 06510, Univ. Rochester Sch. of Med., Rochester, NY 14641.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) damages the nigrostriatal dopamine (DA) pathway and produces parkinsonism in monkeys and humans. Since transplantation of fetal DA neurons into the caudate nucleus (CN) appears to reverse the MPTP-induced motor abnormalities in monkeys, the concentrations of DA and homovanillic acid (HVA) in the CN of monkeys following MPTP treatment and fetal transplants have been measured to provide neurochemical quantitation of both MPTP-induced deficits and of the dopaminergic influence of transplanted fetal neurons.

Adult male African green monkeys were treated with MPTP (2-3 mg/kg IM over 5 days). Some animals showed no gross parkinsonism after MPTP ("asymptomatic"), while others became severely parkinsonian ("symptomatic"). These monkeys were compared with untreated control monkeys. Two symptomatic monkeys subsequently received fetal substantia nigra neuron transplants aimed at the CN, with sacrifice 7 months later. Areas were punch-dissected from coronal brain slices and assayed for catecholamines (by HPLC) and metabolites (by GC-MS).

In the MPTP-treated monkeys a large decrease in DA concentration was observed in the caudate nucleus; the reduction was greater in the symptomatic (98-99%) than in the asymptomatic group (88-98%). DA content of the central substantia nigra was markedly reduced in the symptomatic group (75-95%), but not significantly decreased in the asymptomatic animals. MPTP-treatment was found to reduce DA and/or norepinephrine concentration in anterior cingulate cortex and septum but not in medial orbital frontal cortex, suggesting differences in susceptibility to MPTP within the DA mesolimbic and mesocortical systems.

At some sites in the CN, MPTP-treated animals with nigral grafts had increased DA and HVA concentrations which exceeded those of the symptomatic animals and approached those of the asymptomatic group. The DA concentrations in the central substantia nigra of the grafted and symptomatic animals confirmed comparable damage by MPTP. Related behavioral and anatomical data will be presented by Redmond, Sladek, and Taylor *et al*.

These results extend the biochemical similarities between idiopathic Parkinson's disease and MPTP-induced parkinsonism and indicate that fetal neuronal grafting may restore diminished neurochemical measures of DA function in localized areas.

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403.6 EFFECT OF FETAL SUBSTANTIA NIGRA TRANSPLANTS ON ACQUISITION AND PERFORMANCE DEFICITS IN MPTP-TREATED MONKEYS PERFORMING OBJECT RETRIEVAL/DETOUR TASK. J.R. Taylor, R.H. Roth, T.J. Collier, J.R. Sladek, Jr., D.E. Redmond, Jr. Yale Univ. Sch. of Med., New Haven, CT 06510 and Univ. Rochester Sch. of Med., Rochester, N.Y. 14641.

To assess functional correlates of dopamine system deficits produced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and possible restoration following fetal substantia nigra transplants, we studied the acquisition and performance of an object retrieval task in monkeys. The task requires retrieval of a banana slice ("reward") from a transparent box open on one side and fastened to a tray attached to the front of the cage. Successful performance requires suppressing a tendency to reach directly at the reward ("detour") as (a) orientation of the open side, (b) position on the tray, and (c) position of the banana in the box are manipulated to vary the cognitive and motor difficulty of the trial. Each test session consists of 20 trials delivered and analyzed to detect and dissociate cognitive and motoric deficits. The frequency of "awkward reaching," a more complex motor movement with the hand furthest from the opening, was recorded. Lesions of the dopaminergic dorsolateral prefrontal cortex reveal performance deficits on this type of task, as well as dopamine-depleted "asymptomatic" and obviously motorically impaired monkeys after MPTP treatment.

Thirty adult male African green monkeys *(Cercopithecus aethiops sabaeus)* were treated with MPTP (2-3 mg/kg cumulative doses over 5 days) or sham-treated as controls. After MPTP treatment alone subjects showed retarded acquisition of the task. They made errors on sets of trials that were related to cognitive complexity, performed awkward reaches and displayed motor impairments not found in control subjects. Either before or after training, some monkeys were transplanted with fetal neurons (of variable gestational age) from the substantia nigra or other brain regions as controls. Both the motor and cognitive impairments were modified by fetal nigral cell transplants into the caudate nucleus, which improved performance in some monkeys. This task appears sensitive to MPTP-induced deficits in dopamine systems and to the specific site of placements of fetal neuronal transplants, perhaps separating cognitive from motor recovery.

Supported by N.I.N.C.D.S. grant PO1 NS24032, the Axion Research Foundation/St. Kitts Biomedical Research Foundation, St. Kitts, W.I., S.E.R.C. fellowship to J.R.T. and Research Scientist Award KO5-MH00643 to D.E.R.

403.7 REVERSAL OF MPTP-INDUCED PARKINSONISM UP TO 7 MONTHS AFTER FETAL NEURON TRANSPLANTS IN GREEN MONKEYS. D.E. Redmond, Jr., T.J. Collier, P.N. Foster, V.O. Lewis, J.P. Blount, R.H. Roth, J.R. Sladek, Jr. Yale Univ. Sch. of Med., New Haven, CT 06510 & Univ. Rochester Sch. of Med., Rochester, N.Y. 14641.

Transplantation of fetal substantia nigra (SN) neurons into the caudate nucleus (CN) appeared to reverse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced parkinsonism for 10 weeks in 2 monkeys, but not in a third with less mature neuroblasts transplanted outside the striatum. This previous study raised questions about the effect of fetal age, other placement sites, trophic factors vs. structural integration of grafts, the limits of graft survival, and duration of recovery.

Six *Cercopithecus aethiops sabaeus* (green monkeys) have now been treated with MPTP (2-3 mg/kg i.m. over 5 days) and 4-5 weeks later transplanted with neurons from 8.5, 11, and 21 cm. crown-rump length fetuses in further pilot studies. Small solid grafts were placed through cannulae into the caudate nucleus, lateral ventricle, and cerebral cortex. SN cells were implanted in four animals and cerebellar cells in two. Behaviors were scored, rated, and videotaped by blind observers, and CSF samples were drawn throughout the experiment. Monkeys were sacrificed 5 months or 7 months after transplantation. The morphological, biochemical, and cognitive data are reported by Sladek, Elsworth, and Taylor *et al* in companion papers.

After MPTP treatment, severe parkinsonism was seen in four monkeys, and moderate in two, requiring extensive individual feeding, nursing care, and physiotherapy before and after the transplants. Four required nasogastric tube feeding. After transplantation, behavioral improvement was variable, but slower in all animals than seen previously with less impaired animals and with later gestational-age donor cells. There were clear behavioral differences between some monkeys which improved and some which did not. Others showed intermediate effects. There was also variability in dopamine (DA), homovanillic acid (HVA), and HVA/DA ratios between striatal punch sites, brain regions, and animals, as well as in the specific graft sites, structural integration, and patterns of graft development.

Fetal neural grafts survive in monkeys up to 7 months without adverse behavioral effects, show structural integration with the host brain, restore DA neurochemical activity in localized areas, and restore behavioral function in some parkinsonian monkeys. The influence of several important factors remains unclear. (Supported by PO1 NS24032, Axion Res. Found., St.Kitts Biomed. Res. Found., West Indies, and RSA KO5-MH00643 to DER).

403.8 LONG TERM SURVIVAL OF TRANSPLANTED FETAL NEURONS IN MPTP TREATED AFRICAN GREEN MONKEYS. J.R. Sladek, Jr., T.J. Collier, J.D. Elsworth, R.H. Roth, and D.E. Redmond, Jr. Depts. of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642 and Depts. of Pharmacology and Psychiatry, Yale Univ. Sch. of Med., New Haven, CT 06510

As part of our ongoing investigation of the effects of fetal nerve cell grafts on the experimental Parkinsonism induced by the toxin, MPTP, in primates, we have extended the post surgical survival periods in order to test the continued and progressive therapeutic value of neuronal grafts. This study also has incorporated control grafts of cerebellar cortex as a source of non-dopaminergic (DA) neurons from the same donor tissue that supplied DA grafts of substantia nigra. Moreover, various gestational stages have been utilized to further examine survival potential over a wide range of neuroblast development. Twelve, young adult male African green monkeys from the St. Kitt's colony were treated with 2-3 mg/kg of MPTP as a cumulative dose delivered over 5 days. Following the development of parkinsonian signs after 4-5 weeks, we stereotaxically grafted neurons from 3 fetal African green monkey donors (crl 8.0, 11.0, 21.0 cm) into the caudate nucleus of 6 treated monkeys. One donor supplied cerebellar or nigral tissue to each of two pairs of hosts, and one donor provided nigral tissue to one pair. Multiple "solid" grafts were placed into each animal at either 4 or 6 penetration points. Behavioral and biochemical indices of functional improvement were monitored and are reported in the accompanying abstracts by Redmond and colleagues. At 5 months after transplantation, 3 animals were perfused with 4% paraformldehyde and were stained immunohistochemically for tyrosine hydroxylase (TOH). The other 3 monkeys were prepared after 7 months for co-analysis of DA and metabolite content, neurochemically, and TOH distribution, morphologically, by combining "punched" tissue procedures with immunohistochemistry. Morphological analysis revealed the presence of bilateral "plugs" of fetal tissue in each host monkey. These grafts were positioned both deep within the head of the caudate nucleus and also were seen more dorsally where they extended into and expanded in size within the lateral ventricles. Grafts of cerebellar cortex were devoid of TOH perikarya, whereas such neurons were common features of nigral grafts. In some instances, TOH fibers appeared to radiate from nigral grafts into the host striatum, suggestive of growth of neuronal processes, perhaps favored by the extended survival times in comparison to our earlier study (i.e. 10 weeks). Evidence of graft rejection was not observed, however some erosion of superficial cortical tissue was noted in some animals in and adjacent to the cannula tracts. Survival of grafted neurons over a wide range of gestational ages supports the view that the optimal "window" for graft viability is considerably wider in primates than in rodents and survival and growth of grafted neurons over 5 to 7 months suggests that long term functional improvement may coincide with these observations.

Supported by PHS grant NS 24032.

403.9 EFFECTS OF MPTP ON SOME INDEXES OF ANTIOXIDATIVE PROTECTION IN THE MOUSE SUBSTANTIA NIGRA. R.G. Fariello, O. Ghilardi*, T.N. Ferraro, G.T. Golden, A. Peschechera*, M.T. Ramacci*, and L. Angelucci*. Research & Neurology, VAMC, Coatesville, PA; Neurology, Thomas Jefferson University, Philadelphia, PA; Univ. of Rome and Sigma Tau, Rome, Italy

The administration of MPTP to mice causes initial selective dysfunction of nigral neurons. Searching for mechanisms and cellular sites of action of this potent neurotoxin we have administered a single dose of 30 mg/kg MPTP i.p. to C57 black mice and sacrificed them at 30 min, 1 h, 6 h, or 24 h afterwards. Nigrae, neostriata, and cerebelli were dissected and frozen within 2 min 45 sec after decapitation. Levels of tocoferols (cytosolic, liposoluble antioxidant) and reduced (quinol) and oxidized (quinone) forms of Q9 and Q10 ubiquinoles (mitochondrial antioxidant) were measured by reverse phase HPLC with electrochemical detection with dual electrode. A statistically significant depletion of Q10 in its reduced form with a shift of the reduced/oxidized ratio toward oxidation was found only in the nigra at 1 h after MPTP, with a return to baseline value at 6 and 12 h. Levels of tocoferols remained unchanged at all times. In another series of experiments 30 mg/kg MPTP was administered i.p. daily for 5 days and the mice sacrificed 24 h after the last MPTP injection. Brain regions were dissected and analyzed as in the acute experiments. Again only the Q10 ubiquinol was selectively depleted in the nigra and not in the other regions. These data suggest that MPTP has an early site of action at the inner mitochondrial membrane of nigral neurons at the ubiquinone step, a crucial point linking the respiratory chain to the oxido-reducing systems. Multiple administrations are necessary to produce a long lasting ubiquinol depletion. (Supported by Sigma Tau Pharmaceuticals and the Veterans Administration.)

403.10 MPTP AND [^{3}H]2-DEOXYGLUCOSE (2DG) AUTORADIOGRAPHY IN THE SUBSTANTIA NIGRA OF MICE. Arthur Hess, Dept. of Anatomy, UMDNJ, Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

It is assumed that the process by which MPTP causes dopaminergic neurotoxicity is as follows: MPTP binds to extraneuronal tissue, probably neuroglia, where it is metabolized by MAO-B; the resulting toxic product MPP^{+} then enters the dopaminergic neurons through the dopamine uptake system. Hence, MAO-B inhibitors and dopamine uptake blockers, as expected, are effective in preventing the dopamine depletion caused by MPTP.

MPTP (30mg/kg, 1-2 hours after administration) causes an increase in 2DG uptake in the zona compacta of the substantia nigra and adjacent ventral tegmental area. It is possible that this increase in 2DG uptake, induced very rapidly, is an initial step and the first sign of the degenerative process resulting from MPTP administration.

The MAO-B inhibitor deprenyl (10mg/kg, 30 min preceding MPTP) prevents 2DG increase. Deprenyl, irreversible and long acting, does not block increase in 2DG if administered overnight before MPTP. Clorgyline (10mg/kg), an irreversible MAO-A inhibitor, or mazindol (10mg/kg), a dopamine uptake blocker, have no effect on the 2DG reaction induced by MPTP when injected 12 hrs or 30 min before MPTP. Thus, there are discrepancies in the correlation between the presumed mechanism of neurotoxicity and the 2DG increase in the substantia nigra induced by MPTP.

Further studies are under way to determine the significance of the 2DG reaction induced by MPTP. Dose response studies have shown so far that weaker doses of MPTP (½ dose, 15mg/kg; ¼ dose, 7mg/kg for 2 hours) still produce increase in 2DG uptake. Time response studies have revealed thus far that there is no increase in 2DG uptake if 30mg/kg MPTP is administered 15 minutes or overnight before 2DG injection.

The effects of MPTP on 2DG increase in the locus coeruleus will also be presented; similarly, the effects of various neurotropic substances in blocking this increase will be illustrated.

Some comparative effects of analogs of MPTP on 2DG uptake will also be discussed.

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403.11 NEURAL BASIS OF CEREBRAL VASOSPASM: HYPOTHESIS AND EXPERIMENTS. S.H. Tsai, M.S. Greenberg, J.M. Tew, and M.T. Shipley. Dept. of Anatomy/Cell Biology and Neurosurgery, Univ. of Cincinnati College of Med., Cincinnati, OH 45267.

Subarachnoid hemorrhage (SAH) has devastating secondary effects on cerebral circulation and massive cerebral infarction is produced by delayed vasospasm. Cerebral arteries are innervated by perivascular nerves containing classical transmitters and neuropeptides. Both classes of neuroactive molecules influence vascular diameter and regulate cerebral circulation. We hypothesize that imbalance in the action of these vasoconstrictive and vasodilatative neurotransmitters on the wall of cerebral arteries following subarachnoid hemorrhage (SAH) is a major factor in the cause of cerebral vasospasm. We have examined the changes of transmitters and peptides in perivascular nerves following experimentally induced SAH in rats.

The quantity and distribution of nerves containing norepinephrine (NE) (using antisera to dopamine beta-hydroxylase [DBH], the key synthetic enzyme for NE), neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP) and calcitonin gene related peptide (CGRP) in perivascular nerves were studied by injection of 0.3 ml non-heparinized autologous arterial blood into the cisterna magna. Rats with saline injection into cisterna magna and rats without any manipulation served as controls. Animals were processed for ICC at different time intervals after injection. The entire circle of Willis was dissected free of the brain and split in the middle parasagittally. One half was processed for vasoconstrictive molecules (DBH or NPY) and the other half was for vasodilatative molecules (VIP or CGRP).

Cerebral arteries from non-injected and saline injection rats showed no change in either DBH/NPY or VIP/CGRP. In SAH rats, significant decreases of vasodilatative VIP and CGRP fibers were found at 2 and 3 days after SAH. In contrast, the vasoconstrictive NPY and DBH fibers were unchanged. One week after SAH, all the vasoactive fibers returned to the levels as normal controls.

Delayed vasospasm has been demonstrated 2 to 3 days after SAH in rats. The present results suggest that vasoconstrictive transmitters are relatively unaffected by SAH but vasodilatative transmitters are drastically reduced at precisely the time that the animals develop vasospasm. Loss of vasodilatative function system coupled with the survival of vasoconstrictive function could lead to unchecked vasoconstrictive control of the vascular walls, leading to vasospasm and failure of cerebral perfusion. (Supported by NINCDS 23348, US Army DAMD-86-C-6005 and DOD DAAC-83-60064.)

403.12 DISEASES IN ASSOCIATION AREAS FOUND IN SCHIZOPHRENIA USING $C^{15}O_2$, $^{15}O_2$ AND ^{11}C-GLUCOSE PET. H.Kishimoto, S.Ohno*, H.Sato*, S.Endo*, O.Takazu*, K.Sakurai*, T.Ishii*, M.Matsushita* and M.Iio*. Department of Psychiatry, Yokohama City University, Yokohama 232 and Nakano National Hospital, Nakano, Tokyo 165, JAPAN.

The authors describe new findings in the impaired cortical areas of the brain in patients with schizophrenia using $C^{15}O_2$, $^{15}O_2$ and ^{11}C-glucose PET. It is important to point out that PET images of ^{11}C-glucose showed amino acid pools whereas ^{18}F-deoxy-glucose showed the utilization of glucose.

Ten chronic schizophrenic patients (5 men, 5 women, mean age 36) who were out-patients on a chronic course without medication at the Yokohama City University Hospital, and five control subjects (3 men, 2 women, mean age 36) who agreed to give written informed consent participated in this study. Diagnoses were based on DSM-III criteria and were confirmed by another psychiatrist. Seven to ten minutes after the administration of $C^{15}O_2$, $^{15}O_2$ and ^{11}C-glucose, three to six scans parallel to the orbital meatal (OM) line were done as quietly as possible. The PET instrument was a Headtome-II which was made in Shimazu, Japan. For the present analysis, OM 40, 49, 58, 67, 75 and 84 mm slices were chosen. The authors also took brain X-ray-CTs at the same slice line as the PET and evaluated each part of the brain using the human brain map for computerized tomography by Matsui and Hirano, 1978 (Igaku Syoin , Japan) and identified Brodmann's areas 10, 40 and others. In chronic schizophrenic patients there were three types of PET images when using $C^{15}O_2$, $^{15}O_2$ and ^{11}C-glucose to inspect the cortical area of the brain. The first (type A) was a hypofrontal PET image. The second (type B) was a right side hypoparietal PET image in right-handed schizophrenic patients. The third (type C) was a left side hypotemporal PET image in right-handed schizophrenic patients.

The lowest count area in the brain in type A schizophrenia was Brodmann's area 10, in type B chronic schizophrenia, Brodmann's area 40 and in type C chronic schizophrenia, Brodmann's area 22-38. The percentage of the pixel count reduction in the frontal gyri (Brodmann's 10) in type A schizophrenia using ROI (region of interest, the mean of 25 pixels, 16x16mm) was 34 ($p<0.01$), in the non-dominant side parietal gyri (Brodmann's 40) in type B schizophrenia using ROI was 24 ($p<0.025$), and in the dominant side temporal gyrus (Brodmann's 22-38) in type C schizophrenia using ROI was 20 ($p<0.05$) in each gyri of the brain.

These areas of Brodmann 10 and 40 are the association areas which are important parts of the brain for organizing the personality and for integrating all of the information from the outside and inside of the body in human beings. Supported Grant 86-10-11

404.1 **SEGREGATION OF X AND Y AFFERENTS INTO AREAS 17 AND 18 OF CAT VISUAL CORTEX.** David Ferster, Department of Neurobiology and Physiology, Northwestern University, Evanston IL 60201.

I have been investigating the convergence of X and Y afferents onto single cortical cells with intracellular recording and electrical stimulation of the optic nerve (ON). X and Y axons in the optic nerve have very different electrical thresholds. By virtue of their non-overlapping diameters, one may stimulate Y axons alone at low stimulus currents, and X and Y axons together at high stimulus currents. The origin, X or Y, of PSPs evoked in cortical cells from the ON may therefore be identified by their thresholds.

Each ON was fitted with a cuff-shaped electrode and X and Y thresholds determined from optic tract (OT) recordings. As the stimulus current was raised from zero, a short-latency field potential appeared and grew in the OT, associated with recruitment of Y axons. With further increases in the stimulus current, this early potential reached maximal amplitude, and a second, longer latency potential associated with recruitment of X axons appeared and grew in amplitude. In a control experiment, geniculate X and Y cells were stimulated visually with sine-wave gratings and identified from their non-linearity of spatial summation. The threshold of Y-cells to ON stimulation always fell below the threshold of the later OT potential; X-cell thresholds were always above. Potentials evoked in cortical cells by stimulating the ON at the lower stimulus currents must therefore be mediated by Y axons, and those evoked at higher currents by X axons.

While single cortical neurons have been found with pure Y, pure X, and mixed input (Ferster, Neuroscience Abstr. **12**:128), the distribution of these neurons was surprising. I have found X-mediated synaptic input from the LGN throughout the layers of area 17, but I found Y input only in neurons of area 18. Mixed X and Y input to single cells was found only near the border between the two areas. The failure to find Y input to any of the 35 neurons recorded in area 17 is unlikely to reflect a sampling problem since current source density analysis confirms the pattern: No significant current sources were evoked in area 17 by activating Y-cells from the ON. Conversely, in area 18 only Y-activated sources were found. The latencies of the earliest sink and of the earliest PSPs in area 18 preceded those in area 17 by 1.1 ms (1.9 ms vs. 3.0 ms).

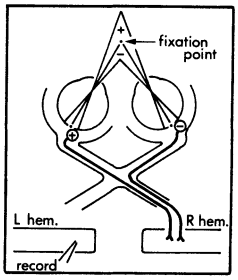
This result is in agreement with Spitzer and Hochstein (J. Neurophysiol. **53**:1245), who could find no Y-like non-linearities in the receptive fields of neurons in area 17. While a segregation of X and Y afferents into areas 17 and 18 would bear on the function of the two afferent types as well as on the function of the two distinct cortical areas, this result clearly requires confirmation given the weight of evidence for Y input to area 17.

404.2 OCULAR DOMINANCE AND BINOCULAR DISPARITY TUNING IN CAT VISUAL CORTEX. Simon LeVay and Thomas Voigt*. Salk Institute, PO Box 85800, San Diego, CA 92138

It has long been suspected that the cortical ocular dominance system is part of the mechanism for stereoscopy. To investigate this issue further, we have used quantitative, automated techniques to determine ocular dominance ratios (OD; 0 = contra, 1 = ipsi), disparity sensitivity (DS; (max. response - min. response)/min. response), best disparity (BD; 0 = fixation plane, + = near disparity, - = far) and best orientation for 272 units in areas 17 and 18 of paralyzed, anesthetized cats. Disparity was varied orthogonal to the cell's best orientation. Zero disparity was determined with a reference electrode at the border of layers A and A1 at the area centralis representation in the LGN.

Both DS and BD values formed continuous distributions. Confirming previous studies, we found no correlation between a cell's best orientation and its disparity tuning properties. We also found no correlation between ocular dominance and disparity sensitivity. There was however a relationship between ocular dominance and best disparity: units with BDs near the fixation place (within 1° of zero) had OD ratios clustered near 0.5 (equal dominance), confirming Ferster, '81, while units with BDs 1° or more from zero had a broad distribution of OD ratios (significance of difference of variance = 0.002, F-test). For units with receptive fields near the vertical meridian (VM) a further correlation emerged: the OD ratios of units in the 'far' range (BDs $\leq -1°$) were biased towards the contralateral eye (median OD = 0.39, n = 37), while those in the 'near' range ($\geq +1°$) were biased towards the ipsilateral eye (median OD = 0.60, n = 37). The difference of medians was significant at p = 0.003 (2-tailed Mann-Whitney). (Cells oriented near horizontal were excluded from this analysis.)

The results confirm a relationship between ocular dominance and disparity tuning. The unexpected contra-far, ipsi-near relationship for units near the VM is explicable in terms of visual geometry and the pattern of decussation in the visual pathway: the required excitatory and inhibitory inputs for the less-frequently encountered cell types (e.g. the ipsi-dominated far cell as sketched here) are directed to the hemisphere opposite to the side being recorded. One might expect interhemispheric connections to supply the missing inputs. Our results, however, suggest that callosal connections do not fully replace, for units near the 17-18 border, the intrinsic horizontal connections within one hemisphere.



(Supported by EY-05551)

404.3 STIMULUS-SPECIFIC NEURONAL OSCILLATIONS IN THE CAT VISUAL CORTEX: A CORTICAL FUNCTIONAL UNIT. Charles M. Gray* and Wolf Singer, Max Planck Institute for Brain Research, 6000 Frankfurt/M. 71, F.R.G., (SPON: J.E. Skinner)

It is well established that the cortical column constitutes the fundamental unit of neocortical structure and function. However, relatively few studies are available which provide a measure of the temporal dynamics of columnar activity. In the course of investigating experience-dependent changes in cortical function in waking kittens, we have observed that neuronal responses are associated with a stimulus-dependent oscillation of the local extracellular field potential (LFP) in the range of 40-65 Hz. Thus, we sought to determine the stimulus specificity of these oscillations and their relation to the underlying neuronal activity.

Experiments were performed on 4 adult cats (6 months of age) previously implanted with a linear array of 8 microelectrodes in area 17 of the visual cortex. The animals were lightly anesthetized with Rompun (Xylazine) 5 mg/kg or nitrous oxide supplemented with Nembutal (1 mg/kg/hr), and fixed in a head holder previously attached to the animals' skull. Drifting light bars, of constant velocity and 5 separate orientations, were passed separately across the receptive fields of isolated unit activity at each electrode site. The LFP and multiunit activity were sampled from each electrode and subjected to off-line analysis of frequency spectra, LFP-unit cross correlation, and conditional firing probability.

Analysis of the LFP power spectra revealed a brief and significant increase in amplitude of activity in the range of 40-65 Hz during the passage of the light bar across the receptive field at the optimal orientation. Cross correlation and conditional pulse probability analyses showed that the neuronal firing probability oscillated in the same frequency range of 40-65 Hz, and showed a steep nonlinear dependence on the extracellular field negativity. Non-optimally oriented stimuli produced corresponding amplitude reductions in neuronal and LFP oscillatory responses. Thus, the data dmeonstrate that orientation-specific neuronal responses exhibit a nonlinear transition to an oscillatory active state in the range of 40-65 Hz, and suggest that a local nonlinear oscillator may provide a good model of the cortical columnar functional unit.

404.4 FACTORS DETERMINING THE VERNIER ACUITIES OF NEURONS IN AREA 17 OF CAT VISUAL CORTEX N.V. Swindale* and M. Cynader (SPON:I. A. Meinertzhagen) Dept. of Psychology, Dalhousie University, Halifax, N.S. Canada, B3H 4J1

We have previously shown (Nature,**319**,591-593,1986) that many cells in cat visual cortex are sensitive to the presence of a Vernier break in a moving bar stimulus, and that this sensitivity lies in the hyperacuity range when expressed relative to spatial resolution. Here we consider how this acuity is distributed amongst cortical cells and what factors determine high and low acuities. Sensitivity was measured as the percentage depression in firing rate caused by a given Vernier offset.

For any one cell, sensitivity was largely unaffected by changes in stimulus parameters such as velocity, bar length or the position of traverse across the receptive field. There was a wide and continuous variation in sensitivity between different cells: some showed no sensitivity at all, while in the most sensitive, response could be halved by an offset equal to a fifth of receptive field width. The highest absolute sensitivities of complex and simple cells were similar, although the majority of cells with little or no sensitivity were complex. Sensitivity was negatively correlated with receptive field width, measured as twice the standard deviation of a Gaussian fitted to the receptive field profile.

A measure of length summation was obtained by comparing the responses to each component bar of a Vernier stimulus with the response obtained when both bars moved in alignment across the field. This measure was defined as $H = 1 - (R_a + R_b)/R_{ab}$ where R_a and R_b are the responses to bars a and b individually, and R_{ab} is the response to both bars in the aligned condition. H is zero when length summation is linear; is > 0 when there is a facilitatory interaction between the two bars and is < 0 when there is an inhibitory interaction. Vernier acuity, expressed relative to receptive field width, was correlated with H, being highest in cells with large values of H and least in cells where H was negative.

A linear model of the simple cell receptive field, together with a threshold, can explain the correlation between Vernier acuity and length summation. However such a model, based on measurements of receptive field profiles made with stationary flashed bars, fails to predict tuning curves as sharp as those actually observed in many simple cells.

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404.5 **THE SPATIAL SPREAD OF ADAPTATION IN NEURONS OF CAT AREA 17.** Stuart G. Marlin* and Max S. Cynader, (SPON: G. Eskes) Department of Psychology, Dalhousie University, Halifax, N.S., Canada, B3H 4J1.

We have investigated the effects of adaptation of small regions of striate neuron receptive fields on responsivity across the entire receptive field. During adaptation, a bar of light (0.2 to 1° wide) was flashed on and off at one point in the receptive field twice per second for a period of one minute. Immediately following this adaptation period, responses to lined stimuli at each of 15 receptive field positions were tested. Line-weighting functions (LWFs) were obtained for the responses to stimulus onset and stimulus offset separately and the post-adaptation LWFs were compared with those obtained prior to adaptation. The spread and location of the adaptation effects were related to the spatial properties of the cell's receptive field. The spatial wavelength, , of a simple cell receptive field was determined from the location of the on and off subregions, and from the results of double-line interaction experiments in complex cells (Baker & Cynader, 1986).

In both simple and complex cells **decreased** responsivity was observed at the point of adaptation. The degree of adaptation decreased as the distance from point of adaptation increased until at some point (approx. 0.4) the adaptation effects were negligible. The spread of response decrement around the point of adaptation thus averaged 0.8 . In many neurons, in addition to a focal line of decreased responsiveness, there was an area of the receptive field surrounding the zone of reduced response in which **increases** in responsivity were observed. This was true in both simple and complex cells and was independent of the initial site of adaptation. This area of increase often occurred on both sides of the point of adaptation and was strongest approximately 1.0 away from the center of the response decrement zone. These areas of increased responsivity which surround the zone of adaptation may reflect inhibitory interactions between adjacent subunits in cortical receptive fields. Since LGN cells do not show these adaptation effects and binocular cortical cells can show interocular transfer of this spatial adaptation, the adaptation effects observed here appear to reflect cortical mechanisms rather than more peripheral ones.

404.6 **THE DISTRIBUTION OF INHIBITION IN THE TWO DIMENSIONAL SPECTRAL RESPONSE OF SIMPLE CELLS.** R. A. Stepnoski*, A. Gottschalk* and L. A. Palmer. (SPON: J. Saunders) University of Pennsylvania, Philadelphia, Pa. 19104

We have previously shown that the 2D spectral response of simple cells in cat is well described as an elliptic Gaussian and that the parameters of this Gaussian are predicted from independently obtained 2D spatial response profiles. Our data are consistent with the notion that simple cells are linear and functionally independent. However, other workers using conditioning-and-test paradigms have demonstrated inhibition by grating stimuli lying outside the excitatory spectral response of a cell. These results imply that the spectral response of simple cells depend in part on local interactions among cells tuned to a range of spatial frequencies and orientations. This inhibition was not apparent in our earlier experiments since only one grating was presented at a time and most cells had virtually no spontaneous activity.

We have developed a method for mapping both excitatory and inhibitory responses over the full 2D spectral domain. A band-limited white noise stimulus is used to elevate the discharge rate while single sinusoidal gratings are simultaneously presented in random order. The d.c. and fundamental component of cyclegrams obtained for each grating stimulus are plotted as a surface and reveal inhibitory zones surrounding the excitatory spectral response. Significantly, this inhibition is maximal at orientations near those which excite the cell and minimal or nonexistent for orthogonal orientations. Further, spatial frequencies above those which excite the cell elicit inhibition; the result for lower spatial frequencies is unclear.

These observations suggest that nearest neighbor inhibitory interactions may operate within the cortex, possibly refining the simple cell spectral response. A simulation exploring this possiblilty is under development and will be compared with experimental results.

Supported by BNS-8420402.

404.7 EFFECTS OF AREA 17 AND 18 ABLATIONS ON LINE ORIENTATION DISCRIMINATION IN THE CAT. J.M. Sprague, E. Vandenbussche*, G.A. Orban, B. Gulyas* and P. De Weerd*. Laboratorium voor Neuro- en Psychofysiologie, K.U. Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium, and Dept. of Anatomy, Sch. of Med., Univ. of Pennsylvania, PA 19104-6058.

From our study on velocity sensitivity we have concluded that area 17 is more suited for analysis of stationary objects than 18 (Orban et al., J. Neurophysiol., 43:1043,1981). In addition area 17 not 18 contains receptive field types of which the preferred orientation shows meridional anisotropies (Orban, Springer-Verlag, 367p., 1984; Schall et al., J. Neurosci., 6:123, 1986). Normal cats show an oblique effect in orientation discrimination (Vandenbussche and Orban, Behav.Brain Res., 9:237, 1983. One would therefore expect lesions of area 17 to have more effect on orientation discrimination measured with stationary lines and to reduce the oblique effect in orientation more severely than area 18 lesions. We tested these predictions in 8 cats trained for up to 1 year to discriminate the orientation of long (12°) lines at a principal and oblique reference orientation. The contrast log $(\Delta I/I)$ of the lines ranged from 0.3 to 2.5 and the width from 0.2 to 1.2°. In six cats area 17 was removed with variable involvement of area 18 while in two cats area 18 and most of 19 was removed. The extent of the lesion was evaluated physiologically by recording in areas bordering the lesion, and histologically by reconstruction of the lesion. This evaluation showed that the size of the 17-18 lesion ranged from a near complete ablation of area 17 (smallest lesion) to an almost complete lesion of both areas 17 and 18 (largest lesion). The effects of the lesions were measured up to one year after the surgery. The effect of the 17-(18) lesions depended on the size: the smallest lesion only increased the jnds in orientation for narrow widths (0.2°) and low contrast; the intermediate lesion increased the jnds in orientation for narrow widths even at high contrast, and the large lesion impaired even the high contrast, large width condition. In all but the smallest lesion the oblique effect in orientation was severely reduced. Our results support the view that area 17 is crucial for fine resolution of the orientation of single lines, especially at low contrast. They also indicate that area 18 supplements 17 at greater line widths and higher contrasts. Most of the oblique effect in orientation is dependent on area 17 cells. The results underscore the importance of varying the stimulus conditions (line width, contrast) to reveal deficits after cortical ablations. (Supported by Univ. Penn. Research Foundation; NIH-EY04906).

404.8 ORIENTATION-SPECIFIC VISUAL MASKING OF SINGLE UNIT RESPONSES IN CAT STRIATE CORTEX. S.B. Nelson Vision Center Lab, Salk Institute and UCSD Dept. of Biology, San Diego, CA 92138

Psychophysical studies have demonstrated that the perception of a brief visual stimulus may be suppressed by a preceding stimulus. This effect is called visual masking. The suppression is orientation-specific and has been correlated with a reduction in amplitude of visually evoked potentials (Tootle and Berkley, J. Neurophys. 53 (1985)1287). In an attempt to study masking at the single neuron level I have presented pairs of briefly flashed "mask" and "test" stimuli to paralyzed, anesthetized cats while recording extracellular responses of single units in area 17. The stimuli were light bars of optimal size and orientation presented consecutively for 200 msec each, separated by a 200 msec inter-stimulus interval (ISI). Both stimuli were presented at the most responsive region within the receptive field (RF). In 30 of 71 cells studied, the mask stimulus caused a significant suppression of the response to the test stimulus (T-test comparison of peak firing rates, P-.05). The degree of suppression varied from cell to cell. In 9 cells the response to the test stimulus was abolished completely. 11 of the 41 cells not showing suppression at a 200 msec ISI were suppressed when the ISI was decreased to 100 or 50 msec. Shorter ISIs were not tested. For 10 cells the suppression extended beyond 200 msec (maximum of 900 msec). No clear correlation was found between masking and cell type (simple, complex) or laminar position.

Psychophysical masking is orientation-specific. I studied this at the cellular level by varying the orientation of the mask stimulus while continuing to present the test stimulus at the cell's preferred orientation. Of 20 cells tested, 2 showed equal masking at all orientations. For the remaining 18 cells the suppression was maximal at the cell's preferred orientation and minimal at the orthogonal orientation. The suppression was however more broadly tuned for orientation than were the cell's excitatory responses. It fell to half-maximal at a mean of 40 deg. from optimal, compared with a mean of 21 deg. for the excitatory responses. For many cells, mask stimuli of 30 deg. from optimal failed to elicit any excitatory response but still caused substantial suppression. This suggests that masking was not due to fatigue of the cell.

These data are consistent with the hypothesis that the inhibition caused by optimally oriented stimuli lying outside the classical RF (Blakemore and Tobin, Exp. Brain Res. 15(1972)439 and Nelson and Frost, Brain Res. 139(1978)359) is also present within the RF. The orientation selectivity of the suppression observed in this study is consistent with the recent finding that intracellularly recorded IPSPs are strongest at the cell's preferred orientation and absent at orthogonal orientations (Ferster, J. Neurosci. 6(1986)1284).

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404.9 A NETWORK MODEL FOR CORTICAL ORIENTATION SELECTIVITY IN CAT VISUAL CORTEX. C. Koch. Division of Biology 216-76, Caltech, Pasadena, CA 91125.

The vast majority of cells in the cat's visual cortex are orientation selective. Two classes of models have been proposed to account for this phenomena. Hubel and Wiesel (1962) have orientation selectivity arising from a row of geniculate neurons with appropriately aligned receptive fields. However, various experiments (e.g. Sillito, 1979) underscore the crucial nature of intracortical inhibition in shaping orientation selectivity. Thus, a number of models have been proposed (e.g. Heggelund, 1981; Koch and Poggio, 1985), such that inhibitory non-oriented interneurons prevent the cell from responding at non-optimal orientations. However, no population of non-oriented cells has been found in area 17 of the cat.

I propose a model of orientation selectivity, based primarily on the interactions between inhibitory interneurons, in which cells of the same orientation inhibit each other in such a manner that the entire network acquires orientation selectivity. Thus, interneurons in the "vertical" column inhibit their neighbors to the "left" and "right," but not those to the "top" and "bottom," while horizontal cells inhibit each other along the top-bottom axis (relative to the diagram plane). In addition, cross-inhibition between cells of differing orientation enhances the orientation tuning. This network model explains how orientation selectivity arises within the input layer to striate cortex and predicts cross-inhibition between neighboring interneurons of similar orientations. It can be tested using multiple-electrode techniques. A fundamental property of the model is its reliance on massive feedback among its elements, in contrast to previous models which are of the feedforward type. I will demonstrate a computer implementation of this model based on anatomical and electrophysiological properties of cortical cells.

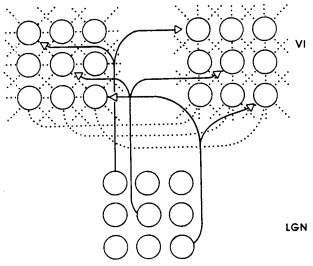

Figure illustrating the model. Only some of the excitatory geniculocortical projections (solid lines) and the cross-inhibitory connections between interneurons of different orientations (dotted lines) are drawn in. Cells in the left (resp. right) group have vertical (resp. horizontal) oriented receptive fields.

404.10 HOW DO STRIATE NEURONS REPRESENT CURVED STIMULI ? G.A. Orban, M. Versavel*, and L. Lagae*. Laboratorium voor Neuro- en Psychofysiologie, K.U.Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

Curved lines such as half circles contain many different local orientations, while chevrons contain only two orientations. From the receptive field properties of endfree and endstopped cells (Orban et al., J.Neurophysiol., 42:833, 1979) one would expect endstopped cells to respond far better to curved lines than to long straight lines. However it is not known whether this ability depends on the mean orientation difference or on the many orientation differences contained in a curved line, and whether endstopped cells can encode curvature, signalling both the degree and sign (concave, convex) of the curvature. In order to address these questions, responses of striate neurons to convex and concave curved lines and to chevrons were compared in anesthetized and paralyzed cats. Chevrons were made out of two 6° long line segments intersecting at different angles. Curved stimuli were made of circles of different radii. For small radii half circles were used, while for large radii only 12° long segments of circles were used. Endstopping was assessed by measuring the responses to straight lines, ranging in length from 0.5 to 12°. Our results show that all striate neurons, including strongly endstopped cells, fail to respond to chevrons with small angular differences. This observation fits with the known orientation tuning properties of the discharge region and the endzones (Orban et al., J. Neurophysiol., 42:833, 1979). Endfree cells respond to a wide range of curved lines except the most curved ones. Endstopped cells respond best to moderate or large curvatures. Degree of endstopping and optimal curvature are correlated : the stronger the endstopping, the larger the optimal degree of curvature. These results indicate that endstopped and endfree cells together are able to sample all degrees of curvatures but only a limited range of orientation differences. Our results further suggest that endstopped cells could not only sample curved stimuli but could also encode curvature. Distinction of concave from convex curvatures seems to require the endstopped cells to have two more properties : direction selectivity and narrow RFs. While our results suggest that curvature can be coded at the level of the striate cortex by a local mechanism using all orientations in the curved line, the results are also compatible with the hypothesis that curvature is encoded, in addition, further along the visual pathway by a multilocal mechanism based on the difference in mean orientation at different loci (Koenderink and van Doorn, Biol. Cybern., 55:367, 1987).

404.11 **EXTRACTING 3-D CURVATURES FROM IMAGES OF SURFACES USING A NEURAL MODEL.** Sidney R. Lehky * and Terrence J. Sejnowski. (SPON: G. F. Poggio). Department of Biophysics, Johns Hopkins University, Baltimore, MD 21218.

Many neurons in primary visual cortex have oriented receptive fields, and consequently are often interpreted as "bar detectors" or "edge detectors". However, in addition to edges, there is significant information contained in the continuous gradations of shading within an image. We have investigated this by constructing a computer model of a neural network that extracts curvatures from the shading information contained in images of simple geometrical surfaces. Specifically, the network determines the principal curvatures (largest and smallest curvatures) at the center of the surface, and their orientation, independent of illumination direction, and the precise location of the surface within the overall receptive field of the network. The network, which is meant to reflect processing occuring within a single cortical column, has model neurons arranged in three layers. Each unit within a layer is synaptically connected to every unit of the next layer. The input layer is an hexagonal array of overlapping, circularly-symmetric, center-surround units with both ON and OFF centers. The output layer is a set of units in which each unit is broadly tuned to both principal curvature, and the orientation of that curvature. Therefore, the activity of a single output unit is ambiguous, and the output information is contained in the joint activities of the output units. Finally, there is the middle, or "hidden unit" layer, which transforms the retinotopic coordinates of the input layer to the curvature-orientation coordinates of the output layer.

We used the "back-propagation" learning algorithm[1] as a design technique to construct a network with the desired characteristics. The network was presented with many sample images, and for each presentation, the actual responses of the output units were compared with the correct output. Then the synaptic weights throughout the network were slightly modified to reduce the error. Following this procedure, the individual units within the hidden layer formed a variety of receptive fields, mostly oriented but a few non-oriented. The response properties of the units were mapped out using "simulated neurophysiology". Using bars of light as stimuli, the oriented hidden units appeared to have characteristics of simple cells. The output units, which receive input from many hidden units, appeared to have some properties of complex cells. We conclude that neurons which can extract curvature can have receptive field properties similar to those which previously were interpreted as bar or edge detectors. Yet other interpretations may be possible. The receptive field properties of a sensory neuron are necessary but not sufficient to determine its function within a network.

[1]Rumelhart, D., Hinton, G., and Williams, R. (1986) *Nature* 323, 533-536.

404.12 RESPONSE PROPERTIES OF STRIATE CORTEX NEURONS IN BEHAVING MONKEY: INSIGHTS FROM STABILIZING THE RETINAL IMAGE. D.Max Snodderly and Moshe Gur*.
Eye Research Institute of Retina Foundation, Boston, MA, USA, and Department of Ophthalmology, Harvard Medical School; and *Department of Biomedical Engineering, Technion-Israel Institute of Technology, Haifa, Israel.

Responses of single neurons in the striate cortex of a Macaca fascicularis monkey were studied while the monkey performed a fixation task and eye position was monitored by a double Purkinje image eyetracker. For most trials, the stimulus was stabilized on the retina. On other trials the stimulus remained stationary in space while the eye movements of fixation moved the stimulus on the retina. Both drifts and small saccades excited the neurons when they resulted in appropriate retinal image motions. Cells with receptive fields 1 to 6 deg. eccentricity were studied.

The receptive fields of cortical neurons move in space with the eye. When the image is not stabilized, estimates of receptive field activating areas are influenced (usually inflated) in unpredictable ways. When more precise control of retinal stimulus position is achieved by image stabilization, responses are vigorous and reliable. Response rates are often more than one hundred spikes per second with less than one log unit of stimulus contrast. There is a gradient of response rate with retinal eccentricity so that stronger responses occur in the fovea.

Receptive field subregions like those described in anesthetized animals can also be characterized in a behaving monkey when the image is stabilized. In addition, powerful inhibitory fields outside the activating regions shape the stimulus selectivity of the cell. These inhibitory fields are strikingly apparent in the behaving animal because of the high response rates that they cancel. When an appropriate stimulus is correctly identified, it stimulates the activating regions without eliciting the powerful inhibition. This means that the absolute response rate becomes an important indicator of the degree to which the experimenter has been able to establish the optimal stimulus for a neuron. Such a criterion has not been applied in the past because of inability to specify the physiological state of anesthetized animals, and inability to be confident of stimulus placement in behaving animals. The results from using a stabilized image with a trained, behaving monkey suggest that we have succeeded in controlling the major experimental variables. We refer to this as a fully calibrated experiment in visual neurophysiology.

404.13 A NETWORK MODEL USING BACK PROPAGATION LEARNING SIMULATES THE SPATIAL TUNING PROPERTIES OF POSTERIOR PARIETAL NEURONS. D. Zipser and R.A. Andersen. Inst. for Cognitive Science, UCSD, La Jolla, CA 92093; Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

Area 7a in the posterior parietal lobule of the monkey is involved in visual spatial representation. Neurons in Area 7a respond to both the retinal location of a visual stimulus and the position of the eyes but are virtually insensitive to the qualities of the stimulus such as size, color, or shape. By combining these two afferents Area 7a neurons code the spatial location of external objects; however, the responses of Area 7a neurons are not directly correlated with spatial location, so additional decoding is required. We have developed a network model that not only demonstrates how spatial information can be decoded from Area 7a neurons but also accounts for their observed quantitative response properties.

The modeling technique we employ makes use of a powerful network learning procedure called "back propagation." Back propagation is able to program the computation carried out by a network using only examples of input and output. Back propagation accomplishes this programming by adjusting the strengths of the synapses within the network. The networks generated by back propagation are optimized to reduce error by a gradient-descent algorithm.

The experimental data that must be accounted for was previously collected in studies with awake behaving monkeys. The retinal receptive fields observed for Area 7a neurons are large and often complex in shape. The receptive fields have one peak or a few peaks that form a smoothly changing, hilly landscape. Most of the eye-position gain fields (which represent the effects of different eye positions on the magnitude of the visual response for retinotopically identical stimuli) are planar and exhibit a characteristic nonlinear interaction between eye position and retinal information.

Our model consists of a three-layer network of neuron-like units. The first layer supplies the retinal and eye-position input information. The middle, or hidden, layer represents the neurons in Area 7a, and the third, or output, layer is trained to represent spatial location. We have found that, after training, the output layer accurately represents the true spatial location of the stimulus. What is more, the hidden unit responses re-create the retinal receptive fields of Area 7a neurons, and also their eye-position gain fields, complete with the characteristic nonlinear interactions between retinal and eye-position signals. There is no topographic organization of the hidden units for spatial tuning, eye position, or retinal position. This lack of topography has also been found for the cells in Area 7a.

LEARNING AND MEMORY: ANATOMY VI

405.1 ADDITIONAL NEURAL AND COGNITIVE EVIDENCE IN PATIENT DRB. A.R. Damasio, H. Damasio, D. Tranel, K. Welsh, and J. Brandt. Div. of Behav. Neurol., U. of Iowa Col. of Med., Iowa City, IA 52242.

Patient DRB has now been studied with advanced magnetic resonance imaging allowing for detailed neuroanatomical mapping of his lesions. In the left hemisphere these include the entorhinal cortex, the hippocampus, the amygdala, areas 38, 20, 21, and part of area 37. Damage in the right hemisphere is comparable but slightly more extensive within area 37; it undercuts the occipitotemporal junction. The basal forebrain region is destroyed bilaterally and so is the anterior insula. The medial aspect of the orbital frontal regions is partially destroyed bilaterally. Thus, virtually all the principal cortical and subcortical limbic structures have been ablated in DRB, and so have several of the key higher-order association cortices. In this light, the extent of DRB's preserved cognitive processes deserves special attention.

DRB's acquisition of new memory remains confined to covert forms of learning, e.g., (a) in rotor pursuit and mirror-tracing tasks DRB is comparable to controls although he has no declarative knowledge of the tasks; (b) in a priming task using word stems DRB shows evidence of priming, albeit at a lower rate than controls; (c) in a forced choice recognition of the words used in the priming task DRB performs at an 80% level; (d) daily observation of his behaviors in a constrained setting reveals covert learning of affect-laden stimuli.

In both the anterograde and retrograde periods of his history, DRB remains unable to recognize or recall at overt level any unique episode or stimulus and entirely unable to retrieve the specific temporal or spatial reference of any knowledge (this is true even for key items of his autobiography). By contrast, his access to "non-episodic" knowledge acquired prior to his illness is largely intact although the degree of preservation depends on the conceptual domain to which the stimulus belongs (natural kinds are less well preserved than man-made or ontologic kinds). DRB's linguistic knowledge (assessed with fine linguistic experiments), reveal the intactness of fundamental phonemic, phonetic, morphological, syntactical, and intonational representations and rules of language.

In order to study DRB's profile outside traditional memory assessment, he was administered several projective personality tests. On the Rorschach test, both the qualitative and quantitative features of his response protocols were normal. He uses typical determinants (predominantly form but also texture, color, etc.), and the responses contain an appropriate mixture of wholes and major and minor details. The stories that DRB created for the Thematic Apperception Test did not reveal "psychopathology." (They are sparse and unimaginative but contain appropriate temporal sequencing, and contingent actions and relationships between characters. Inconceivable events or relationships are not described).

405.2 ALZ-50 DEMONSTRATES THE DESTRUCTION OF HIPPOCAMPAL PROJECTION NEURONS IN ALZHEIMER'S DISEASE. W.G. Tourtellotte, G.W. Van Hoesen, B.T. Hyman, L.J. Kromer* and A.R. Damasio. Depts. of Anatomy and Neurology, University of Iowa College of Medicine, Iowa City, IA 52242.

The hippocampal formation is a major target for pathological changes that occur in Alzheimer's disease (AD), such as neurofibrillary tangles. The Sommer sector has been implicated consistently, especially in its more medial or subicular part, which is the source of projection neurons that send axons to both telencephalic and diencephalic areas. By contrast, comparatively less pathology has been reported in the adjacent CA1 part of Sommer's sector, a field that is connected powerfully with the subiculum and shares many extrinsic projections with it. We have examined and compared the extent of subicular and CA1 pathology in AD using both conventional pathological stains, such as Congo red and thioflavin S, and a highly sensitive monoclonal antibody, Alz-50, which recognizes an antigen found in high concentrations in AD. Both conventional stains and Alz-50 immunocytochemistry reveal pathological involvement of the subiculum and CA1 zones. However, the pattern of immunoreactivity using Alz-50 reveals that nearly the entirety of the CA1 zone is targeted in many AD brains as well as some neurons in the CA3 and CA4 zones. These findings are of interest because they reveal that all neurons that form Sommer's sector are undergoing alterations in this disorder. Left unaffected, is only a partial population of CA3 and CA4 hippocampal neurons which project to the septal region (many reports suggest that the cholinergic neurons of the septem, may themselves be targets for pathologic involvement in AD). The CA1 and subicular pyramids are highly interconnected allocortical areas and they project massively to layer IV of the entorhinal cortex. Since the latter is also targeted in AD, it is clear that both intrinsic connections as well as extrinsic connections of the hippocampal formation are explicitly affected, with both the cell of origin for axons within Sommer's sector affected as well as its postsynaptic target. This leads to a nearly total deefferentation of the hippocampal formation. Combined with significant alterations in hippocampal input due to both entorhinal and basal forebrain pathology, there seems little doubt that the integrity of hippocampal neural systems are highly compromised in AD. In fact, it is likely that these changes have no less devastating functional consequences for memory processes than hippocampal destruction itself. (Supported by: NS 14944, PO NS 19632 and the Mathers Foundation. We thank P. Davies and B. Wolozin, for providing Alz-50).

405.3 COVERT DISCRIMINATION OF FAMILIAR STIMULI OTHER THAN FACES IN PATIENTS WITH VISUAL RECOGNITION IMPAIRMENTS CAUSED BY OCCIPITO-TEMPORAL DAMAGE. D. Tranel, A.R. Damasio, and H. Damasio. Div. of Behav. Neurol., U. of Iowa Col. of Med., Iowa City, IA 52242.

Using the electrodermal skin conductance response (SCR) as a dependent measure, we have previously demonstrated that subjects with severe defects of face identity recognition can discriminate, at a covert level, stimuli that they fail to recognize overtly. Those patients often show recognition impairments in other categories of stimuli, such as cars or buildings, although this has received little systematic investigation. The current study probed the recognition performance of three subjects, in the categories of cars, buildings, personal effects, and faces, using SCRs and verbal ratings as dependent measures of covert and overt levels of processing. The data were related to detailed neuroanatomical analysis of each subject, based on magnetic resonance imaging.

For each category (cars, faces, etc.), the set of stimuli consisted of 40 items, 10 of which were targets (items the subject was highly familiar with) and 30 of which were nontargets (items the subject had never encountered before). Targets and nontargets were randomly mixed and presented one at a time, while skin conductance was recorded from the palm of each hand. During a second presentation, the subject used a preassigned scale to make a verbal rating of the degree of familiarity of each stimulus.

Based on their verbal ratings, all three subjects showed marked difficulty in the overt recognition of target items in the four classes of stimuli. However, the subjects often produced discriminatory SCRs to the targets they could not overtly recognize. For example, subjects #1 and #2 showed significantly larger SCRs to target faces, buildings, and cars, and subject #2 even showed this effect for familiar personal belongings. Neuroanatomical findings were as follows: Subject #1 has bilateral subcalcarine damage in areas 18/19 and unilateral right hippocampal damage; Subject #2 has bilateral damage to areas 20/21, 37, and the hippocampal region; Subject #3 has right subcalcarine damage in 18/19, and left parahippocampal damage.

The results suggest that both in relatively "pure" visual agnosics (whose lesions are placed in "early" visual association cortices), as well as in so-called global amnesics (whose lesions involve higher-order association cortices and the hippocampal region), the brain "recognized" stimuli to which it had been previously exposed. Given the diverse placement of the lesions, this indicates that the multi-component records necessary for recognition of unique familiar stimuli are laid down at multiple levels of CNS and can be accessed by different routes. For all 3 subjects, access of those records was capable of triggering autonomic responses, but failed to lead to the evocation of pertinent factual representations which are necessary for "conscious" recognition.

405.4 MEMORY DEFICITS IN CALLOSAL AGENESIS. I. Brown and F. Wood.* Bowman Gray Sch. of Med., Winston-Salem, NC 27103.

Agenesis of the corpus callosum (ACC) often occurs in conjuction with a complex of serious CNS malformations which are symptomatic with widely varying degrees of severity. Thus, in agenesis, when cognitive deficits are noted, they tend to be attributed to the accompanying anomalies rather than to the agenesis, per se. Indeed, the consensus has been that when agenesis occurs alone, symptoms are absent or so mild as not to attract clinical attention.

Compensatory mechanisms hypothesized to account for this apparent lack of symptoms in acallosals have included overdeveloped ipsilateral pathways and assumption of information transfer by remaining interhemispheric commissures. Additionally, it has been suggested that a more parsimonious explanation may be that we have not applied sufficiently sensitive measures to study the question adequately. For example, eliminating cross-cueing, Gazzaniga found deficits in acallosals similar to those shown by commissurotomy patients. Milner concludes that both cognitive and skilled performances probably suffer as a result of agenesis, but that there are great individual differences. Our experience with two adolescent boys supports this view.

Case 1 had onset of seizures at age 16 months, but despite this, early development progressed normally and the boy demonstrated advanced verbal skills. By age 10, his grades had declined, he was easily disoriented, and memory problems were severe. At age 11, magnetic resonance imaging (MRI) revealed complete ACC and enlarged anterior cerebral arteries, but no other abnormalities.

Case 2 has had no seizures, but was slow in achieving developmental milestones and has a history of academic difficulty. Parents report that he has poor motor skills and poor short-term memory. CT scan revealed complete ACC with dilated ventricles, but no other abnormalities.

Both boys have WISC-R scores in the low average range. Scores on neuropsychological tests were somewhat inconsistent across the two profiles except for a substantial deficit in memory for structured verbal and visual material as measured by the Cowboy Story and the Rey-Taylor figure.

Nakamura and Gazzaniga have suggested that while a single hemisphere may perform as well as a whole brain on a simple short-term memory task, each hemisphere may have certain load limits which, if exceeded, will result in memory deficit. The imposition of structure onto a memory task such as the Cowboy Story and Rey-Taylor figure may add to the memory load so that a limited capacity becomes overloaded. Further, if, as they speculate, a major commissural function is that of suppressing competing motor responses, such responses may act as a source of interference in memory tasks. These cases lead us to suspect that it is premature to assume that ACC is asymptomatic and that memory deficit in ACC must be explored.

405.5 LONG TERM MEMORY DISTURBANCES IN BRAIN-DAMAGED ADOLESCENTS AS ESTABLISHED BY A COMPREHENSIVE MEMORY TEST BATTERY. H.J. MARKOWITSCH* and E. HOFMANN* (SPON: ENA). Dept. Psychology, Univ. Konstanz, D-7750 Konstanz, Fed. Rep. Germany.

Brain-damaged adolescents with memory disturbances as a principal symptom were tested with the help of a newly developed memory battery. This consisted of 1) self-assessment scale, 2) a questionnaire on general orientation and remote memory, 3) a prose text, 4) remembering a name, 5) remembering a short route and message, 6) a word list (Buschke), 7) a cube test, 8)-11) memory for faces, tonal sequences, odors, tactile stimuli, 12) prose (after long delay), 13)remembering a name, 14) remembering a route and message (after long delay), 15) a word list (after long delay), 16) the tower of Hanoi problem, 17) paired-associate learning, 18) the Benton test, 19) selective visual-spatial reminding (Muramoto), 20) digit span, 21) episodic remote memory, 22) a delayed-match-to-sample task, 23) the Wisconsin Card Sorting Test, 24) paired associate learning under a sentence condition, 25) solving the tower of Hanoi problem after a long delay, 26) keeping a diary, 27) a verbal short term memory test. Furthermore, 28) a personnel assessment of the patient's memory was filled out by the testing psychologist.

In building the test battery emphasis was laid on including memory tests in several sensory modalities, on episodic and skill memory, and such tests emphasizing everyday memory situations.

Of the 8 patients one had suffered from cerebral hypoxia, one from a hypophysis-adenome with fronto-basal damage; 2 others had fronto-temporo-basal damage; 2 had traumata (in the temporo-parietal cortex in one case and in the occipital cortex, with additional encephalitis, in the other); one patient had an occlusion of the internal carotis with left basal ganglia changes; and the last had a rupture in the middle of the corpus callosum with possible fornix damage. The behavior of these patients was compared with that of a matched control group.

While the test profiles varied between individuals, the following findings were consistent: Largest deficits were obtained in tests using long delay periods, while short delays usually had little effect; IQ points generally were superior to the patients' performance in everyday situations. There was - compared to normal controls - a poor adaptation of strategies and a slow speed in processing memory tasks.

405.6 RADIOLOGICAL (CT) FINDINGS IN PATIENTS WITH KORSAKOFF'S SYNDROME AND THEIR RELATIONSHIP TO MEMORY IMPAIRMENT. A.P. Shimamura*, T.L. Jernigan and L.R. Squire. V.A. Med. Ctr. San Diego, CA 92161 and Dept. of Psychiatry, UCSD, CA 92093.

We obtained computed tomography (CT) scans from 7 patients with Korsakoff's syndrome, 7 age-matched alcoholic subjects, and 7 age-matched healthy control subjects. Quantitative analyses were performed by using CT values to estimate fluid volume and tissue density in several brain regions. We assessed fluid volume bilaterally in six regions, defined as frontal lobe, Sylvian fissure, vertex, third ventricle, superior quadrageminal cistern, and medial cerebellum. Brain density was estimated by averaging CT values in small (5 mm x 5 mm) areas sampled bilaterally in defined areas: thalamus, caudate nucleus, putamen, anterior white matter, posterior white matter, and centrum semiovale.

Compared with healthy controls, both patients with Korsakoff's syndrome and alcoholic subjects exhibited increased fluid volume in the frontal lobe ($ps < .05$) and Sylvian fissure ($ps < .08$). Compared with alcoholic subjects, patients with Korsakoff's syndrome exhibited increased fluid volume in the third ventricle ($p < .08$), low density values in the thalamus ($p < .01$), as well as marginally low density values in the caudate nucleus ($p = .09$). Moreover, they exhibited even greater fluid volume in the Sylvian fissure ($p = .06$) than alcoholic subjects. No other structural measures approached statistical significance. In summary, cortical regions were generally affected by alcoholism, whereas diencephalic regions were particularly affected by Korsakoff's syndrome.

We investigated the relationship between structural abnormalities and memory impairment in the 7 patients with Korsakoff's syndrome. A series of Spearman rank order correlations was performed between structural measures and each of 12 independent measures of anterograde amnesia. Because each brain region provided one measure for the left hemisphere and one for the right hemisphere, we obtained a total of 24 independent correlations for each region. Only two structural measures yielded a significant number of positive correlations with memory measures. These were the thalamus density measure (median $r = .34$, 18/24 positive correlations, $p < .025$) and the frontal fluid measure (median $r = .43$, 18/24 positive correlations, $p < .025$). No other correlational analysis approached statistical significance. Thus, the memory impairment observed in patients with Korsakoff's syndrome was correlated with thalamic and frontal lobe damage but not with other structural impairment.

405.7 Cognitive Deficits in Patients with Frontal Lobe Lesions: Significance for the Amnesia of Korsakoff's Syndrome
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Patients with Korsakoff's syndrome typically exhibit disorders of cognitive function in addition to severe anterograde and retrograde amnesia. These deficits include impaired verbal fluency, failure to release from proactive interference (PI), and impaired metamemory. These deficits are often absent in other etiologies of amnesia and are dissociable from the recall and recognition deficits of amnesia. It has been suggested that these deficits may depend on frontal lobe damage. We studied three patients who had lesions of the right frontal lobe, two who had lesions of the left frontal lobe and two who had bilateral lesions. The lesions were caused by a cerebrovascular accident or neurosurgery for abscess. All patients exhibited normal IQs and normal performance on the Weschler Memory Scale, but were impaired on the Wisconsin Card Sort Test.

Verbal Fluency: Patients with left or bilateral (but not right) frontal lesions were markedly impaired in verbal fluency as measured both by the Benton Verbal Fluency Test and by the Initiation and Perseveration subtest of the Dementia Rating Scale (DRS, ps<.05). This finding is consistent with the idea that the deficit in verbal fluency in patients with Korsakoff's syndrome is due to frontal lobe pathology.

Proactive Interference: Previous studies showed that a shift in the semantic category during list learning will improve recall (release from PI). Patients with Korsakoff's syndrome do not benefit from a category shift. All of the patients with frontal lobe lesions demonstrated release from PI, and their performance did not differ from that of control subjects. These results suggest that frontal lobe damage alone may not be sufficient to impair release from PI.

Metamemory: Patients with Korsakoff's syndrome are poor at judging how accurate they will be on a subsequent memory task We found that patients with frontal lobe lesions also show a deficit in metamemory performance compared to age-matched control subjects (p<.05). This finding suggests that metamemory is dissociable from the ability to remember new information and may depend on the integrity of the frontal lobes.

In summary, some cognitive deficits (verbal fluency and metamemory) are common to amnesic patients with Korsakoff's syndrome and patients with frontal lobe disease. Patients with frontal lobe disease do not exhibit severe memory impairment. These cognitive deficits may therefore be related to frontal lobe pathology in patients with Korsakoff's syndrome.

405.8 HIPPOCAMPAL LESIONS, BUT NOT FORNIX OR MAMMILLARY BODY LESIONS, PRODUCE LONG-LASTING AMNESIA IN MONKEYS.
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We have used a battery of tasks sensitive to human amnesia to characterize in monkeys the severity, pervasiveness, and duration of memory impairment following damage to specific medial temporal and diencephalic structures implicated in the human amnesic syndrome. One traditional idea has been to suppose that amnesia results when damage occurs within a functional neural circuit that includes most prominently the hippocampal formation, fornix, and mammillary bodies.

To clarify whether these anatomically related structures contribute equally to memory function we administered six memory tasks to separate groups of monkeys with bilateral lesions of the hippocampus (H), fornix (FX), and mammillary bodies (MB), and to a group of normal monkeys (N). The H lesions damaged the hippocampus proper, dentate gyrus, subiculum, posterior entorhinal cortex, and most of the parahippocampal gyrus. On the first task, delayed nonmatching to sample, the H and MB groups, but not the FX group, were impaired. The H group was impaired on all subsequent amnesia-sensitive tasks (see Table), and the impairment endured for at least 1.5 years from surgery. The FX and MB groups were unimpaired on all subsequent tasks (except delayed response, on which the FX group was impaired).

SUMMARY OF BEHAVIORAL FINDINGS

	1 Delayed NMTS	2 Object Retention	3 8-Pair Concurrent	4 Delayed Response	5 Pattern Discrim.	6 Motor Skill
H	+	+	+	+	-	-
FX	-	-	-	+	-	-
MB	+	-	-	-	-	-

Tasks 1-4: tasks sensitive to human amnesia; Tasks 5-6: tasks analogous to those amnesic patients do not fail; NMTS = nonmatching to sample; + = impairment; - = normal performance.

These findings, together with previous anatomical findings suggest that the fornix and mammillary bodies may not be as prominent in the neuropathology of amnesia as once thought. In contrast, damage to the hippocampal formation and adjacent parahippocampal gyrus produces a significant and long-lasting memory impairment.

405.9 NEW EVIDENCE OF BRAIN INJURY IN THE AMNESIC PATIENT N.A. BASED ON MAGNETIC RESONANCE IMAGING.
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N.A. has been amnesic since 1960 when he sustained a penetrating brain injury with a miniature fencing foil. The amnesia occurs as a strikingly circumscribed disorder, primarily affecting verbal material. Previous CAT scans demonstrated a lucency in the region of the left mediodorsal nucleus, but no additional damage was revealed. In 1986 and 1987 N.A., now 48 years old, was studied at the Magnetic Resonance Institute at UCSD. Initially, contiguous 3mm sections through the forebrain, diencephalon and mesencephalon were imaged using a T1 weighted sequence. Separate studies were carried out in the coronal, horizontal, and sagittal planes. Subsequently, other studies were conducted with 5mm sections and T1, T2 or balanced sequences. Three major areas of damage were identified. In the left thalamus there is a prominent linear defect. Its path approximates the internal medullary lamina, and it extends from within the third ventricle to nearly the lateral limit of the thalamus. The defect extends for approximately 15mm anteroposteriorly and likely involves the mediodorsal thalamic nucleus as well as the ventral anterior, ventral lateral and midline nuclei; it also likely interrupts the trajectory of the mammillothalamic tract. On the basal surface of the left anterior hypothalamus the T2 weighted images show an apparent mass that is interpreted as either gliosis, microcystic encephalomalacia or a highly proteinaceous cyst. There is extensive damage to the posterior hypothalamus which is both bilateral and symmetric. One important finding is that the mammillary nuclei are not visible in images from any of the three planes. Finally, the right anterior temporal lobe is replaced by signal that resembles CSF. This image represents either an actual parenchymal defect or macrocystic encephalomalacia, which could have occurred during the craniotomy that was done following N.A.'s injury. This damage extends from the temporal pole anteriorly, to a level approximately midway through the amygdaloid complex; the hippocampal formation on the right side appeared entirely intact. In summary, damage was found in left diencephalic structures, bilateral mammillary nuclei and right anterior temporal lobe. The significance of this damage for understanding N.A.'s circumscribed memory impairment will be discussed.

405.10 MULTIPLE RELATIONS BETWEEN FACT-LEARNING AND PRIMING IN GLOBAL AMNESIA.
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Despite their fact-learning deficit, globally amnesic patients can sometimes be influenced in a normal fashion by prior exposure to a stimulus, when subsequently asked to perform a task with that stimulus (priming). Until recently, the preservation of priming appeared to be unrelated to the severity and etiology of amnesia or to the locus of brain lesion. More recently, however, Graf and Schacter (1985) found that priming of new word associations was intact only in mildly amnesic patients. This link between an aspect of fact-learning (reflected in severity of amnesia) and some aspect of priming (for new word associations) led us to consider whether there are a variety of relationships between fact-learning capacities and different kinds of priming. In order to address this question, we examined the status of different kinds of priming (stem completion, homophone-spelling, lexical decision, fragment completion, naming of words and nonwords) in patients with amnesias of varying severity. These sorts of priming were selected because they have been dissociated from some measures of fact-learning in normal subjects.

In one experiment, we tested 4 patients with amnesias due to bilateral lesions of limbic-diencephalic structures. Two of them had severe amnesias (H.M. and a patient with Korsakoff's syndrome), and two had mild amnesias (a patient with bilateral stroke and another with encephalitis). Following exposure to a list of words, all four patients were impaired relative to normal control subjects in recall and recognition of the words, but the latter two patients were less impaired then the former. Nevertheless, all four patients showed similar and normal levels of stem-completion priming with the same words. In contrast, other studies with H.M. revealed an association between his fact-learning and priming performance on several tasks.

Thus, severity of amnesia interacts selectively with some but not other kinds of verbal priming. This finding suggests that some forms of verbal priming include a component mediated by neural systems subserving fact-learning. Further, these results call for reconsideration of some dissociations between priming and fact learning in normal subjects.

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405.11 FAILURE TO IMPROVE A SKILL FOR MENTAL ROTATION IN GLOBAL AMNESIA. L. M. Parsons*, J.D.E. Gabrieli, and S. Corkin. Department of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139. (SPON: K. Nakayama).

Patients with global amnesia have been able to acquire and retain a variety of motor, perceptual, and problem-solving skills despite an impairment in fact learning. These patients demonstrated intact learning by gradually improving the efficiency of their performance across days. No explicit account exists, however, of the mechanisms of skill learning that specify what skills may be acquired by the neural systems that appear to mediate these patients' normal learning for such tasks as mirror reading and mirror tracing. By current understanding, it seems that an amnesic patient ought to possess and to be able to improve mental rotation skill. Observers often mentally rotate one object to the orientation of another when required to discriminate between identical and slightly different shapes seen at different orientations. The time to rotate an object mentally is usually a monotonically increasing function of the angle of rotation. The slope of the reaction-time/orientation function indicative of mental rotation can decline greatly with practice. Such decline in slope occurs gradually and does not appear to depend upon fact-learning processes.

In our study, the subjects were H.M., whose global amnesia followed bilateral medial temporal-lobe resection, and three normal control subjects of similar age and education. They pressed one button marked "normal" to indicate that an R was in the normal form, or another button marked "reversed" to indicate that an R was in the mirror-reversed form. Stimuli were presented at each of 12 rotations, 30° apart. Subjects performed 72 trials in each of two sessions (one hour apart) on three successive days.

As expected, H.M. had impaired memory for having performed the task or having seen the shapes in prior sessions. His initial performance was slow, but showed evidence of mental rotation comparable to that of normal subjects. Surprisingly, he showed no improvement in the mean reaction time of his judgment and no decrease in the slope of the reaction-time/orientation function. He did make fewer errors with practice, but the improvement could not be dissociated from a possible speed-accuracy tradeoff. Control subjects showed moderate improvements in all performance measures.

To the extent that these results indicate that fact-learning ability is necessary for improving skill in mental rotation, our finding suggests that we need to reformulate our conception of what skills amnesic patients can learn and what neural systems underlie mental rotation skill in normal subjects.

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405.12 ACQUISITION OF PROBLEM-SOLVING SKILLS IN GLOBAL AMNESIA. J.D.E. Gabrieli, M. M. Keane*, and S. Corkin. Department of Brain and Cognitive Sciences, and Clinical Research Center, MIT, Cambridge, MA 02139.

The presence of spared learning capacities in global amnesia indicates that distinct neural systems subserve different kinds of memory. Understanding the functions of these neural systems requires knowledge about the cognitive mechanisms that support preserved learning. The present studies followed a report that an amnesic patient, H.M. (whose amnesia followed bilateral medial temporal-lobe excisions) could acquire and retain a skill for solving the Tower-of-Hanoi problem (Cohen and Corkin, 1981). In the Tower-of-Hanoi protocol followed by Cohen and Corkin, a prescribed set of questions was posed to subjects throughout the sessions in order to encourage all subjects to adopt the same effective strategy. In order to assess the role of those questions in H.M.'s learning, we readministered the problem to H.M. in a protocol in which examiner-subject discussions were minimized by not posing these questions. Under these conditions, H.M. failed to improve his solution efficiency across days. Unlike his performance in 1981, H.M. selected his moves very rapidly. His quick responses made it impossible to readminister the problem in the original fashion because there were no intervals in which an examiner could pose questions. Also remarkable was H.M.'s mastery, from the start of the current sessions, of some portions of the puzzle and his poor performance in other portions; his ability to extract himself from novel positions not encountered during efficient solutions was especially impaired. On a second test of problem-solving, the Missionaries-and-Cannibals problem, H.M. and two other amnesic patients (with amnesia due to Korsakoff's syndrome and bilateral stroke) demonstrated increasing efficiency across days.

These results are interpreted as being consistent with the idea that amnesic patients can learn problem-solving skills, but the role of examiner-patient interaction in shaping that skill merits further consideration in order to characterize the nature of that learning. Such consideration may be a prerequisite for understanding the theoretical significance of preserved problem-solving capacities in global amnesia.

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SPECIFICITY OF SYNAPTIC CONNECTIONS AND SYNAPTOGENESIS

406.1 GROWTH CONES ISOLATED FROM IDENTIFIED *APLYSIA* NEURONS: ANALYSIS OF PROTEINS AND GLYCOPROTEINS. J. Protic* and R. T.Ambron, Dept. Anatomy and Cell Biology, Columbia University, New York, N.Y. 10032

Efforts to identify molecules that mediate synaptic interactions have been aided by the use of dissociated cell cultures. A particularly advantageous preparation utilizes *Aplysia* neurons, many of which are large and amenable to analysis by a variety of techniques. We have been using identified neuron L7, which innervates auricle muscles (Alevizos and Koester, '86), and the RUQ neurons that innervate muscles of the vasculature (Rittenhouse and Price,'86) When the individual neurons are grown in culture with their appropriate muscle cells, the growth cones firmly contact the target and in some cases induce spontaneous contractions. Intracellular stimulation of L7 causes contractions of the auricle muscles *in vitro* and evokes EJPs.

We presume that the molecules involved in target recognition are present on the surface of the L7 and RUQ growth cones. RUQ cells have extraordinarily large growth cones in culture that can be isolated and analysed directly (Flaster et al.,1986). We first used a general labeling procedure to survey how many membrane constituents are present and then focused on the glycoproteins. Isolated growth cones were iodinated using either ^{125}I-Bolton-Hunter reagent or the chloramine-T method and a crude membrane fraction was obtained by centifugation. The radiolabeled proteins were resolved by 2D PAGE followed by fluorography. Over 50 polypeptide species were detected, including 7 components that are rapidly transported along axons of *Aplysia* neurons *in vivo* . These then constitute a group of synaptic polypeptides that are also present at growth cones.

The oligosaccharide moieties present in the glycoproteins on the RUQ growth cone surface were fractionated using a combination of lectin affinity chromatography, gel filtration, and HPLC. This protocol has successfully resolved nine classes of glycopeptides from the glycoproteins of individual *Aplysia* neurons. RUQ cells were grown in the presence of ^{3}H-glucosamine for 24h and the growth cones collected. ^{3}H-glycopeptides, released from the glycoproteins by pronase treatment, were analyzed and six species were found to be enriched in the growth cones. None of these glycopeptides bound to Concanavalin A, but at least one binds to wheat germ agglutinin (WGA). The localization of this species was confirmed by the extensive binding of FITC-WGA to growth cone membranes. Interestingly, analyses of several neurons,including L7, have shown that similar WGA binding glycopeptides are constituents of axonally transported glycoproteins. These may then constitute a class of glycopeptide that is present at all growth cones. We do not yet know if there are differences in the composition of the glycopeptides among the cells. To see if these glycopeptides will bind to target cells, we used WGA-agarose to isolate the glycopeptides from neurons of 180 ganglia. The bound material was coupled covalently to 0.9um fluorescent beads which were then added to muscle cells in culture. We found intense labeling of certain muscle types. The nature and specificity of the binding is now under investigation. Supported by The MDA and NIH.

406.2 **SELECTIVE SYNAPTOGENESIS AND MODULATION OF JUNCTIONAL CONDUCTANCE IN CULTURED NEURONS OF *APLYSIA*.** G.M. Carrow and I.B. Levitan. Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254.

Neurons grown in primary culture after dissociation often exhibit selectivity in the formation of synapses. For example, pairs of neurons obtained from homologus ganglia or cell clusters ("homologous" pairs) in the mollusc, *Aplysia californica*, show different patterns of connectivity than cell pairs derived from heterologous ganglia ("heterologous" pairs). We have now found that selectivity in formation of electrical synapses between cultured *Aplysia* neurons is expressed as a difference in junctional conductance.

We examined electrical synapses in homologous and heterologous pairs of neurons derived from juvenile buccal and pleural ganglia as well as from bag cell clusters in the adult abdominal ganglion. The neurons were dissociated after treatment of the ganglia with dispase and were grown in a modified L-15 medium plus hemolymph at 19°C. After the neurons had grown overlapping neurites, each cell of a pair was voltage clamped with a single microelectrode by switching between current injection and voltage monitoring. In order to obtain a direct measure of junctional conductance, both cells of a pair were clamped to the same membrane potential, transjunctional voltage pulses were applied, and the resulting junctional current monitored.

Homologous pairs of cells were coupled with a high junctional conductance in the range 10 to 25 nS, similar to that previously shown for pairs of buccal neurons (Bodmer et al., 1987, *J. Neurosci.*, in press). Although heterologous pairs of cells were also coupled when grown in the presence of hemolymph, the junctional conductance in these cases was low, ranging from 1 to 5 nS. As previously found for buccal pairs, both classes of junction were non-rectifying, ungated by voltage, and resistant to treatment with 1 mM octanol. These shared properties indicate that the various cell types have similar junctional channels. Thus, the observed dichotomy in junctional conductance may result from modulation of a single type of junctional channel. A further indication that these channels may be modulated is our finding that the effect of Concanavalin A on promoting electrical coupling between heterologous cells (Lin & Levitan, 1986, *Soc. Neurosci. Abstr.* 12:540) likely results from an increase in junctional conductance induced by the lectin.

The observation of strong electrical coupling *in vitro* between neurons with a common ganglionic origin is consistent with observations of intraganglionic electrical coupling *in vivo* for each of these cell types. By contrast, interganglionic electrical coupling has rarely been observed in *Aplysia*. It remains to be determined how these cells are able to distinguish homologues from non-homologues. The ability to quantify and modulate electrical coupling in the simple system described here should facilitate study of this and other problems of connectivity. [Supported by NSF grant BNS84-00875 to IBL and NRSA fellowship F32-HD06739 to GMC.]

406.3 IN VITRO ANALYSIS OF POSITION-DEPENDENT SELECTIVITY DURING THE INITIAL STAGES OF SYNAPTOGENESIS. D.H. Sanes*, D. Lo, and M.-m. Poo. Section of Molecular Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

It has been proposed that the location of a cell within the pre- and postsynaptic populations may endow it with a unique molecular identity. This positionally derived information could be of use in establishing the appropriate pattern of synaptic connections. We have begun to test this hypothesis using an in vitro preparation of neurons and myocytes obtained from disparate axial positions of the Xenopus embryo.

Fertilized eggs were injected with rhodamine- or fluorescein-conjugated dextran. When the embryos had progressed to Nieuwkoop and Faber stages 22-24 they were prepared for primary culture. The neural tube and myotome were dissected free and sectioned into 4-6 equivalent pieces. The most rostral segments from rhodamine labeled animals, and those segments located approximately 75% caudally from fluorescein labeled animals, were dissociated and cocultured for 1-2 days at room temperature. Myoballs were manipulated into contact with neurons of same or different label (i.e. axial position), and the quality of induced synaptic transmission was monitored using whole-cell recording techniques.

Both rostrally and caudally derived myoballs were capable of eliciting transmitter release from the same neurite. There was no apparent difference in frequency or amplitude of the spontaneous end-plate currents over the course of 1-10 minutes. In addition, we have observed synaptically elicited contractions for positionally unrelated nerves and myocytes that had been in contact for several hours. We are currently investigating these more extended time points in a quantitative manner. The ability of rostrally or caudally derived myoballs to adhere with a neurite does not appear to be influenced by position either, although our assay is not quantitative. When a positionally unrelated myoball is withdrawan from a neurite after 10 minutes in contact, the adhesion is capable of lifting the neurite off of the substratum. We tentatively conclude that there does not appear to be an initial preference for synaptic transmission or adhesion between cholinergic neurons and myocytes based upon their original axial position within the embryo. Supported by NS-22764 and NS-12961.

406.4 SENSORY NEURONS THAT INNERVATE A SPECIFIC TARGET ARE GENERATED OVER A PROTRACTED DEVELOPMENTAL PERIOD. B. Mendelson and E. Frank. Dept. of Neurobiology Anatomy and Cell Science, University of Pittsburgh, Pittsburgh, PA 15261.

The times of last cell division (birthdays) of sensory and motor neurons that innervate the triceps brachii muscles of the bullfrog forelimb were determined to learn if neurons that innervate a specific target are generated at a specific developmental time. Previous investigators, examining the generation of unidentified sensory and motor neurons, have suggested that large muscle sensory afferents may develop at earlier times than smaller neurons innervating cutaneous targets. We have combined ^{3}H-thymidine (^{3}H-TdR) autoradiography with HRP histochemistry to focus specifically on the development of neurons that innervate a particular target.

A labeling paradigm was implemented such that ^{3}H-TdR was available continuously throughout a specific developmental period. After the tadpoles metamorphosed, triceps neurons were labeled by filling them retrogradely with HRP. Any HRP-filled triceps neuron generated during the period of ^{3}H-TdR administration thus had a ^{3}H-labeled nucleus. The retinae of the same frogs were also examined since retinal neurons are known to be added continuously around the circumference of the eye during development. The pattern of labeled retinal neurons served to verify the sharp onset and continuous availability of ^{3}H-TdR during the period of labeling. Retinal cells born after the termination of ^{3}H-TdR injections were weakly labeled compared to those generated during the administration of ^{3}H-TdR.

All neurons that innervated the triceps muscle were generated prior to metamorphosis. However, triceps sensory neurons were generated over a protracted period of larval development, from early limb bud through early pre-metamorphic stages. Their time course of generation parallels that previously described for all other brachial sensory neurons. We also observed no strict relationship between sensory soma size and birthdate; large and small triceps neurons were labeled at both early and late developmental times. Thus, the time of origin of triceps sensory neurons is not a discrete developmental event; triceps afferents appear to be generated at the same time as neurons that innervate other brachial muscles and those that innervate cutaneous targets.

Triceps motoneurons were born earlier than the sensory neurons. Fewer than 5% of the triceps motoneurons were labeled after ^{3}H-TdR injections performed at early limb bud stages, and no triceps motoneurons were labeled at later developmental times. Thus, triceps motoneurons are born only during embryonic and early larval stages.

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406.5 SPECIFICITY OF DEVELOPING SENSORY PROJECTIONS TO THE SPINAL CORD. Carolyn L. Smith Dept. Neurobiol. Anat. & Cell Sci., Univ. Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Sensory neurons have highly stereotyped central projections. Neurons in each ganglion project to specific segments of the spinal cord and arborize in specific laminae. However, following removal of dorsal root ganglia from tadpoles, neurons in adjacent ganglia can project to areas of the spinal cord they do not normally innervate (Frank and Westerfield, J. Physiol. 324: 495, 1982; Smith and Frank, Brain, Behav. Evol., in press). A possible explanation of this plasticity is that developing sensory neurons project more widely than sensory neurons in adults and that partial deafferentation of the spinal cord allows the retention of these widespread projections.

To assess the specificity of sensory projections during their development, I used HRP to label central projections from dorsal roots in tadpoles at different developmental stages. Neurons in both thoracic and limb-level ganglia are generated throughout the first half of the larval period. Therefore, sensory fibers must grow into the spinal cord during these stages. Nevertheless, fibers from each ganglion arborize only at appropriate levels of the spinal cord. For example, thoracic sensory neurons form dense neuropils in the thoracic spinal cord and the brain stem. Although their axons pass through the brachial region, they rarely branch or form boutons at this level. This is true even at stages when no other sensory neurons arborize in the brachial region. Developing sensory neurons also project selectively to appropriate laminae. Thoracic sensory neurons, most of which are cutaneous afferents, arborize in the dorsal horn but not in the intermediate gray matter. Conversely, hypoglossal sensory neurons, which are muscle afferents, arborize in the intermediate gray matter but not in the dorsal horn. This specificity of developing sensory projections suggests that the novel projections formed by sensory neurons following removal of adjacent ganglia are actually *induced* by the operation. Since sensory neurons also innervate wider areas of the periphery than normal after removal of adjacent ganglia, one possibility is that they are induced to form novel central projections by their novel peripheral targets. An unexpected finding was that afferents from the limbs only begin to arborize in the regions around their own dorsal roots several stages *after* they form projections to more rostral and more caudal regions of the spinal cord. These differences in the stages at which projections to different spinal levels develop suggest that local properties of the spinal cord may control the timing of sensory fiber arborization.

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406.6 **Specific Synaptic Connections Between Muscle Sensory and Motor Neurons Form in the Absence of Coordinated Patterns of Muscle Activity.** E. Frank, Dept. of Neurobiology, Anatomy & Cell Sci., Univ. Pittsburgh, Pittsburgh, PA 15261.

The synaptic connections between stretch-sensitive muscle sensory afferents and spinal motoneurons are highly specific, but the mechanisms responsible for assuring the specificity during development are unknown. One possibility is that the temporal correlation (or anti-correlation) of electrical activity in pre- and post-synaptic neurons reinforces appropriate synaptic connections. To test this possibility experimentally, I disrupted the normal motor activity patterns of limb muscles in tadpoles during the developmental period when sensory-motor connections are made centrally, and then assessed the effects of this disruption on the pattern of these connections after they had formed.

The motor innervation of forelimb muscles was disrupted unilaterally in bullfrog tadpoles at midlarval stages (Taylor & Kollros XIV to XVII) by removing a 0.5-1.0 mm segment of the second ventral root, which contains the axons of all motoneurons innervating the forelimb. Motoneurons subsequently reinnervated muscles in a non-specific manner (Farel, PB, *JCN* 254:125, '86). Sensory fibers were not disturbed. Because sensory-motor connections normally begin to form at Stage XVII, many synapses probably formed while the muscles had no motor input, and even after reinnervation had occurred, the motor activity was uncoordinated since each muscle was innervated by different kinds of motoneurons. After the tadpoles completed metamorphosis, the amplitudes of monosynaptic EPSPs from triceps muscle sensory afferents in identified limb motoneurons were measured by intracellular recording. The degree of disruption of motor innervation was checked in each frog by retrogradely labeling those motoneurons that reinnervated the triceps brachii muscles with HRP.

Whenever the reinnervation of triceps muscles was non-specific (as judged by HRP labeling), the projections of triceps sensory neurons onto limb motoneurons were functionally inappropriate. Motoneurons innervating triceps muscles, which normally have large triceps EPSPs, frequently received very little triceps input whereas motoneurons innervating other muscles, which normally receive little triceps input, often had large triceps EPSPs. However, different populations of triceps sensory afferents selectively innervated the same subpopulation of limb motoneurons; there was a strong positive correlation between the strengths of inputs from medial vs. internal-external triceps afferents onto individual motoneurons, even though these strengths did not correlate with the functional identity of the motoneuron, as they do in normal frogs.

The selective innervation of a specific subpopulation of motoneurons by triceps sensory afferents suggests that the mechanisms for assuring this selectivity have remained intact despite drastic changes of motor activity. This result is consistent with a chemoaffinity mechanism where connections are determined by matching chemical markers on sensory and motor neurons. Triceps sensory afferents would innervate "triceps" motoneurons even though some of them now project to non-triceps muscles, and "non-triceps" motoneurons would receive little triceps input even though some of them now project to triceps muscles. However, mechanisms dependent on correlated patterns of electrical activity in sensory and motor neurons would not produce selective patterns after such manipulations.

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406.7 **CORRESPONDING SPACIAL GRADIENTS OF TOP MOLECULES IN DEVELOPING RETINA AND OPTIC TECTUM.** D. Trisler and F. Collins. Lab. Biochem. Genet., NHLBI, NIH, Bethesda, MD 20892 and Dept. Anat., Univ. Utah Sch. Med., Salt Lake City, UT 84132

The topographic map of cell position in avian retina is inverted in its projection to the optic tectum. Dorsal retinal ganglion cell axons project to ventral tectum and ventral retinal ganglion cells project to dorsal tectum. Topographic gradients of TOP molecules along the dorsoventral axes of retina and tectum also are inverted. In the retina the highest level of TOP detected with α-TOP monoclonal antibody was present dorsally; in optic tectum TOP was most abundant ventrally. Ten-fold gradients of TOP were detected in both the retina and tectum of 5-day embryos.

Immunofluoresence studies of α-TOP antibody binding to tissue sections from retina and tectum revealed a ring pattern of fluoresence around most or all cells from the pial surface to ventricular surface of tectum and from the vitreal surface to the pigmented epithelium of retina. All cells in any region along the dorsoventral axes expressed similar levels of TOP, but the amount of TOP per cell varied continuously along the axis of the gradient in both retina and tectum. Thus, TOP can be used to identify cell position along the dorsoventral axis of developing tectum as well as retina.

TOP was present in retina and tectum at all ages tested from embryonic d.3 to adult. The level detected in tectum decreased during this period while that in retina increased. Five-fold more TOP was detected in ventral than in dorsal half tectum of d.3 to d.8 embryos. The level of TOP in tectum after d.10 was too low for us reliably to detect dorsal-ventral differences. TOP gradients were present in both retina and tectum when retinal axons arrive at the tectum on day 6 of development and were present during the initial retinal-tectal interactions as retinal axons are growing across the tectum. The presence of corresponding TOP gradients in retina and tectum at this time in development suggests a possible role for the molecule in orienting the dorsoventral axis of the retinal projection onto the tectum possibly by homophilic interactions between TOP molecules.

406.8 NEONATALLY DEAFFERENTED TRIGEMINAL INTERPOLARIS CELLS EXPRESS CERVICAL RECEPTIVE FIELDS WITHOUT CERVICAL PRIMARY AFFERENT SPROUTING. L.A. Morse*, B.G. Klein & M.F. Jacquin (SPON: K.R. Smith). Dept. of Neuroscience, N.Y. Coll. of Osteopath. Med., Old Westbury, NY 11568.

Trigeminal (V) brainstem subnucleus interpolaris (SpVi) contains an anatomically and functionally diverse population of neurons. In normal adult rat, local circuit neurons have small receptive fields (RF), each reflecting input from 1 class of receptor organs (e.g. 1 whisker). Thalamic- or cerebellar-projecting SpVi cells have larger RF's and correspondingly larger dendritic trees; yet convergence is restricted to 1 class of receptor organs (e.g. multiple whiskers without intervening guard hairs or skin). However, these intramodality convergent RF's never include receptor surfaces innervated by cervical primary afferents (PA's). We have previously shown that neonatal infraorbital nerve section results in a sparse infraorbital PA projection to SpVi in adulthood, and that surviving deafferented cells often have altered RF's which incorporate a variety of V receptor organs (Stennett et al., Soc. Neurosci. Absts. 12:543, 1986). We now report that some neonatally deafferented SpVi cells express intermodality-convergent RF's spanning V and cervical fields. Intra- and extracellular recording, electrical stimulation, and RF mapping procedures were used to study the responses of SpVi neurons. In 23 Nembutal-anesthetized rats subjected to left infraorbital nerve section at birth, 141 cells were isolated in left SpVi. Cells recorded in the medial half of SpVi, while often exhibiting unusual forms of V convergence, never expressed cervical inputs. However, of 86 cells isolated in the lateral half of SpVi, 12 (6 thalamic-projecting, 3 cerebellar-projecting, 3 local circuit neurons) had RF's which included V vibrissae, guard hairs, and/or skin, as well as ipsilateral cervical ear, neck, shoulder, arm, forepaw glabrous and/or hairy skin. Their latencies to V ganglion, thalamic, or cerebellar shocks did not differ from normal. In 4 cases, the V component was restricted to the extreme caudal mandibular vibrissa and/or guard hairs, though in the remaining 8 cells it extended well into the whisker pad. Mechanical displacement thresholds for discharging these cells from each of these regions were equivalent.

In 4 similarly lesioned adults, HRP was injected subcutaneously and bilaterally into forepaw, arm, and shoulder regions. The distribution of PA terminals in cervical dorsal horn and dorsal column nuclei was identical on the 2 sides. In 1 case, 1 labeled axon penetrated lateral SpVi on the left side; otherwise, SpVi was free of label. Thus, it is unlikely that cervical PA sprouting into SpVi will account for cervical RF's in deafferented SpVi cells. An alternative mechanism is postsynaptic morphological changes. 5 SpVi cells with cervical RF's were stained with HRP; 4 of these had morphologies which were indistinguishable from normal. Cervical RF's in deafferented SpVi cells may, therefore, reflect plasticity in descending systems or activation of normally ineffective synapses.

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406.9 THE COMBINED EFFECTS OF UNILATERAL ENUCLEATION AND LIGHTING CONDITIONS ON THE SYNAPSE-TO-NEURON RATIOS IN THE RAT VISUAL CORTEX. K.S. Bedi* (SPON: L. Crnic). Department of Anatomy, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland.

Unilateral enucleation of rats soon after birth or rearing animals in dark conditions during early postnatal life have both been shown to affect the morphology of the visual system. One of the questions which arises from these studies is whether combined dark rearing and neonatal enucleation interact with each other in their effect on the visual centres. This study was designed to examine this question.

Rats whose right eye was removed on day one after birth and non-enucleated rats were raised in either light or dark conditions from birth until 39 days of age when they were killed. Stereological procedures were used to estimate the synapse-to-neuron ratios in layers II to IV of both the right and left visual cortices of each rat.

Light reared non-enucleated rats had about 16100 synapses-per-neuron in both right and left cortices. Rearing non-enucleated rats in the dark reduced this value to between 11000 and 12000. There were no significant differences in the synapse-to-neuron ratios in the right or left visual cortices between enucleated and non-enucleated rats raised in the light. However dark reared enucleated rats showed significant deficits in the ratio in both visual cortices. The extent of this deficit was more marked in the contralateral than the ipsilateral (to the enucleated eye) visual cortex. The decrease in the contralateral visual cortex was not significantly greater than that observed in the corresponding cortex from dark reared non-enucleated rats.

These results provide useful information on the combined and separate effects of unilateral enucleation at around birth and dark rearing during early life on the interneuronal connectivity of both the ipsi- and contralateral visual cortices of rats. They also show the vast importance of visual stimulation for the normal development of the subcortical visual centres.

406.10 **DEVELOPMENT OF PERFORATED SYNPASES IN THE ECTOSTRIATUM OF NORMAL AND MONOCULARLY DEPRIVED ZEBRA FINCHES.** Barbara Nixdorf*, University of Bielefeld, Department of Biology, Postfach 8640, 4800 Bielefeld, FRG

An electron-opaque postsynaptic density (PSD) is one of the defining characteristics of synapses. Siekevitz (1985) has suggested that this feature is functionally important and may participate in plastic changes in brain structure and function. Perforations are frequently observed in the PSD. Nieto-Sampedro et al. (1982) have proposed that synapses with perforations may represent intermediate stages in an ongoing cycle of synapse turnover that is part of the normal maintenance and adaptation of the nervous system. Thus, the incidence of perforations may be an indication of synaptic turnover and synaptogenesis.

I studied the incidence of perforated synapses at several early ages in the ectostriatum (the telencephalic projection area of the tectofugal pathway) of zebra finches which were visually normal or had been monocularly deprived. Normal finches were prepared for EM at ages 5, 10, 20 and 100 days (n=3 each;details in Nixdorf & Bischof, JCN 250: 133, '86). Micrographs covering more than 4000 μm^2 were scanned at each age. At 5 and 10 days, ectostriatum contains about 7 perforated synapses/ 1000 μm^2. This rises to about 19 at 20 days ($p<.05$) and stays high at 100 days. Although synapses with a *single* perforation increased only slightly in number ($p=0.1$) from 10 to 20 days, synapses with *two or more perforations* increased by 600% ($p<0.05$). At 100 days, the number of multiple perforated synapses decreased again ($p=0.02$), however the number of perforated synapses of all sorts per unit area remained constant.

Monocular deprivation affects the incidence of perforated synapses. In birds with one eye occluded from hatching to day 20 (n=5), the incidence of perforated synapses is 30% lower on the deprived than the nondeprived hemisphere ($p<.01$). The nondeprived hemispheres are similar to those of controls. After 100 days of deprivation (n=5), the number of synapses with single perforations is similar across groups. The number of synapses with multiple perforations is higher in the nondeprived hemispheres than either the deprived hemispheres or the control hemispheres ($p<.02$) Thus monocular deprivation seems to have a twofold effect on the development of perforated synapses: in juvenile birds the ultrastructural effects are found on the deprived side, whereas in the adult bird the effects appear on the nondeprived side.

If perforated synapses indicate intermediate stages of synapse turnover, then the highest activity in normally reared birds would be found at the 20 day stage when they are just fledged and start to fly. Visual deprivation could cause a delay in synaptic development in deprived areas and subsequent hypertrophy in nondeprived areas. [Supported by grants from the DFG (Bi 245/2.3) and by the Studienstiftung des deutschen Volkes] Present address: Psych. Dept.,Cornell Univ., Ithaca, N.Y. 14853

406.11 THE SYNAPTIC COMPOSITION OF NEOCORTICAL TRANSPLANTS, A QUANTITATIVE ANALYSIS. B.C. Cree*, L.M. Smith, F.F. Ebner. Center for Neural Science, Brown University, Providence, R.I.

The synaptic composition of normal (5 week, age-matched control) BALB/c mouse parietal neocortex was compared to that of transplanted E-14 and E-18 embryonic cortex after maturation in adult host neocortex. Electron micrographs were quantified for the type and frequency of axon terminals and synaptic contacts. Stereological techniques were used to determine axon terminal, synapse, and cell soma densities. Several striking features of synaptogenesis under transplant conditions are that the ratio of round to flat to unknown vesicle types, the ratio of asymmetric to symmetric to unknown membrane differentiation, and the ratio of dendritic shaft to dendritic spine contacts are almost identical to the ratios found in age-matched normal neocortex. Therefore, the types of axon terminals and synapses in the transplants appear to be closely regulated by the transplant neurons since thalamocortical and commissural fibers from the host brain do not grow into intact block transplants in normal hosts.

Both transplants and controls developed the same density of axon terminals; however, the axon terminals in the transplants formed fewer synapses than controls. Furthermore, the numerical density of E-18 cell bodies was comparable to the controls while the E-14 transplants exhibited a substantially higher cell body density. As a result, the synapse to neuron and axon terminal to neuron ratios were significantly lower in the E-14 transplants than in controls and E-18 transplants.

These results suggest that use dependant (sensory experience) and state dependant (changes in the level of excitation) mechanisms do not influence the density of axon terminals and the types of synapses; however, these factors seem to influence synapse density. Finally, donor age significantly affects the number of neurons present in the transplants and therefore strongly influences the synapse to neuron ratio (supported by NIH grant #NS-13031).

CATECHOLAMINES V

407.1 RADIOAUTOGRAPHIC DEMONSTRATION OF THE INCREASING IMPORTANCE FROM RODENT TO PRIMATE, OF DOPAMINERGIC AS COMPARED TO SEROTONERGIC INNERVATION OF THE CEREBRAL CORTEX. B.Berger*, S.Trottier*, C.Verney*, P.Gaspar*. INSERM U106, Hopital Salpetrière, 75651 Paris cedex 13, and U97, 2ter Rue d'Alésia, 75014 Paris FRANCE (sponSOTELO)

Biochemical(Brown & al.)and catecholamine fluorescence studies (Levitt & al.),immunodetection of tyrosine hydroxylase(TH)(Lewis & al.)have suggested an extensive dopamine(DA)innervation in the primate cerebral cortex.To specifically visualize DA and serotonin (5HT) afferents to the neocortex in Cynomolgus monkeys,we developed an in vitro approach using uptake of tritiated amines.Vibratome sections were incubated with pargyline 10-4M and with: either DMI 5.10-6M and (3H)DA 2.10-7M for DA-axons labeling or cold norepinephrine 5.10-6M and (3H)5HT 5.10-7M for 5HT-axons labeling.Addition of specific uptake inhibitors:benztropine or fluoxetine prevented the visualization of DA or 5HT axons respectively.

The 3 motor areas (4,6,SMA) exhibited the densest DA innervation with a characteristic pattern of cluster-like accumulations in layer III(Berger & al.Neurosc.Let.1986,72,121).In the other areas,the presence or absence of a layer IV was paralleled by a distinct pattern of laminar distribution.In the agranular anterior cingulate cortex, DA fibers reached all layers but more densely layer I and V-VI. On the contrary,the granular prefrontal,parietal,temporal and posterior cingulate cortices exhibited a bilaminar-like pattern with two dense bands in deep layer I and in layers V-VI. A variable number of labeled axons crossed layers II to IV,sometimes forming small ascending fascicles with an occasional cluster in layer III. In the transition zone between the primary motor area 4 and the postcentral area 3: area 3a where a layer IV is hardly visible,much more DA fibers ran through layer III than in the other parts of the granular parietal cortex. The primary visual cortex (a.17) displayed the weakest DA innervation almost confined to layer I whereas in area 18,both layers VI and V also received a slight innervation.

A widespread 5HT innervation characterized all cortical areas. Contrary to the DA innervation,its regional density followed a general rostrocaudal increasing gradient,the highest density being observed in the visual areas 17 & 18,with the characteristic laminar distribution described by Morrison & Foote using immunocytochemistry and the lowestdensity in the motor areas.

When compared to the restricted DA terminal fields in the rat, this extensive DA innervation of the cerebral cortex in monkey and probably also in man as shown by TH and DBH immunodetection,suggests an increasing and diversified functional role of this amine in phylogeny. Whether these DA afferents constitute different systems with distinct origin and timing of development as shown in the rat is now worthwhile further analysis.

(Supported by INSERM and Fondation Singer-Polignac)

407.2 **DOUBLE LABELING USING FLUORO-GOLD REVEALS NEUROTRANSMITTER IDENTITY OF AFFERENTS TO LOCUS COERULEUS.** V.A. Pieribone, G. Aston-Jones, M. Bohn[1] and H. Bernstein-Goral[1] (Spon: D. Schuster), Dept. Biol., New York Univ., NY 10003 and [1]Dept. Neurobiol. Behav., SUNY-Stony Brook, NY 11794.

We have reported that retrograde transport of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) reveals major afferents to LC in paragigantocellularis (PGi) and prepositus hypoglossi (PrH), in the area of C1 and C3 adrenergic neurons in rostral medulla, respectively. To study the neurochemical identity of these afferents to locus coeruleus (LC), we have developed a sensitive retrograde method using small deposits of Fluoro-Gold (FG). We agree with Schmued and Fallon (1986) that this tracer offers many advantages over others. We have found that (i) FG can be microiontophoresed to produce dense focal deposits, (ii) cellular recordings from injection pipettes allow physiologic identification of injection sites, (iii) FG is not taken up by fibers of passage, (iv) FG is as sensitive as the WGA-HRP/TMB method, and (v) FG can readily be combined with immunofluorescence to identify neurotransmitter(s) of afferent neurons.

Dense iontophoretic deposits of FG, restricted to LC, were made from micropipettes (10 um tips). Single cell recordings from the injection pipette were used to locate LC. After 5 days animals were perfused, and brain sections were subjected to rhodamine-immunofluorescent detection of phenyl-N-methyl transferase (PNMT), a marker of adrenergic cells, or serotonin (5-HT). Alternate fluorescent illumination revealed cells in the same section that fluoresced for FG, rhodamine, or both.

The most common LC afferent neurons in PGi and PrH were medium-sized (380 um^2) fusiform and triangular cells, but a more lateral group of large (700 um^2) multipolar LC-projecting cells could also be identified in the PGi area. LC afferents were closely intermingled with PNMT-positive cells in PGi and PrH. In PGi, 21% of FG-labeled neurons were also immunoreactive for PNMT. Doubly labeled neurons appeared to be preferentially located in rostral and medial PGi, while the larger LC afferent neurons in lateral PGi were devoid of PNMT. In PrH, only 4% of LC afferent neurons also stained for PNMT. Initial results indicate that a minority (<10%) of LC-projecting neurons in these areas exhibit 5-HT immunoreactivity. These results reveal that LC receives adrenergic and serotonergic inputs from its two major afferents, but that the majority of inputs from PGi or PrH supply neither transmitter; thus, multiple neurotransmitter systems innervate LC from each of these areas. Additional studies are underway to determine the neurochemical identity of other LC afferents in PGi and PrH.

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407.3 Effects of Stress on the Estimated Activity of Discrete Populations of Mesoamygdaloid Dopamine Neurons. C.D. Kilts, T.D. Ely* and C.M. Anderson* (Spon: N. Cant). Dept. of Psychiatry and Pharmacology, Duke Univ. Med. Center, Durham, NC 27710.

Populations of dopamine (DA) neurons have been demonstrated to be selectively activated or inhibited by stress. In spite of the fact that the amygdala has been shown to be critically involved in neuroendocrine, autonomic and gastric responses to stress, the effect of stress on mesoamygdaloid DA neurons has not been well examined. Mesoamygdaloid DA neurons are organized into distinct populations innervating the discrete component nuclear groups of the amygdaloid complex and exhibit a composite of mechanisms of regulation and signal transduction strikingly different from that of other DA systems. We have undertaken a systematic study of the effects of physiological and psychological stress on the biochemically estimated activity of mesoamygdaloid DA neurons with the initial results being presented here. Groups of rats were subjected to 60 min of restraint stress and then immediately sacrificed and the amygdaloid and other brain nuclei of interest were micropunch dissected. The content of DA, DOPAC and HVA were determined by on-line trace enrichment HPLC with electrochemical detection. Consistent with previous reports (Demarest et al., Neuroendocrinology 41:437, 1985), restraint stress decreased the estimated activity (DOPAC, DOPAC/DA) of tuberoinfundibular DA neurons. Mesoamygdaloid DA neurons were differentially responsive to restraint stress with the activity (DOPAC/DA) being decreased in the lateral and cortical, but not central or basal amygdaloid nuclei.

These results suggest that DA neurons may modulate the stress response mediated by amygdaloid mechanisms and further support the functional heterogeneity of populations of mesoamygdaloid DA neurons. The response of mesoamygdaloid DA neurons to stress is opposite that of other mesotelencephalic DA neurons and resembles that of tuberoinfundibular DA neurons. The examination of the response to other forms of stress as assessed by distinct techniques of biochemical estimation will further test these preliminary observations and are in progress.
(Supported by MH 39967)

407.4 IN VIVO NEUROCHEMISTRY OF STRESS: BIOGENIC AMINE METABOLITES IN FRONTAL NEOCORTEX OF ESCAPABLY VS. INESCAPABLY STRESSED RATS WITH BRAIN MICRODIALYSIS PERFUSION. F. Petty and G. F. Kramer.* VA Med Ctr and Univ of TX Health Science Ctr, Dallas, Texas 75216

We here report use of the new technique of brain microdialysis perfusion to study biogenic amine metabolism in frontal neocortex of rats before, during and after exposure to stress. Probes for microdialysis perfusion were implanted in frontal neocortex of anesthetized rats under stereotaxic guidance one day prior to stress exposure. A yoked experimental design was used in which pairs of rats received identical amounts of tail-shock (0.8 mA) while confined to small plexiglass boxes equipped with wheels, which one rat could turn to terminate the tail-shock (coping). Eighty unsignaled shocks were delivered during the 90-minute stress session. Control rats were similarly confined and had electrodes attached but no shock delivered. Perfusion of the microdialysis probe was maintained before and after shock presentation. Samples of perfusate were collected at 20-minute intervals and injected immediately onto a electrochemical HPLC. Levels of DOPAC and HVA rose rapidly to 150-200% of baseline during the shock presentation, and returned to baseline soon after shock offset. Levels of 5-hydroxyindolacetic acid (5-HIAA) increased only slightly during shock. One day after the stress session, perfusion was reestablished, and the rats were tested for stress-induced behavioral depression (learned helplessness). No neurochemical differences were found between escapably vs. inescapably shocked rats during stress presentation and immediately after stress. On the day after stress there were no significant differences among the three experimental groups in levels of DOPAC or HVA, but animals receiving escapable shock had a significant higher level of 5-HIAA in cortical perfusate than either controls or animals receiving inescapable stress.

In a separate experiment, diazepam administered intraperitoneally was found to block the stress- induced rise in dopamine metabolism in frontal neocortex in vivo, in a dose-dependent manner. These findings support the possibility that benzodiazepines may exert their effects in protecting against harmful effects of stress at least in part through an interaction with the mesocortical dopaminergic system. The results also support an involvement in frontal neocortical serotonergic metabolism in the development of behavioral depression after exposure to inescapable stress.

407.5 BILATERAL MICRODIALYSIS MONITORING OF STRIATAL DOPAMINE METABOLISM IN THE UNILATERALLY NIGROTOMIZED RAT: RESPONSES TO L-DOPA. P.J.Knott[1,2,5], T.S.Brannan*[3], H.C.Kaufmann*[3], C.Harden*[3], L.K.H.Leung*[4] and J.G.Young[2,4]
Departments of Neurobiology[1], Psychiatry[2], Neurology[3], Pediatrics[4] and Pharmacology[5]. The Mount Sinai School of Medicine, New York, N.Y. 10029, U.S.A.

We have used the microdialysis perfusion technique to bilaterally monitor the changes of striatal dopamine metabolism in the extracellular fluid (ECF) of unilaterally nigrotomized rats in response to L-DOPA administration both in the presence and absence of a peripheral decarboxylase inhibitor.

Male Sprague-Dawley rats (200-220g), unilaterally lesioned with 6-OHDA were tested one week after lesioning with apomorphine (1.0mg/kg). Rats demonstrating contralateral circling were selected for microdialysis studies under chloral hydrate anesthesia which were performed 4 weeks after lesioning. Bilateral microdialysis samples were obtained from striata at 20 min intervals using previously described probes (Clemens,J.A. and Phebus,L.A. Life Sci., 35: 671, 1984) and subsequently analysed by HPLC for dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and L-DOPA. L-DOPA (50mg/kg i.p.) was administered immediately after obtaining three basal samples from each side in control (n = 9), or carbidopa-treated rats (100mg/kg i.p., injected one hour previously, n =5). Over the baseliine period, values for DA, DOPAC and HVA in dialysates from the lesioned side were always lower than from the intact side. In the absence of carbidopa, L-DOPA induced increases of extracellular DOPAC and HVA were found in the lesioned striatum to be about 50% of those observed from the intact side. In carbidopa-pretreated rats however, increases of ECF DA metabolites were comparatively minor when compared to those observed in the intact striatum (less than 10%). In all studies striatal tissue measurements of DA showed that lesions were near complete. These data indicate that in the absence of carbidopa, significant quantities of L-DOPA are metabolised peripherally to DOPAC and HVA and are able to reach the centrally implanted dialysis probes where they contribute significantly to to ECF concentrations of DA metabolites. We also noted that ECF L-DOPA concentrations were very similar in both lesioned and non-lesioned sides although tissue L-DOPA concentrations (presumably intracellular) were generally higher in the lesioned striatum. These findings have important implications to studies of precursor loading on DA metabolism, measured either by microdialysis or in striatal tissue.

407.6 MECHANISM(S) OF THE NICOTINE-INDUCED RELEASE OF NEWLY SYNTHESIZED DOPAMINE FROM RAT STRIATAL SLICES. T.C. Westfall, L. Vickery* and L. Naes*. Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104.

Studies of nicotinic-cholinergic receptors in the mammalian brain have revealed high concentrations of nicotinic-high affinity binding sites in both midbrain and target areas of dopamine containing neurons. We have previously observed that high concentrations of nicotine (100-10,000 µM) release dopamine from striatal slices by either displacement of the amine in a manner similar to amphetamine or to activation of an atypical receptor since the response was not antagonized by classical nicotinic-cholinergic antagonists and was independent of extracellular calcium levels. In the present investigation we report the effect of low concentrations of nicotine and studies which have examined the mechanism by which low concentrations of nicotine release dopamine. Male Sprague Dawley rats were decapitated and striata rapidly removed over ice. Striata were dissected with glass manipulators, sliced sagittally with a MacIlwain tissue chopper at a thickness of 0.5 mm. The slices were placed in isolated chambers and continuously superfused with a buffer containing ^{3}H-tyrosine. Superfusate effluents were continuously collected by means of a fraction collector and ^{3}H-dopamine separated from other ^{3}H products by amberlite and alumina column chromatography and measured by liquid scintillation spectrometry.

Nicotine (1-100 µM) produced a concentration dependent release of newly synthesized ^{3}H-dopamine. The evoked release of ^{3}H-dopamine by 1 µM nicotine was attenuated by mecamylamine (10^{-5}M) or d-tubocurarine ($5x10^{-5}$M), was dependent upon extracellular calcium and reduced in the presence of tetrodotoxin (10^{-5}M).

Attempts were made to determine if cyclic AMP acted as a second messenger in mediating the nicotine-induced release of dopamine. The effect of 8-bromo cyclic AMP, dibutyryl cyclic AMP, 3-isobutyl-1-methylxanthine (all of which increase intracellular cyclic AMP levels) as well as 2',5'-dideoxyadenosine (known to inhibit adenylate cyclase) alone or in the presence of nicotine on the release of dopamine were examined. The cyclic AMP analogues as well as 3-isobutyl-1-methylxanthine released dopamine by themselves and dibutyryl cyclic AMP was found to produce an additive effect with nicotine. 2',5'-dideoxyadenosine failed to reduce the nicotine-induced release of dopamine.

It is concluded that low doses of nicotine release dopamine by activation of nicotinic cholinergic receptors. It does not appear that cyclic AMP is a second messenger in mediating this response.
(Supported by NIH grants DA02668 and NS16215.)

407.7 ESTRADIOL STIMULATES DOPAMINE RELEASE FROM STRIATAL TISSUE IN VITRO. J.B. Becker, C.J. Moore*, J.-H. Cha* and B.L. Firestein*. Psychology Department and Neuroscience Laboratory Bldg. The University of Michigan, Ann Arbor, MI 48104-1687.

The systemic administration of estradiol potentiates amphetamine-stimulated striatal dopamine (DA) release and amphetamine-induced rotational behavior (Becker & Beer, Behav. Brain Res., 19:27, 1986). However, it is not known where estrogen acts to induce changes in these indicies of striatal DA activity. The absence of genome-activating estradiol receptors in the striatum has lead some investigators to hypothesize that these effects are indirectly mediated by estrogen's action on other brain areas and/or the pituitary. Nevertheless, local application of estradiol to the striatum is found to induce behavioral effects in ovariectomized (OVX) female rats, suggesting that estradiol can directly influence neural activity in the striatum (Becker et al., Pharmacol. Bioch. Behav., 1987, in press). In the experiments to be reported, we examine the influence of estradiol on the release of DA from striatal tissue in superfusion.

Striatal tissue from OVX adult female rats was dissected, sliced ($1mm^3$) and placed into superfusion chambers (Becker et al., J. Neurosci. Meth., 11:19, 1984). A modified Krebs-Ringer phosphate medium continuously flowed through the chamber at a rate of 100µl min, and samples were collected over 10 min intervals. In one experiment, after the collection of baseline samples, estradiol (0, 10,100, or 1000 pg/ml was infused for 30 min followed by infusion of 60mM K^+ for 2.5 min. In a second experiment, after collection of baseline samples, estradiol was infused in pulses [10 min estradiol (90 pg/ml); 20 min without estradiol] for 3 pulses (90 min). This was compared with the effect of estradiol infused continuously (30 pg/ml) for 90 min. Control superfusion chambers received infusions of vehicle (0.0015% ethanol in medium). DA in the effluent was measured by HPLC-EC.

The prior presence of estradiol in the superfusion medium at concentrations of 10,30,90 or 100 pg/ml (but not 1000 pg/ml) potentiated the subsequent responsiveness of striatal tissue to K^+ stimulation. The continuous infusion of estradiol had no effect on the basal efflux of DA at any concentration. In contrast, when estradiol was infused in pulses, estradiol alone stimulated the release of DA from striatal tissue slices in addition to potentiating the subsequent response to K^+-induced depolarization.

We conclude that estradiol can act directly on striatal tissue to both stimulate the release of DA and increase the responsiveness of DA terminals to stimulation. Since genome-activating receptors for estradiol are not present in the striatum, this suggests that estrogen is able to influence neural activity through an alternative mechanism or receptor. (Sponsored by NSF: #BNS84-11763).

407.8 REGULATION OF STRIATAL DOPAMINE SYNTHESIS: LACK OF DEPENDENCE ON PRECURSOR AVAILABILITY. N. Lake and J. Commissiong. Dept. of Physiology, McGill Univ., Montreal, Quebec, Canada H3G 1Y6.

Neurotransmitter in monoaminergic neurons may be regulated by the two following mechanisms: 1) the released transmitter is returned to the Ca^{2+}-dependent pool of releasable transmitter by an active uptake process, and re-released. 2) The released transmitter is catabolized, the synthetic enzymes are activated, and transmitter synthesis is enhanced. Two corollaries of mechanism #2 are that transmitter metabolites should rise, and in the homeostatic control of transmitter synthesis, precursor availability may become a limiting factor during release. Experiments were done using ♀ Sprague Dawley rats, 250 g, anaesthetized with Brietal (50 mg/kg i.p.). In the first experiment, the left medial forebrain bundle (MFB) was stimulated electrically (15 Hz; 400 µA; 20 min) using a stereotaxically-placed (L -1.4; H -1.9;AP+2.8) bipolar electrode. Under these conditions, striatal dopamine (DA) concentrations remained stable, and there was no increase in DOPAC or HVA, the major metabolites of DA. After the infusion of TTX (1.5 µl; 3.0×10^{-7} M) into the left MFB there was a sustained increase in the synthesis and metabolism of DA in the left striatum, which peaked at 4 hrs. At this time DA, DOPAC and HVA were all significantly increased ($p < 0.001$). This effect of TTX is linear over the 1.0×10^{-9} M to 1.0×10^{-5} M range. None of the precursors of DA, phenylalanine (PHE), tyrosine (TYR) nor L-aspartyl-L-phenylalanine methyl ester (aspartame or APM), each given at 250 mg/kg caused any significant increase in the TTX-induced synthesis and metabolism of DA, versus the saline-treated control. Valine, a member of the class of large, neutral amino acids (LNAAs), shares a common transport mechanism into the brain with PHE and TYR. It was administered at 400 mg/kg, i.p. to the TTX-treated rats, and is expected to block the transport of PHE and TYR into the brain, leading to a reduction in the TTX-induced increased synthesis of DA. However, after 4 hr, there was no statistically significant difference between the saline-saline and saline-valine groups, or between the TTX-saline and TTX-valine groups, for DA, DOPAC and HVA. The results suggest that 1) increased DA metabolism is not a necessary accompaniment of increased DA release. 2) The mechanisms that control dopamine homeostasis during episodes of real, increased synthesis are robust, and that within limits, neither increased nor decreased precursor availability exerts a significant effect on the enhanced synthetic process. Reuptake, not de novo synthesis, appears to be the important variable. Despite the wealth of published data, the mechanisms that regulate changes in monoamine release, synthesis and metabolism in the brain are still somewhat obscure.

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407.9 THE EFFECT OF CHRONIC COCAINE TREATMENT ON CATECHOLAMINE DISPOSITION IN DIFFERENT BRAIN REGIONS IN RAT: A SPECIFIC TARGET EFFECT ON FRONTAL CORTEX DOPAMINE. F. Karoum, R. Fawcett* and R.J. Wyatt. Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

Cocaine is a non-amphetamine stimulant with poorly understood central catecholamine effects. Efforts to compare cocaine behavioral properties with changes in central catecholamine steady state concentration and metabolism generally reveal poor correlations. Nevertheless, the prevailing view in the literature favor a tendency for cocaine to preferentially influence central dopamine (DA). Paradoxically, in spite of a reported long-term reduction in tyrosine hydroxylase (Trulson et al.: Expt. Neurol. 94:1986) in the substantia nigra and caudate nucleus following 1 week chronic cocaine treatment, dopamine metabolism in the mesolimbic and caudate nucleus was reported to remain unchange or increased following acute (Bagchi: Neurosci. Abst. No# 38.17:1986) and chronic cocaine (Roy et al.: Neuropharmacology 17:1978) treatments. In an attempt to gain a better understanding of cocaine's effects on central catecholamines, we chronically administered cocaine (10 mg/kg twice daily) to rats for 1,2 and 3 weeks. Their hypothalami (Hy), hipocampi (HP), septa (SP), caudate nuclei (CN) and frontal cortex (Fx) were removed for analysis of norepinephrine (NE), DA and their metabolites 1 hour after the last treatment. In another experiment, rats were treated chronically for 1 week and their brains analyzed 6 weeks later. All biochemical analysis were carried out by mass fragmentography. There were five rats in each study.

One hour after an acute dose of 10 mg/kg, cocaine produced no reduction in NE, DA or their metabolites in any of the 5 brain regions analyzed. During the course of 1,2 and 3 weeks chronic treatment with cocaine (10 mg/kg twice daily) no change in NE or 3-methoxy-4-hydroxyphenylglycol (MHPG) concentrations was found in the above 5 brain regions. DA concentration was reduced by about 50% in the frontal cortex while remained unchanged in the Hy, HP, SP and CN during the course of the 3 weeks chronic cocaine treatment. The concentration of 3,4-dihydroxy-phenylacetic acid (DOPAC) was slightly (about 20%) but significantly reduced in the Hy, CN and SP. In contrast DOPAC concentration was markedly (about 50%) reduced in the Fx.

The results of brain analyses 6 weeks following the discontinuation of 1 week chronic cocaine treatment, indicated a continued long-term suppression of DA metabolism in the Hy, CN and Fx. Consistent with effects observed during chronic cocaine treatment, the Fx was the area most affected. NE and MHPG concentrations remained unchanged.

The results of the present study suggest a preferential effect of cocaine on central dopaminergic systems. Of the various regions assessed chronic cocaine appears to exert its best effects on Fx and Hy DA turnover or metabolism. These 2 brain regions may therefore play important roles in the long-term effects of cocaine.

407.10 BRAIN CATECHOLAMINE CONCENTRATIONS FOLLOWING SYSTEMIC TRETINOIN AND/OR HALOPERIDOL IN RATS. G.M. Straw*,F. Karoum (SPON: P. Oliver). Neuropsychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

Previous work in this laboratory has suggested that behavioral habituation to arousal may be altered by retinoic acid administration, in synergism with co-administered haloperidol. A corollary hypothesis is that brain catecholamine activity is altered by such treatment. Evaluation of two areas of the brain was done in male Sprague-Dawley rats. All animals were dosed intraperitoneally. Control animals, Group 1 (N=5), received vehicle only. Group 2 (N=5) received haloperidol 1 mg/kg/day. Group 3 (N=4) received tretinoin 5 mg/kg/day. Group 4 (N=4) received haloperidol 1 mg/kg/day and tretinoin 0.5 mg/kg/day. Group 5 (N=4) received haloperidol 1 mg/kg/day and tretinoin 5 mg/kg/day. Four hours after the last injection the animals were sacrificed by decapitation and the hypothalamus and the right caudate nucleus were removed and assayed for catecholamines and metabolites by mass fragmentography (Karoum, F., in Methods in Biogenic Amine Research, ed. S. Parvez et al., 237-255, 1983). Dopamine (DA), homovanillic acid (HVA), and dihydroxyphenylacetic acid (DOPAC) were measured in the caudate nucleus; and DA, HVA, norepinephrine (NE), and 3-methoxy,4-hydroxyphenethyl glycol (MHPG) were measured in the hypothalamus. Multivariate analysis of variance results were consistent with significant changes in caudate DA ($p<.03$), caudate HVA ($p<.002$), caudate DOPAC ($p<.02$), and hypothalamic DOPAC ($p<.0001$). No significant changes were measured in hypothalamic DA, NE, or MHPG. Comparisons of group means by Tukey's method ($p<.05$) showed that the combination of haloperidol and tretinoin raised caudate DA and lowered both caudate HVA and caudate DOPAC. Tretinoin alone tended to lower caudate HVA, and haloperidol alone tended to raise caudate DOPAC, but neither effect reached significance. Haloperidol with tretinoin at 5 mg/kg/day caused a clear increase in hypothalamic DOPAC ($p<.05$) over all other conditions. A trend for tretinoin alone to increase hypothalamic DOPAC, but no effect from haloperidol alone is seen. These results are consistent with the hypothesis of a synergistic effect of tretinoin and haloperidol on central catecholamine activity. A dose dependency is seen for tretinoin in the hypothalamus.

407.11 DOES CYCLO(HIS-PRO)(CHP) ACT LIKE A DOPAMINERGIC AGONIST? C.PRASAD. Section of Endocrinology, Department of Medicine, Louisiana State University Medical Center, New Orleans, LA

CHP is a cyclic dipeptide that is ubiquitously distributed throughout a number of tissues and body fluids of both man and animals. Administration of exogenous CHP to animals has been shown to elicit a variety of endocrine as well as central nervous system-related biologic activities. Although the mechanism through which CHP acts is not understood, some of its biologic activities (e.g. attenuation of prolactin secretion, inhibition of dopamine (DA) uptake by striatal synaptosomes, and hypothermia in rats) suggest the potential involvement of a dopaminergic mechanism. To further examine the mechanism of action of this peptide, we have investigated relationship between CHP and DA using two different behavioral paradigm.

First, experiments were conducted to explore whether dopaminergic drugs modify CHP-induced hypothermia. Subcutaneous injection of CHP to cold-exposed (5°C for 12-14 hrs.) rats led to a dose-and time-dependent decline in rectal temperatures. Hypothermia increased with increasing CHP dosage reaching to a maximum of -0.91°C at 10 mg/kg. Fifty minutes following treatment with 10 mg/kg CHP, rectal temperature declined to a nadir of 0.91°C and then steadily rose to control levels by 90 min. Pretreatment of rats with DA-antagonists (0.05 mg/kg SCH23390 or 10 mg/kg sulpiride, in vitro antagonists of D_1- and D_2- receptors respectively) led to a significant attenuation of CHP-induced hypothermia (control, -0.63 ± 0.07; +SCH23390, -0.35 ± 0.04; sulpiride, -0.40 ± 0.03; $p < 0.05$). Furthermore, chronic oral treatment of rats with CHP (1.96 ± 0.4mg/kg/day for 24 days) led to a significant ($p < 0.01$) increase in the ability of apomorphine (APO, 1mg/kg) to induce hypothermia.

Next, we chose to investigate the effect of this peptide on stereotypic behavior (SB), a behavior associated with the activation of postsynaptic DA receptor, induced by APO or amphetamine (AMP). APO elicits this behavior by direct stimulation of postsynaptic DA receptor, whereas AMP acts indirectly by making more DA available at postsynaptic site. Thirty min. pretreatment of rats with 0.5 mg/kg CHP led to a significant augmentation of median SB induced by 5mg/kg AMP, but not APO (AMP, 1.85 ± 0.1; AMP + CHP, 3.60 ± 0.2; $p < 0.0025$ and APO, 2.0 ± 0.1; APO + CHP, 2.1 ± 0.18; $P > 0.10$). However, administration of large dosage of CHP alone to naive rat (50 mg/kg i.p. or s.c.; 5mg/kg i.c.v.) does not induce any motor activity change. In conclusion, our data suggest that within hypothalamus (thermoregulation) and/or basal ganglia (SB) CHP may act via a dopaminergic mechanism.

407.12 BRAIN IRON/FERRITIN ASYMMETRIES IN HEMI-PARKINSON RATS DEMONSTRATED BY MAGNETIC RESONANCE IMAGING. S. Hall*, J. N. Rutledge* and T. Schallert (SPON: E. Bigler). Dept. of Psych and Inst. Neurol. Sci., Univ. Texas, and St. David's Hospital, Dept. of Radiology, Austin, TX. 78712.

High concentrations of iron accumulates in the brains of several species, including rat, monkey and man. Because iron exists in areas that correspond roughly to the distribution of catecholamines and is necessary for dopamine (DA) metabolism, it has been suggested that iron may be involved, at least in part, in the function of DAergic neurons in the brain.

The distribution of iron can be demonstrated either by Perls' histochemical stain for ferric iron or by magnetic resonance imaging (MRI). Heavily T2-weighted images produced by MRI exhibit a decreased signal (T2*) caused by iron, probably in the form of ferritin.

Investigators studying MR T2-weighted images of patients with Parkinson's Disease and related disorders have reported both increased and decreased iron in the nigrostriatal system. To explore this issue further, brain iron distributions in rats were measured using T2-weighted MR images and Perls' method. Severe unilateral catecholaminergic depletions were produced by infusing 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle or striatum of one hemisphere. Sham-control infusions were placed in the contralateral hemisphere of these same animals. Rats with bilateral-sham operations and naive controls were also examined.

Postoperatively, animals with unilateral lesions exhibited sensorimotor asymmetries which recovered in about 5 months. The behavior of control and bilateral sham-operated rats was normal. Asymmetries in iron/T2* effect were found in all unilaterally- lesioned rats scanned by MRI; however, some demonstrated an increased iron/T2* effect in the contralateral nigrostriatal region while others showed an increased iron/T2* effect on the ipsilateral side. Bilateral sham-operated and control rats had no asymmetries with either iron analysis.

MRI provides an analysis of brain iron unique from Perls' method that may be more relevant to studying diseases of catecholaminergic dysfunction. The finding that the side of catecholamine depletion did not predict the side of T2*/iron effect may bear importantly on conflicting reports of increased vs decreased iron in diseases of the extrapyramidal system.

AGING AND DEMENTIA: ANATOMY

408.1 PATHOLOGY IN BRAINSTEM CHOLINERGIC NEURONS IN ALZHEIMER'S DISEASE. E.J. Mufson and D.C. Mash. Inst. for Biogerontology Res., Sun City, AZ 85351 and Univ. Miami Med. Sch. Miami, FL.

Alzheimer's disease (AD) is characterized neuropathologically by neurofibrillary tangles (NFT) and senile plaques in the cerebral cortex, hippocampus and amygdala. Brainstem cortical projection neurons (e.g., serotonergic dorsal raphe and noradrenergic locus coeruleus) also contain NFT in AD. Recently, we have shown that all cholinergic neurons located within the monkey mesencephalic tegmental nuclei cuneiformis and tegmenti pedunculopontinus (Ch5) contain choline acetyltransferase as well as acetylcholinesterase (AChE) and these cholinergic perikarya project to widespread cortical areas (Mufson et al., Neurosci. Abstr. 8:135, 1982). The purpose of the present study was to determine whether these cholinergic neurons also contain NFT in AD. Both AChE and nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase histochemistry delineate tegmental cholinergic neurons (Vincent et al., Neurosci. 17:186, 1986). We used these histochemical procedures to localize cholinergic perikarya in the tegmentum of 4 AD (70-78yrs.) and 3 normal (66-78yrs.) patients.

Tissue reacted for either AChE or NADPH-diaphorase revealed positive neurons within a region of the deep tegmental grey corresponding mostly to the nuclei cuneiformis and tegmenti pedunculopontinus according to Olszewski and Baxter (1954). AChE stained neurons appeared brown and NADPH-diaphorase positive cell bodies dark blue. These enzyme positive neurons were arranged into a compact lateral and a diffuse medial subdivision. Both AChE and NADPH-diaphorase positive perikarya were seen to penetrate into adjacent fiber bundles. In order to demonstrate that NADPH-diaphorase and AChE co-localized within the same neuron, tissue was simultaneously stained for NADPH-diaphorase and AChE. Virtually all NADPH-diaphorase containing cell bodies also contained AChE.

Cortical and brainstem tissue stained with Thioflavin-S revealed many tangles and plaques in the AD samples and only a few in the normal aged brains. Tissue reacted individually or concurrently for NADPH-diaphorase and AChE was counterstained with Thioflavin-S. Tangles were found in many of these cholinergic neurons as well as within the locus coeruleus, dorsal and ventral raphe nuclei, medial parabrachial nucleus and pontine central grey. Plaques were found throughout the mesencephalic and medullary tegmentum as well as in the inferior colliculus. These results demonstrate that cholinergic neurons within the mesencephalic tegmentum which project to cortex exhibit neurofibrillary degeneration similar to that seen in noradrenergic and serotonergic cortical projection systems in AD. Supported by an ADRDA Faculty Scholar Award (EJM).

408.2 MAPPING OF TANGLES AND ALZ-50 IMMUNOREACTIVE CELLS IN AGED HUMAN BRAINS WITH AND WITHOUT ALZHEIMER'S DEMENTIA. J.L. Price, P. Davis* and L. Robins* (Spon: L. Berg). Depts. of Anat. & Neurobiol. and Psychiatry, Washington Univ. Sch. Med., St. Louis, MO 63110.

Previous studies have shown characteristic patterns of neurofibrillary tangles in the hippocampal formation of brains of Alzheimer's Disease patients (M.J. Ball, Acta Neuropath., 42:73, 1978; Hyman et al., Ann. Neurol., 20:472, 1986), and other reports indicate tangles in further structures of the limbic forebrain, including the amygdala, olfactory cortex and nucleus basalis. The present project is designed to precisely map tangles and other neuropathological markers in these areas and adjacent parts of the brain. Alternate series of sections were stained for plaques and tangles with the Bielschowsky silver method and thioflavin-S, or were stained immunohistochemically with the Alz-50 monoclonal antibody, which reacts with a protein closely associated with tangles (Wolozin et al., Science. 232:648, 1986). The distribution and density of tangles and Alz-50 immunoreactive cells (A50-IC) were mapped throughout the limbic forebrain and correlated with a clinical assessment of the degree of cognitive impairment in each individual, based on the clinical record and on a structured interview with a collateral informant. To date, six cases have been examined, ranging from 59 to 83 years of age, and from normal to severely demented in cognitive status.

Tangles and A50-IC have similar patterns of distribution in all cases. Affected cells are consistently found in the perirhinal and entorhinal cortices and the subiculum, although the degree of involvment varied from very slight in the unimpaired individuals to extensive in the severely affected individuals. The para- and presubiculum contain almost no neuropathological markers even in the most severe cases. Elsewhere, the amygdaloid nuclei and the anterior olfactory nucleus consistently contain tangles and A50-IC, although at lower densities. The nucleus basalis, and the temporal and frontal cortex are only slightly affected except in the most severely demented case.

In general the A50-IC have the same distribution as tangles, but are more plentiful. However, a major difference is seen in the caudate/putamen. Very few tangles are stained in these nuclei with silver or thioflavin in any of the brains, but substantial numbers of A50-IC are found in the unimpaired, mild and moderately affected cases. These reactive neurons in the striatum are stained relatively lightly, unlike the more dense, filamentous staining seen elsewhere. Also, in the mild to moderately affected cases Alz-50 staining is seen along the pial and ventricular surfaces apparently in reactive astroglia.

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408.3 DISTRIBUTION OF MAP-2 IMMUNOREACTIVITY IN THE HIPPOCAMPAL FORMATION OF NORMAL AGED AND ALZHEIMER'S DISEASE BRAIN. A.C. McKee* and N.W. Kowall (SPON: E.T. Hedley-Whyte). Dept. Neuropathology and Neurology, Massachusetts General Hospital, Boston MA 02114.

Microtubule-associated protein 2 (MAP-2) is restricted to neuronal perikarya and dendrites in the hippocampal formation of rodents. Using a well defined monoclonal antibody raised against MAP-2, we investigated the morphology and distribution of MAP-2 immunoreactive neurons in the hippocampal formation of 8 patients with pathologically verified Alzheimer's disease (AD) and 6 elderly controls. Normally, MAP-2 in the dentate gyrus is confined to granule cell neurons whose distal brush-like dendritic branches are especially prominent. In the AD cases, these distal branches, which receive inputs from the entorhinal cortex via the perforant pathway, are much less prominently stained. In field CA4, densely immunoreactive dendritic arbors and less intense neuronal perikarya are found that are not altered in the AD cases. In CA1-3 of control cases both pyramidal and basket cell perikarya and dendrites are positively stained. The long apical dendritic processes of the pyramidal neurons emerge in orderly radial register. In AD, the normal orientation of the pyramidal neurons is lost and their dendritic processes appear bulbous, tortuous, and truncated. Abnormal dendritic proliferation is occasionally seen suggesting that aberrant regeneration may occur. These changes are more severe in CA1 than CA 3. Pyramidal neurons , normally found in all layers of the subiculum are also severely affected in the AD cases. Densely immunoreactive Cajal-Retzius neurons and layer IV pyramids are found in entorhinal cortex. Intense fiber staining is evident in layer I and surrounding layer II cell clusters. Prominent infracortical MAP-2 neurons are evident throughout the parahippocampal gyrus. In periallocortical regions of the parahippocampal gyrus, pyramidal neurons are prominent in layers II-III and V-VI. These neurons are also morphologically abnormal in some cases.

Our findings show that MAP-2 immunoreactive neurons in the hippocampal formation are morphologically altered in AD. Further studies of this distinctive subset of cortical neurons will help define the nature and extent of cytopathological changes affecting AD cortex.

408.4 [^{3}H]-IMIPRAMINE BINDING IN THE HUMAN HYPOTHALAMUS AND NUCLEUS BASALIS OF MEYNERT: AGE AND ALZHEIMER RELATED CHANGES

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This laboratory has previously reported age-and AD-related changes of serotonergic synaptic markers in both hypothalamus (Sparks et al. Ann Neurol 20:124, 1986) and nucleus basalis of Meynert (Sparks et al. Ann Neurol 19:602, 1986). Serotonergic changes in nucleus basalis of Meynert (nbM) correlated well with both cholinergic changes in nbM and cognitive dysfunction in AD. Serontonergic changes in sub-regions of hypothalamus correlated well with regional pathology in AD, and suggested a possible relationship to a number of non-cognitive abnormalities observed in the disease.

We have also found in both hypothalamus and nbM that the apparent turnover of serotonin (5HIAA content/5HT content) is increased in aging, and it is increased further in AD. To explore the possibility that altered serotonin (5HT) uptake could account for changes in 5HT turnover, we have investigated the binding of [^{3}H]-Imipramine to membrane preparations from rat and human hypothalamus and from human nbM.

Imipramine binding to rat hypothalamus was significantly inhibited by Flouxotine and Norelignine. Scatchard constants for this binding (Kd=3.3 nM; B max=35.7 f mol/mg wet) were consistent with previous reports (Severson, Neurobiol Aging 7:83, 1986).

Imipramine binding to human hypothalamus was also inhibited by Flouxotine, but not appreciably by Norelignine. Scatchard constants for this binding (Kd=1.5 nM; B max=26.5 f mol/mg wet) were also consistent with previous reports (Rehavi et al., Life Sci 26:2273, 1980).

In human nbM we found two Imipramine binding sites; a high affinity site (Kd=1.5 nM; B max=24.6 f mol/mg wet) and a lower affinity site (Kd=5 nM; B max=34.8 f mol/mg wet). The high affinity site appeared similar to that found in the human hypothalamus, and the lower affinity site appeared similar to that found in rat hypothalamus.

Age and AD related changes will be presented.

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408.5 ARGYROPHILIC GRAINS IN THE HUMAN BRAIN CHARACTERIZE A DISEASE ASSOCIATED WITH ADULT ONSET DEMENTIA. H. Braak and E. Braak*. Department of Anatomy, University of Frankfurt, D-6000 Frankfurt 70, Fed. Rep. Germany.

Unusual cytoskeleton abnormalities were found in the brains of individuals afflicted with adult onset dementia. Most conspicuous were spindle-shaped argyrophilic grains loosely scattered throughout the neuropil. Additionally, coiled bodies of silver stained filaments were encountered, mainly located within the white substance. Argyrophilic grains and coiled bodies contained dense accumulations of straight tubules with a diameter of 6-8 nm.

The argyrophilic grains were found, in abundance, within sector CA1 of the Ammon's horn and layer Pre-β of the entorhinal region. A slightly less dense scattering occurred in layer IIIab of the adjoining temporal isocortex, insular cortex and orbitofrontal cortex. The basolateral amygdaloid complex and the hypothalamic lateral tuberal nucleus were the most affected subcortical structures.

Eighty brains of demented individuals were examined. Forty eight of them showed the features of Alzheimer's disease and four those of Pick's disease. Twenty eight cases revealed the abnormalities under consideration. Ten showed exclusively argyrophilic grains and coiled bodies, while 16 also had Alzheimer changes. One of the 28 cases was associated with Parkinson's disease and one showed features of both Alzheimer's and Parkinson's disease.

Twenty brains of non-demented individuals of about the same age and devoid or almost devoid of Alzheimer changes were used as controls. None of the control brains showed argyrophilic grains and coiled bodies. These changes, therefore, are considered the morphological substrate of an unknown disease associated with adult onset dementia.

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408.6 CSF FROM SUBGROUPS OF ALZHEIMER'S PATIENTS CONTAIN ANTIBODIES RECOGNIZING CHOLINERGIC NEURONS IN THE RODENT CENTRAL NERVOUS SYSTEM

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The etiology of Alzheimer's (AD) disease is obscure. However, immunological abberations have been suggested to be critical factors in the pathogenesis of this disease. To this end an investigation was carried out to examine if the cerebro-spinal fluid (CSF) from AD patients and other forms of dementia contain antibodies which recognize neuronal populations in the rodent CNS. Rodents were perfused with a mixture that allows the fixation of acetylcholine (ACh) and dopamine (DA) in the rodent CNS. Immunocytochemical observations indicate that there are antibodies in the CSF of some AD and dementia patients capable of recognizing neuronal populations in the rodent CNS. Moreover these antibodies appear to be recognizing cholinergic or cholinergic-like neurotransmitter molecules for the following reasons: i) the antibodies recognize neurons in the medial septum and spinal motor neurons brain regions previously described as being predominantly composed of cholinergic neurons, ii) the CSF immunoreactivity of these regions was totally eliminated by prior incubation with anti-ACh antiserum and vice versa but not with anti-DA antiserum iii) there is a similarity in the location of neurons in the medial septum region following staining with patient CSF, acetylcholinesterase and anti ACh antiserum, iv) the immunocytochemical reacton was observed only following perfusion with a mixture previously demonstrated to fix ACh in the rodent CNS and v) CSF incubation of sections from rodents perfused with 4% paraformaldehyde instead of gluteraldehyde failed to mark neurons in the medial septum. Furthermore adsorption of the CSF with Protein A sepharose indicated that the immunoreactivity in these regions is due to the subclass IgG3. These observations show the presence of IgG in the CSF of a subgroup of AD and dementia patients that may be of importance for the diagnosis, the investigation of progression and for possible treatment of the disease. It may be speculated that the morphological changes (plaque formation) as well as decreases in presynaptic cholinergic markers in AD may be triggered by abnormalities in the immune system.

408.7 **A MODEL OF SENILE DEMENTIA IN YOUNG ANIMALS?** F. Cattabeni, M.P. Abbracchio*, M. Cimino*, D. Cocchi*, M. Di Luca*, L. Mennuni* and P. Zaratin*. Institute of Pharmacological Sciences, Via Balzaretti 9, 20133 Milan Italy.

Methyl-azoxy-methanol (MAM) is an antimitotic agent which kills cells in the mitotic phase. Its administration to pregnant rats at day 15 of gestation results in a dose-dependent microencephaly in the offspring. A single dose of 25 mg/Kg reduced the telencephalic mass by about 50%; 15 mg/Kg produced a reduction of about 20%, whereas 5 mg/Kg was ineffective in reducing the telencephalic size. Such a reduction is due to the impaired formation by the citotoxic agent of the neurons dividing at day 15 of gestation and eventually leading to the neuronal populations forming cortical interneurons. On the other hand, neurons providing the cortical afferents are not affected since their birth-date in rats preceeds the administration of MAM. This therefore results in animals with a chronic nor-adrenergic & cholinergic hyper-innervation in the cortex, without glial proliferation. In fact, neuronal markers of afferent fibers show higher concentrations with respect to normal animals, whereas markers for intrinsic neurons do not show any difference in concentration, indicating a non-selective loss of these neuronal populations. The only exception seems to be somatostatin, which is reduced (833 pmol/mg prot in controls, versus 450 pmol/mg prot in MAM animals). Interestingly, these microencephalic animals do not show dramatic signs of neurotoxicity, but they show deficits only in tests for learning & memory. For instance, a clear-cut dose-dependent impairment of memory can be observed in a passive avoidance test.

Since it has been recently hypothesized that in senile dementia of Alzheimer's type (SDAT) the cholinergic degeneration is probably a secondary event consequent to neuronal losses in the target area, these microencephalic animals might be a useful model to test such an hypothesis.

408.8 **HIPPOCAMPAL PATHOLOGY IN DOMINANTLY INHERITED ALZHEIMER'S DISEASE.** R.G. Strubleţ, R.J. Polinsky*§, J.C. Hedreenţ, L.E. Nee*§, R.G. Feldman§§, P. Frommeltţţ and D.L. Priceţ. ţNeuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; §Clinical Neuropharmacology Section, NINCDS; §§Dept. of Neurology, Boston Univ. Sch. of Med., Boston, MA 02118; ţţLemgo, Federal Republic of Germany.

Familial Alzheimer's disease (FAD), inherited as an autosomal dominant, is characterized by the presence of neurofibrillary tangles and amyloid deposits in plaques. The gene encoding for amyloid protein has been localized to a region of chromosome 21. In four pedigrees, a marker on chromosome 21 has shown linkage to FAD [St. George-Hyslop et al., Science 235:885-890, 1987]. Although a genetic abnormality on 21 appears to underlie FAD, other factors (including other genes, age, anatomical features, etc.) in brain could modify the distribution of pathology. We have hypothesized that, because cases of FAD presumably represent a homogeneous population, the distribution of hippocampal pathology in these cases should be relatively uniform. Variations within or among families would reflect factors (such as duration of disease, regional responses, etc.) other than the inherited loci. In eight cases of FAD (four from one pedigree and two each from two other pedigrees), the density of plaques and tangles was determined for hippocampal subdivisions CA1-4, subiculum, presubiculum, and dentate gyrus. Onset ranged from 37 to 56 years of age, and duration of disease varied from 4 to 16 years of age. In all families, there was a consistent pattern of pathology in these regions. CA1-2 had the highest density of both plaques and tangles; presubiculum showed little pathology. Plaque size was consistently largest in CA4. Statistical analysis disclosed no differences among families in plaque density, distribution, or size. Individual differences (e.g., plaque type, plaque and tangle density, or cerebellar plaques) did not assort by family. These observations suggest that the expression of hippocampal pathology in FAD is quite stereotyped and that characteristics of the pathology vary as a function of hippocampal region. However, it remains to be determined whether the pattern of pathology occurring in the hippocampus reflects a direct effect of a gene abnormality on chromosome 21 or some other determinant (such as transmitter specificity, connectivity, etc.) of cells in this region.

408.9 **PATHOLOGY IN THE LOCUS COERULEUS AND DEPRESSION IN ALZHEIMER'S DISEASE.** C.A. Rossţ, R.M. Zweig*§, J.C. Hedreen§, C. Steele*ţ, D.L. Price§ and M.F. Folstein*ţ. ţDept. of Psychiatry & Behavioral Sciences, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205; §Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Depression is a frequent complication of Alzheimer's disease (AD). We have recently found that patients with AD who become depressed tend to have a strong family history of affective illness [Pearlson et al., in preparation]. Since AD has a known neuropathology, it may provide a model for studying aspects of the neuropathology of depression. The noradrenergic system has often been linked to depression, and noradrenergic systems in the locus coeruleus (LC) degenerate in some cases of AD (e.g., Bondareff et al. [Neurology 32:164-168, 1982]). The number of neurons in the LC was measured in the brains of patients with pathologically proven AD; cell counts in patients who had depression (DSM-III criteria) were compared to counts in patients who did not have symptoms of depression. All pigmented neurons in the LC (on one side of one 12-µm cresyl violet-stained section at each of three levels of the LC) were counted by two investigators blind to clinical histories. At three levels, numbers of neurons were decreased in depressed AD patients compared to nondepressed AD patients: rostral level, 9.9 ± 3.4 (mean ± SD) (n = 6) vs. 21.9 ± 16.4 (n = 6); middle level, 15.1 ± 7.4 (n = 7) vs. 34.5 ± 21.7 (n = 9); and caudal level, 46.2 ± 6.7 (n = 5) vs. 51.8 ± 15.7 (n = 5). Control cases were: rostral, 54.1 ± 21.4 (n = 7); middle, 139 ± 41 (n = 9); and caudal, 147 ± 35 (n = 9). The difference between depressed and nondepressed AD cases was significant ($p < 0.05$) at the middle level of the LC, the region in which AD pathology was greatest. The difference between depressed and nondepressed AD cases was unlikely to be due to differences in age of onset or severity of dementia shortly prior to death, since forebrain pathology (blind ratings of neuronal loss, plaques, and tangles in amygdala, hippocampus, neocortex, and nucleus basalis) was indistinguishable in depressed vs. nondepressed individuals with AD. These findings provide evidence for a relationship between the presence of depression and the severity of cell loss in the LC in AD. Thus, AD may provide a disease in which to study aspects of the neuropathology of depression.

408.10 AGE-RELATED LOSS OF NERVE GROWTH FACTOR (NGF) SENSITIVE RAT BASAL FOREBRAIN NEURONS S. Koh, P. Chang* and R. Loy Depart. of Neurobiology and Anatomy, Univ. of Rochester, Rochester NY 14642

Basal forebrain magnocellular neurons contain NGF receptors, take up and retrogradely transport NGF from their terminals in the hippocampus and neocortex, and respond to exogenously administered NGF by increased production of choline acetyl transferase(ChAT). Following transection of the fimbria-fornix, there is a rapid loss of NGF receptor and subsequent cell death in the medial septum (MS) and vertical limb of diagonal band (VDB). This cell death can be prevented by continous intraventricular injection of NGF. Furthermore, chronic infusion of NGF into the subpopulation of behaviorally impaired aged rats improves their swim maze performance, suggesting that degeneration of NGF sensitive neurons in the basal forebrain may play a role in the cognitive loss in aging. We have used 192 IgG (E. Johnson, Washington U.) to immunocytochemically visualize NGF receptors in aged (30 mos) and young (10 mos) male Long Evans rats. The brains were removed from the perfused rats, 30 um thick freezing microtome sections cut, and every third section was collected and processed for immunocytochemistry. Immunoreactive neurons in MS, VDB and nucelus basalis(NB) were counted from every stained section (50 horizontal sections per brain). The entire nuclei spanning 20 sections for MS , 18 sections for DB and 33 sections for NB were included for analysis. A subpopulation of aged rats can be identified in which NGF immunoreactive cells in the basal forebrain appear vacuolated and shrunk, and the neuropill staining is makedly reduced. In these morphologically distinct aged rat brains, there is not only a shrinkage of neurons but an apparent cell loss: 30 % in MS, 26 % in DB and 29 % in NB. Even in the aged rats in which the qualitative change in cell morphology is not apparent, there is a moderate decrease in cell number relative to the young control: 7 % in MS, 17 % in DB and 18 % in NB. While age-related declines in neurochemical markers of the cholinergic system are well-documented, no actual loss of acetylcholine esterase- or Chat-positive basal forebrain neurons has been previously reported in rodents. Our finding is the first indication of apprent cell loss and degeneration of neurons in the basal forebrain in aged rats. Supported by Grant ADRDA PRG-86-041 (RL) and MH-09541 (SK)

408.11 **CHOLINE-INDUCED PLASTICITY IN NEOCORTICAL NEURONS OF THE AGING MOUSE BRAIN: EFFECT ON DENDRITIC BRANCHING AND SPINES.** M. Bedo-Wierdl*, R. Dvorak*, and R.F. Mervis. Div. Neuropathology, The Ohio State Univ. Med. Ctr., Columbus, Ohio 43210.

Loss of dendritic material may be found in advanced normal aging and in Alzheimer's disease. This may reflect deterioration of neuronal membranes and would involve changes in dendritic branches and spines. The resultant disruption of neuronal circuitry and synaptic elements would be the anatomical substrate of geriatric memory impairment. Diets enriched in choline may, however, provide a substrate for maintenance and/or synthesis of new membrane material. Thus, in this study we evaluated the effects of long term dietary enrichment with choline chloride, highly purified (95%) phosphatidylcholine (PC), and a commercial soy-lecithin (Centrolex). Male C57Bl/6NNIA mice were fed diets enriched with these choline-containing compounds at either 4.8 or 10.8 mg choline/gm of chow for 11 months -- from 13 to 24 months-old. Control mice were fed a standard lab chow (Purina) containing 2.3 mg choline/gm. Experimental diets were isocaloric and isonitrogenous. The 24 month-old mice were killed, and their brains stained using the rapid Golgi method. Dendritic branching of the basilar tree of layer V pyramidal cells of frontoparietal cortex was quantified using the Sholl Method of concentric circles. Dendritic spines were evaluated on the terminal tips of the basilar tree of this same neuronal population in terms of density, length, and categorized according to shape.

In these subjects, normal aging resulted in a 26% loss of distal dendritic branches (non-significant). However, high levels of dietary supplementation with choline and PC produced significant new growth in the distal third of the dendritic field. In comparision to age-matched controls, the increase amounted to 166% ($p < 0.006$) and 125% ($p < 0.02$), respectively, for choline and PC.

Analysis of dendritic spines from 20 micron terminal tip segments showed no significant loss of spine density with normal aging, nor was there a significant change in spine length. In view of the significant increase of branch length, these findings suggest that new dendritic branch growth was accompanied by new formation of dendritic spines.

(Supported by the Department of Pathology, the Roessler Foundation, and the Central Soya Company).

408.12 EFFECTS OF PHOSPHATIDYLSERINE ADMINISTRATION ON AGE-RELATED CHANGES OF SEPTAL CHOLINERGIC NEURONS IN THE RAT. M.G. Nunzi, F. Milan*, P. Polato*, D. Guidolin* and G. Toffano. Fidia Research Laboratories, 35031 Abano Terme (PD), Italy

Derangement of the brain cholinergic system has been implicated in the cognitive deficits associated with advancing age. In this study we have investigated age-related structural changes in cholinergic nuclei of the rat septal complex and the possible counteracting effect of prolonged phosphatidylserine (BC-PS) treatment. Phosphatidylserine is a naturally occurring phospholipid, provided with pharmacological activity (Toffano and Bruni, Pharmacol Res Comm, 12:829-845, 1980). In this context, BC-PS has been shown to increase learning and memory functions in old rats (Drago et al., Neurobiol Aging, 2:209-213, 1981) and to stimulate brain cholinergic mechanisms (Pedata et al., Neurobiol Aging, 6:337-339, 1985). Six Sprague-Dawley rats for each of the following groups were studied: 4-month-old rats, 27-month-old rats and old age-paired rats which received BC-PS suspended in tap water (average intake: 50 mg/kg/die) from the age of 15 months. The cholinergic cell population of the medial septal nucleus and diagonal band, identified on 40 μm thick sections by using a monoclonal antibody to choline acetyltransferase (ChAT) and the PAP technique, was studied by means of an interactive image analysis system (IBAS, Kontron Zeiss). In old-untreated animals the mean cell number of ChAT-positive cells is reduced by 19.6% ($P<.05$; Student's t test) with respect to young animals, while in old BC-PS treated rats no significant differences were observed. Furthermore, the mean area and the mean diameter per cell body are significantly decreased in old untreated animals (-18.7% and -8.6%, respectively; $P<.01$) whereas no significant reductions are present in old BC-PS treated rats. In the same groups of rats a densitometric analysis of acetylcholinesterase (AchE) staining in the hippocampus, a major target of the cholinergic innervation from medial septal complex, also revealed a counteracting effect by the phospholipid on age-induced decrease of the AchE staining. This finding may be correlated with the capability of long-term BC-PS treatment to maintain dendritic spine density of hippocampal pyramidal neurons in old rats (Milan et al., Bess et al. (eds), John Libbey Eurotext 408-413, 1986). In conclusion, these data suggest that BC-PS may affect survival and trophic degree of cholinergic septal neurons by ensuring remodelling of synaptic connections throughout advancing age.

CELL LINEAGE AND DIFFERENTIATION V

409.1 THE SEX-DETERMINATION GENE DAUGHTERLESS IS NECESSARY FOR PERIPHERAL NEURON DIFFERENTIATION IN DROSOPHILA EMBRYOS. M.Caudy#, E.Grell*#, L.Jan#, Y.N.Jan#, C.Dambly-Chaudiere*+ and A.Ghysen+; # Howard Hughes Med. Inst., U.C., San Francisco, CA 94143; + Lab. de Genet., U. Lib. de Bruxelles, Belgium..

In order to analyse the genetic regulation of neuronal differentiation in Drosophila embryos, we began to study l(2)II, an embryonic lethal mutation reported to have CNS defects (Campos-Ortega, quoted in Nusslein-Volhard, et. al., (1984), Roux's Arch. Devel. Biol.; 193: 267-282). We found that in addition to CNS defects, l(2)II embryos are missing the entire peripheral nervous system (PNS).

l(2)II maps near the (maternal effect) sex-determination gene daughterless (da, genetic map, 2-41.3), and both lie within the deficiency Df(2L)J27 (chromosome map, 31D-31F). It was recently found (independently by E. Grell and J. Tomkiel) that l(2)II is lethal when in trans with da (as are the lethal da null alleles, da^2 and da^3, Cronmiller and Cline, 1987; Cell 48: 479-487). This suggested that l(2)II is a mutation in the same gene. To test this possibility, we stained da^2 and da^3 homozygotes and found that they also are missing the entire PNS and have (varied) CNS defects. Similar defects were seen in da^2/l(2)II embryos as well. l(2)II, da^2 and da^3 all are EMS induced mutations so that each is likely to be a point mutation rather than a deletion covering more than one gene. Furthermore, the original da hypomorph mutation has been found to show partial defects in PNS formation in many of the embryos in which it is in trans with either l(2)II or the deficiency Df(2L)J-27. Therefore, l(2)II and all of the da alleles are defective in PNS development, and appear to be mutations in the same gene.

In l(2)II and the da null homozygotes, neuron-specific antibodies reveal no sign of peripheral neuron differentiation at any time during (or after) the 9-11 h. stages during which the PNS normally is formed. However, the epidermis continues normal segmentation and cuticle formation at least until the 24 h. stage. Also, the gut continues to undergo morphogenesis well after the normal time of PNS differentiation, and the somatic muscles differentiate in their normal numbers and positions during the 10-14 h. stages. Therefore, it appears that the da+ gene product is not necessary for the differentiation of all tissue types (at least not at early stages), but is necessary for the differentiation of all peripheral neurons. Thus, the da+ gene product appears to have at least two widely different developmental functions: as a maternally derived factor which is necessary for sex determination in the embryo, and as a zygotically derived factor which is necessary for the embryo to form a peripheral nervous system.

409.2 **CELLS IN NEURONAL LINEAGES OF XENOPUS EMBRYOS PREFERENTIALLY SYNTHESIZE PARTICULAR PROTEINS.** S.L. Klein and M.L. King*. Dept. Anat. & Cell Biol. Univ. Virginia Sch. Med. Charlottesville, VA 22908.

The proteins that are synthesized by blastomeres that produce different amounts of the nervous system were compared. ^{35}S-methionine was injected into blastomeres whose progeny will express different fates and allowed to incorporate into newly synthesized proteins for several cell cycles. The proteins were extracted in Tris buffer long before primary embryonic induction and separated by 1-dimensional SDS polyacrylamide gel electrophoresis. Two proteins of about 200,000 daltons were preferentially synthesized by cells that produce a large portion of the nervous system. For example, between the 16-, and 64-cell stage, these proteins were synthesized in 10-fold excess by dorsal animal cells (the progeny of blastomere D1.1) compared to ventral animal cells (the progeny of V1.2); their synthesis was undetectable in ventral vegetal cells (the progeny of V2.2). The amount of these proteins that the cells synthesize is correlated with the proportion of nervous system that the cells produce. D1.1 populates extensively forebrain, midbrain, hindbrain and spinal cord, V1.2 populates dorsal hindbrain and spinal cord, and V2.2 contributes only a few cells to the spinal cord (Moody,S.A. '87. Dev. Biol. 119). These results show that cells that are in neuronal lineages display specific biochemical characteristics long before they display morphological differences.

The synthesis of these proteins was examined after performing manipulations that changed the proportion of cells that entered neuronal lineages. UV irradiation during the first cell cycle produces an embryo with virtually no nervous system and treatment with Li^+ during the fifth cell cycle produces an embryo that consists almost entirely of head structures. These manipulations had no affect on the overall pattern of protein synthesis, but preliminary observations indicate that the manipulations altered the synthesis of the 200,000 dalton proteins. If these results are substantiated they will suggest that these proteins are involved in regulating cell fate.

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409.3 IDENTIFICATION OF RADIAL GLIAL CELLS WITHIN THE DEVELOPING MURINE CENTRAL NERVOUS SYSTEM USING A NEW IMMUNOHISTOCHEMICAL MARKER. J.-P. Misson*, M. Yamamoto, M.A. Edwards,, G. Schwarting, V.S. Caviness Jr. E.K. Shriver Center, Waltham, MA 02254 and Neurology Dept., Mass. General Hosp., Harvard Medical School, Boston, MA.

The monoclonal antibody, RC2, was generated in mouse by conventional hybridoma methodology using rat brain homogenate as immunogen. Immunocytochemistry was performed on fresh-frozen cryostat sections and vibratome sections following fixation with periodate-lysine-parafomaldehyde. From the neural tube stage (E9-10) until the early postnatal period, RC2 delineates in the ventricular zone (VZ) of the CNS a subpopulation of radial bipolar cells similar to those described with another radial glial (RG) marker previously generated in our laboratory, RC1 (Edwards et al., Neurosci. Abst., 12: 182, 1986). A descending process extends to the ventricular margin and an ascending process contacts the external limiting membrane by one or more endfeet expansions. By E11, the radial processes are organized in fascicles. Between E12-E17, they adopt marked regional variations in their patterns of alignment and density, particularly in association with cerebral corticogenesis (Gadisseux et al., Neurosci. Abst., 13, 1987) and with cerebellar and basal ganglia development. A progressive perinatal loss of stained RG is observed which coincides with the appearance of monopolar and multipolar immunoreactive cell forms outside the VZ, as consistent with the transformation of RG into astrocytes. In the cerebellar cortex from E15 to P7, RC2-positive monopolar cells accumulate in the Purkinje cell layer and differentiate as Bergmann glia. Over the same period, morphologically similar RC2-positive cells appear in the cortical plate of the hippocampal formation. Between P7 and P14, immunoreactivity of all glial cell forms becomes undetectable. Antigenicity with RC2 sustains aldehyde fixation, thus allowing high resolution light microscopy and ultrastructural study of RG morphology in plastic sections following Epon-embedding of reacted vibratome sections. Such preparation combined with [^{3}H]thymidine autoradiography allow confident recognition of mitotically-active bipolar RG of the murine cerebrum, consistent with our previous study using RC1 (Misson et al, Neurosci. Abst. 12:1585,1986).

Antigens recognized by RC2 in immunoblots of E15 mouse brain include two discrete protein bands at 95 and 106 kD and a single species of ganglioside or acidic glycolipid. Such antigens have not previously been identified as associated with radial cell forms. Thus, in addition to providing a sensitive and robust marker for developmental studies of RG, the RC2 antibody appears to identify a novel glycoconjugate epitope associated with this cell class in the fetal mouse CNS. [Supported by NATO grant 27B85BE and NIH grants HD05515, HD21018, CA25532, NS12005].

409.4 CO-EXPRESSION OF MYELIN BASIC PROTEIN AND GLIAL FIBRILLARY ACIDIC PROTEIN BY IMMATURE OLIGODENDROCYTES IN VITRO. B.H. Choi. University of California Irvine, Irvine, CA 92717.

Although it is generally believed that oligodendrocytes (OC) originate from poorly defined cells referred to as "glioblasts" the nature of the precursor cells from which OC are derived is still debated. In previous studies from this laboratory, we presented evidence to suggest, in the developing human fetal CNS, (a) that "transitional" cells possessing the light and EM features of both astrocyte and OC appear just prior to the onset of myelination, (b) that immature OC transiently express glial fibrillary acidic protein (GFAP) and (c) that myelin-forming OC are probably derived from astroglial precursor cells.

In order to test this hypothesis further, we have prepared mixed glial cell cultures from neonatal rat cerebra according to the method of McCarthy and de Vellis (J Cell Biol 85: 890, 1985). Small round cells were separated from the mixed glial cell cultures at 7 to 9 days-in-vitro by shaking and plated onto the multi-well culture chambers containing cover slips. Eighteen to 24 hours later, the cultures were studied immunocytochemically by exposing the same population of cells to both antisera for myelin basic protein (MBP) and GFAP by the use of double-labeling (fluorescein or rhodamine-conjugated) indirect immunofluorescent technique. The cells were also double-labeled for galactocerebroside (GC) and GFAP. Mouse monoclonal anti-GFAP and rabbit polyclonal anti-MBP sera were used for double-labeling. Monoclonal anti-GC and polyclonal anti-GFAP sera were also used.

Although there were numerous small round cells on the cover slips expression of both MBP and GFAP was seen in the minority of the cell population that bear the morphological characteristics of OC by light and electron microscopy. There were small round cells showing immunoreactivity only for GFAP while others reacting positively for either MBP alone or GC alone. Many others reacted negatively for all three antisera. The demonstration of both MBP and GFAP in the same OC suggests that these cells correspond to the "transitional" cells that we have described previously and supports the hypothesis that OC probably originate from astroglial precursors.

(supported in part by USPHS grant ES 02928)

409.5 CLONAL ORGANIZATION OF A GLIAL CELL LINEAGE IN VIVO. R.K. Small. Dept. of Zoology and Cell Biology, University College, London WC1E 6BT.

Myelin-forming cells of the rat optic nerve derive from migratory progenitor cells that enter the chiasmal pole of the nerve at embryonic ages (Small et al, 1987). These cells are bipotential 'O-2A' progenitors which give rise at postnatal ages, first, to oligodendrocytes and later, to type-2 astrocytes, the cells shown recently to contribute fine processes to nodes of Ranvier (ffrench-Constant and Raff, 1986).

When first detected in the embryonic nerve, the O-2A cell lineage is present in the chiasmal portion of the nerve as linear chains of cells occurring in the ventral half of the nerve and extending into the optic chiasma. Mitotic figures are frequently observed among the cells within these chains. With time, the number of linear chains increases and these chains spread into the retinal portion of the nerve. At embryonic ages, these cells are not present beyond the optic chiasma in the central continuation of the optic pathway, the optic tract. This suggests that progenitor cells migrating from a source in the vicinity of the chiasma, show directional migration into the optic nerve but do not enter the optic tract. Ultrastructural analyses of embryonic nerves reveal extensive surface contacts between cells of these chains and surrounding ganglion cell axons in the nerve; few contacts have been detected so far, between O-2A cells and the radially-oriented, early glial cell population of the nerve: type-1 astrocytes. The complex intercellular junctions seen between cells within chains at EM level, permit clusters of O-2A lineage cells to be obtained for in vitro analysis after gentle dissociation of embryonic nerves.

Cells of the O-2A lineage appear in the embryonic optic nerve shortly after ganglion cell growth cones reach the optic chiasma, perhaps indicating that these neurites provide a pre-formed substrate guiding progenitor cells into target areas which they eventually myelinate. Thus, the O-2A cell lineage seems to be intimately associated with, and perhaps even dependent upon, the axon surface for its first appearance in the embryonic nerve. This association may persist and form the basis for the later functional specialization shown by the lineage for ensheathing axon surfaces.

409.6 THE DEVELOPMENTAL EXPRESSION OF GLIAL SPECIFIC mRNAs IN PRIMARY CULTURES OF RAT BRAIN VISUALIZED BY IN SITU HYBRIDIZATION. E. Holmes*, G. Hermanson*, R. Cole*, and J. deVellis. Laboratory of Biomedical and Environmental Sciences, UCLA, Los Angeles, CA 90024.

In our model tissue culture system for studying glial differentiation, 1-2 day old rat brain cortex is dissociated and plated in serum-supplemented medium. A stratified mixed glial population results where phase-dark process-bearing cells astride a bedlayer of polygonal astrocytes. To more definitively characterize lineage and differentiation in these cells maintained in culture over time, the expression of mRNAs encoding glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP), respectively astrocyte and oligodendrocyte markers, were mapped by in situ hybridization with ^{35}S-labeled riboprobes. The time points evaluated were 8, 12, 14, and 19 days in culture. GFAP mRNA expression, seen in astrocyte nuclei and cytoplasm as well as in some phase-dark cell nuclei, increased through day 12 and thereafter plateaued amid a concomitant increase in cell density. In contrast, MBP mRNA was barely detectable at day 8 but increased approximately 20 fold by day 12 and 14. As the phase-dark cells matured, labeled isolated round cells extended processes in which MBP mRNA was detected and by day 19, large clumps of labeled cells dominated the culture. Control hybridization with the sense RNA strand was equal to background emulsion. Extending the characterization of these cultures by in situ hybridization for glutamine synthetase (GS) and glycerol phosphate dehydrogenase (GPDH) (other glial markers) is in progress. The population distribution will be computer analyzed.

Supported by AWU/DOE and NIH.

409.7 **NEUROGENESIS OF IMMUNOCYTOCHEMICALLY IDENTIFIED CHOLINERGIC NEURONS IN THE BASAL FOREBRAIN OF RAT.** D.R. Brady, P.E. Phelps and J.E. Vaughn, Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The basal forebrain cholinergic system embodies a heterogeneous group of neurons that are distributed in the telecephalon extending from the medial septal nucleus at the midline through the diagonal band nucleus along the ventral forebrain, and continuing through the nucleus basalis of Meynert to the peripallidal region dorsolaterally. Neurons of this cholinergic system project topographically to the hippocampal formation and cerebral cortex, and have been implicated in the pathophysiology of neurodegenerative disorders such as Alzheimer's disease. This study was conducted to ascertain developmental relationships between cell birthdays and the expression of the cholinergic neurotransmitter phenotype of neurons that comprise the basal forebrain system.

The techniques of tritiated-thymidine autoradiography and ChAT immunocytochemistry were combined to determine the birthdays and neurogenetic gradients of cholinergic cells in this region of rat brain. Pregnant rats received a single intraperitoneal injection of isotope on embryonic days 12-17 (E12-17). Isotopically labeled progeny were processed as adults for ChAT immunocytochemistry followed by autoradiography. For quantitative analysis, ChAT-positive neurons with centrally sectioned nuclear profiles were counted and classified as being heavily radiolabeled or unlabeled according to grain counts over each nucleus.

ChAT-positive neurons in the medial septal and diagonal band nuclei are generated in a caudal to rostral gradient between E13 and E17. In these nuclei, a peak generation of double-labeled cells is observed on E15. A caudal to rostral gradient of neuronal birthdays is observed between E13-E16 in the basal nucleus of Meynert. Production of neurons in this nucleus peaked early (E13) during the generation period, and declined over succeeding embryonic days. These results demonstrate that the generation of cholinergic basal forebrain neurons obeys the neurogenetic gradient of all neurons previously described for this brain region (Bayer, S.A., Int. J. Devl. Neurosci. 3:229-243, 1985). This is consistent with the results of other combined immunocytochemical/autoradiographic studies of cholinergic cells (Phelps et al., Soc. Neurosci. Abstr. 12:767, 1986) that suggest position within a neurogenetic gradient is a more potent determinant of neuronal birthday than a cell's prospective transmitter phenotype. Supported by NIH grant NS 18858.

409.8 REGULATION OF VIP AND VIP RECEPTOR IN SUBCLONES OF SK-N-SH HUMAN NEUROBLASTOMA CELLS: EVIDENCE FOR A POTENTIAL AUTOCRINE FUNCTION OF VIP. J. Waschek*, J.M. Muller*, C.-M. Hsu*, V. Yu*, W. Sadee*, and L. Eiden* (SPON: J. M. Roberts). Unit on Molecular and Cell Neurobiology, Lab. of Cell Biology, National Institute of Mental Health, Bethesda, Md 20892 and Department of Pharmaceutical Chemistry, Univ. of California, San Francisco CA 94143.

The human neuroblastoma cell line SK-N-SH contains distinct cell subtypes, which after subcloning, interconvert at a very slow rate (Biedler and colleagues, J. National Cancer Institute, 1983). The SH-SY5Y and SH-EP subclones have neuronal and epithelial and morphologies, respectively, whereas the SH-IN subclone has a morphology in between the SY5Y and SH-EP. It has been previously shown that the parent cell line SK-N-SH expresses VIP (5-10 ng/mg protein), and that 12-O-tetradecanoyl-phorbol- 13-acetate (TPA) and forskolin induce further this expression. We now report similar studies with the subclones of SK-N-SH. The SY5Y cells contain smaller amounts of peptide (0.3 to 1.0 ng VIP/mg protein) than the parent cells, and can be stimulated to increase VIP levels 3- and 2-fold by treatment for 24 hours with TPA 10^{-8} M and forskolin 2.5×10^{-5} M, respectively. Prolonged (> 4 days treatment) with dibutyryl cAMP results in a 4- to 10-fold increases in VIP. SH-IN cells, on the other hand, contain a much higher basal amount of VIP (approximately 4 ng/mg protein) than SY5Y. Northern blot analysis of mRNA isolated from subclones confirm the differences in basal expression between SY5Y and SH-IN subtypes.

To study the tissue-specific expression and inducibility of VIP at the level of transcription, the VIP gene was isolated from a human genomic library, and a 3.2 kb fragment, containing more than 2 kb of 5' flanking sequence was fused to the gene encoding bacterial chloramphenicol acetyltransferase (CAT). TPA 10^{-8} M and forskolin 2.5×10^{-6} M caused 10- and 3-fold stimulation of CAT transcription from the VIP promoter, respectively. No stimulation above basal was seen with RSVCAT (Rous sarcoma virus promoter fused to CAT) in SY5Y cells, and no stimulation was seen with either the VIP/CAT construction or RSVCAT in HeLa cells, which do not respond to TPA and forskolin with an increase in endogenous VIP.

To study the action of VIP as an autocrine factor, subclones were assayed for the presence of membrane VIP receptors by radioreceptor binding. The EP cell line was found to be rich in high-affinity VIP receptors (>7,000/cell, Kd = 0.5nM), while the SY5Y and SH-IN lines had 7-fold fewer receptors (Kd = 0.5 nM). The differences in membrane receptor levels in different subclones may be due to the presence of VIP (see related abstract, J. Muller et. al.). The results show that there are differences in individual cell types in levels of expression of VIP and presence of membrane receptors. In addition to the possible interrelationship between levels of VIP and its receptor, we suggest that VIP has an autocrine role in the determination of neuronal phenotype.

409.9 INTRINSIC AND EXTRINSIC INFLUENCES ON NEURONAL PHENOTYPE: EXPRESSION OF GAD AND OTHER mRNAs IN THE CEREBELLA OF WEAVER, AND REELER MICE. C.W. Wuenschell[1], A. Messer[4], and A.J. Tobin[1,2,3]. [1]Department of Biology, [2]Molecular Biology Institute, and [3]Brain Research Institute, University of California, Los Angeles, CA 90024; and [4]Wadsworth Laboratories, New York State Department of Health, Albany, NY 12201.

Purkinje cells of normal mice characteristically contain mRNAs encoding glutamic acid decarboxylase (GAD) and the 28 kD calcium binding protein (CaBP, or calbindin D_{28k}). The production of these two mRNAs could result from the unfolding of a program established early in cerebellar development and dependent thereafter only on factors intrinsic to the Purkinje cells themselves. Alternatively, the expression of the genes for GAD and CaBP could depend on factors extrinsic to the Purkinje cells. The genetically altered cerebella of weaver and reeler mice offer the opportunity to distinguish these alternatives.

We have used in situ hybridization to ^{35}S-labeled antisense RNAs to examine the cellular distribution of mRNAs encoding GAD, CaBP, and proenkephalin in normal and mutant mice. In normal mice, Purkinje cells contain both GAD and CaBP mRNAs, but not proenkephalin mRNA. Golgi II cells contain GAD and proenkephalin mRNAs, but not CaBP mRNA.

Despite the nearly complete loss of granule cells in weaver mice, Purkinje cells contain both GAD and CaBP mRNAs. This result supports the view that the expression of these two genes proceeds independently of extrinsic cues.

In reeler, Purkinje cells lie in a variety of microenvironments -- orthotopically between molecular and granular layers, or ectopically within or below the granular layer. We initially identified ectopic Purkinje cells by their size, distribution, and staining. In situ hybridization reveals that both orthotopic and ectopic Purkinje cells contain both GAD and CaBP mRNAs. We do not detect proenkephalin mRNA in either orthotopic or ectopic Purkinje cells. Like our findings with weaver, these data support the autonomy of Purkinje cell development. At least for the aspects of neuronal phenotype that we have examined, the pattern of Purkinje cell cytodifferentiation does not depend on environmental influences from granule cells.

This work was supported by NIH grants to AJT (#NS 20356 and NS 22256) and to AM (#NS 17633). CWW was supported in part by a USPHS Training Grant in Genetic Mechanisms (#GM 7104).

410.1 **THALAMIC AND CORTICAL AUDITORY PATHWAYS CONVERGE IN THE RAT AMYGDALA.** *J.E. LeDoux, C. Farb*, D.A. Ruggiero, and D.J. Reis,* Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Connections between the acoustic thalamus and amygdala play a role in the processing of the emotional significance of auditory stimuli (LeDoux et al, J. Neurosci., 4, 1984, 683-698; Neurosci., 17, 1986, 612-621; Iwata et al, Brain Res., 383, 1986, 195-214). The aims of the present study were to (a) precisely define the projection field of the acoustic thalamus in the amygdala and the thalamic origin of these projections, (b) determine whether the auditory cortex (ACX) and amygdala receive projections from common areas of the acoustic thalamus, and (c) determine whether the acoustic thalamus and ACX project to common areas of the amydgala.

Male Sprague-Dawley rats (n=22) were studied. WGA-HRP was injected iontophoretically under halothane anesthesia while the rats were held in a sterotaxic frame. After survival for 48 h, the rats were perfused and the brains processed histochemically using TMB as a chromagen.

The acoustic thalamus, defined as the projection field of the inferior colliculus, includes the ventral, medial and dorsal divisions of the medial geniculate body (MG), as well as adjacent regions, including the posterior intralaminar nucleus (PIN) and peripeduncular area (PP) (LeDoux et al, J.Comp.Neurol., 242, 1985, 182-213). Following injections in the ventral MG division, labeled cells and processes were confined to the posterior lateral neocortex (primary ACX). Injections centered on the medial MG division and the underlying PIN and PP produced anterograde and retrograde labeling in cortical areas ventral to the primary ACX, including the perirhinal cortex and an area between the perirhinal cortex and primary ACX (suprarhinal cortex). Anterograde labeling was also present in the lateral nucleus of the amygdala (AML) and the amygdalostriatal transition zone (AST). Injections in AML resulted in retrograde labeling in the medial MG, PIN and PP and in the perirhinal and suprarhinal cortical regions. Following injections in primary ACX anterograde and retrograde labeling was seen in the MG (especially in the ventral division) and in the suprarhinal cortical region. Injections involving the perirhinal and suprarhinal areas produced anterograde labeling in AML and AST and anterograde and retrograde labeling in PIN, PP, and medial and dorsal MG.

Acoustic information relayed from the inferior colliculus to the thalamus can thus reach the amygdala by several pathways, each of which converges on AML and AST. All parts of the acoustic thalamus project to cortical areas (primary auditory cortex, suprarhinal cortex, or perirhinal cortex). Perirhinal and suprarhinal areas, in turn, project to AML. Although primary auditory cortex does not project to the amygdala, it can influence this region through local connections with suprarhinal cortex. AML and AST also receive direct inputs from the medial MG, PIN, and PP. These multiple, parallel afferents to the amygdala may constitute pathways by which acoustic stimuli are transformed into emotional signals.

410.2 MORPHOLOGICAL EVIDENCE FOR INHIBITORY INPUTS TO THE INFERIOR COLLICULUS (IC) FROM THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS (DNLL). A. Shneiderman and D. Oliver, Dept. of Anatomy, Univ. Conn. Health Center, Farmington, CT 06032.

The DNLL may provide a significant ascending input to the IC. However, unlike other projections to the auditory midbrain, most neurons in the DNLL exhibit immunoreactivity for GAD antibodies (Adams and Mugnaini, Brain Res. Bull. 13:585-590, 1984). This implies that the DNLL could provide inhibitory inputs to the IC. In this study, the projections of the DNLL to the IC of the cat were characterized with anterograde autoradiographic techniques at the light (LM) and electron microscopic (EM) levels and with analysis of retrograde transport of HRP from the IC to the DNLL.

The bilateral projection of the DNLL to the IC had a topographic order that suggests a tonotopic arrangement of the afferent axons. At the LM level, the contralateral projection was organized into heavily labeled bands alternating with areas of lighter labeling. In contrast, the ipsilateral projection to the IC was more diffuse. If bands were present ipsilaterally, the contrast between the heavily and lightly labeled areas was not as sharp as it was contralaterally.

At the EM level, after axonal transport of 3H-leucine from the DNLL to the contralateral IC, the labeled axonal endings contained pleomorphic synaptic vesicles and made symmetric synaptic contacts. In these samples, labeling was significantly non-random ($p < .001$), and the endings which contained pleomorphic vesicles constituted approximately half of all the endings within pars centralis of the central nucleus. Of these endings with pleomorphic vesicles, 25% were labeled after injections in the contralateral DNLL. In contrast, 25-50% of the endings with round synaptic vesicles could be attributed to projections from the cochlear nuclei (Oliver, J. Comp. Neurol., In Press, 1987). Label in endings with pleomorphic vesicles was over 7 times background. Other tissue compartments were labeled at background levels or below except for unmyelinated (axonal) profiles in one sample. A few boutons with round synaptic vesicles could have been labeled, but they had radioactivity only 1.1 times background. Most labeled axonal endings formed synaptic contacts on medium or large dendrites or sometimes on cell bodies. Preliminary evidence showed the projection from the ipsilateral DNLL to be sparse, and labeled endings were widely distributed. Both types of axonal endings may have been labeled.

Thus, the DNLL could provide a major source of inhibition to the contralateral IC. Due to its proximal synapses and the banded, tonotopic organization of the axons, the DNLL could produce highly localized, significant IPSPs which are influenced by the frequency parameters of acoustic stimulation.

(Supported by NIH grant NS18391.)

410.3 THE MEDIAL GENICULATE BODY OF THE GUINEA PIG: SUBDIVISIONS, TONOTOPY AND PROJECTIONS TO AUDITORY CORTEX. H. Redies* (SPON: B.B. Lee), Max-Planck-Institute for Biophys. Chem., Dept. of Neurobiol., 3400 Göttingen, Am Faßberg, Fed. Rep. of Germany

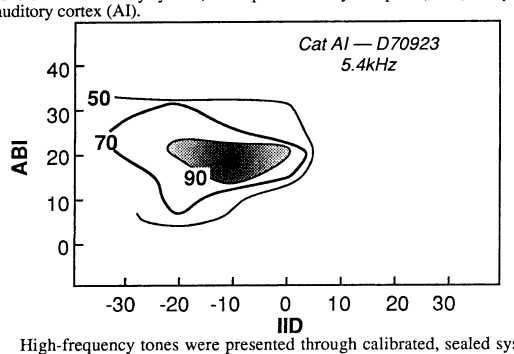
On the basis of microelectrode mapping experiments, cytoarchitectonics (Nissl stained frontal sections) and projections from the medial geniculate body (MGB) to the auditory cortex, a functional division of the MGB was achieved. The efferents of the subnuclei were determined by implanting retrogradely transported tracer substances into electrophysiologically identified fields of the auditory cortex.

(1) The **ventral nucleus** (MGBv) occupies a lateroventral position in the rostral two thirds of the MGB. It projects to the anterior and the dorsocaudal tonotopic cortical fields (see Redies and Creutzfeldt 1986, Neurosci. Lett., suppl. 26). This nucleus is tonotopically organized: in it's posterior half, low best frequencies are located laterocaudally, high frequencies mediorostrally. In the anterior half, the isofrequency laminae are curved, such that the low frequencies surround the high frequencies. The cells are of medium size and densely packed. (2) The **shell nucleus** (MGBs) surrounds the MGBv as a continuous shell dorsally, laterally and ventrally. It projects to a non-tonotopic cortical area situated ventrocaudally in the auditory cortex. A tonotopic order could not be discerned. Cytoarchitectonically, it is difficult to demarcate the MGBs from the MGBv, since the cells are often similar in appearance. (3) The **rostromedial nucleus** (MGBrm) is situated in the medial portion of the rostral part of the MGB. It projects to a small tonotopic cortical field located rostrally to the anterior field. The neurons in the MGBrm are medium sized and much less densely packed than in the MGBv. (4) The **caudomedial nucleus** (MGBcm) lies in the caudal half of the MGB. It occupies a medial position and becomes more and more prominent posteriorly. This nucleus projects to the entire auditory cortex. The neurons are large, not very densely packed and deeply staining.

A comparison of the guinea pig with other species reveals some unexpected differences. The magnocellular (medial) nucleus of the cat lies rostrally in the MGB (MOREST 1964, J. Anat., 98:611-630), while its putative homologue in the guinea pig, the MGBcm, is located caudally. Moreover, it it unclear what might correspond to the guinea pig's MGBrm in other mammals. The shell nucleus of the guinea pig corresponds topographically to the dorsal, marginal and ventrolateral nuclei of the cat's MGB. But in the guinea pig, it is clearly a continuous structure, and there is no ground for subdividing it into several functional units.

410.4 HIERARCHICAL PROCESSING OF BINAURAL LEVEL CUES IN THE ASCENDING AUDITORY SYSTEM. M.N. Semple* and L.M. Kitzes* (SPON: Y. Torigoe). Dept. of Anatomy and Neurobiology, University of California, Irvine, Irvine CA 92717.

In the central nucleus of the inferior colliculus (ICC) of the gerbil, *Meriones unguiculatus*, most high-frequency neurons are influenced jointly by the interaural intensity difference (IID) and the average binaural intensity (ABI) (Semple, M.N. and Kitzes, L.M., *J. Neurophysiol.*, in press). Many such neurons respond nonmonotonically to one or both binaural intensity parameters. Thus, some units have a best IID, some have a best ABI (BABI), and others seem to be influenced by a two-way intensity network (TWIN), responding maximally to a particular combination of IID and ABI. The present study was designed to assess the processing of binaural sound pressures at two other levels of the auditory system, the superior olivary complex (SOC) and primary auditory cortex (AI).



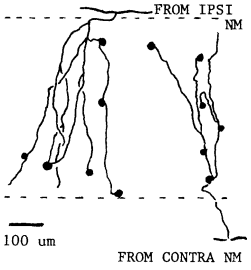
High-frequency tones were presented through calibrated, sealed systems. Single neurons in gerbil SOC were typically influenced both by IID and ABI. BABI responses were common, but none of 56 SOC neurons displayed TWIN tuning. By contrast, TWIN responses accounted for 10% of our sample in gerbil ICC and 47% of AI neurons studied in one cat. A typical TWIN response from cat AI is illustrated, showing iso-rate contours for 50, 70 and 90% of peak discharge rate. In that experiment, BABI responses (27%) were also frequently encountered.

These results reveal that nonmonotonic tuning to IID and ABI is a property common to diverse mammalian species. Moreover, the finding that TWIN responses occur with increasing prevalence at successively higher levels of the auditory system indicates that TWIN tuning is a product of hierarchical processing of binaural information.

Supported by NINCDS grants NS17596 and NS23813.

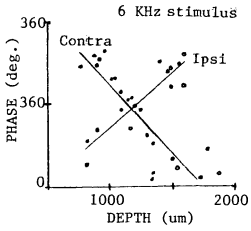
410.5 AXONAL DELAY LINES CREATE MAPS OF INTERAURAL PHASE DIFFERENCE IN THE OWL'S BRAINSTEM. C. E. Carr and M. Konishi. Div. Biology, California Institute of Technology, Pasadena, CA 91125.

Sensitivity to the interaural temporal disparities that underlie sound localization in the barn owl first appears in nucleus laminaris (NL), a brainstem auditory nucleus which is also the first recipient of binaural input. Eighth nerve afferents enter the brain and cochlear nuclei. Nucleus magnocellularis (NM) codes for phase information and projects bilaterally and tonotopically to NL.



The organization of NM afferents in NL provides a morphological substrate for a map of interaural delays. Incoming axons from the ipsilateral NM enter the dorsal surface of NL, while contralateral axons of the same frequency enter from the ventral surface. The afferents interdigitate (Fig. 1), and innervate NL neurons through the dorsoventral extent of the nucleus. If NM afferents act as delay lines, physiological recordings should reveal an orderly change in conduction delay with depth.

Period histograms were used to measure the timing of phase locked spikes in these afferents in over 200 intracellular recordings in NL. Systematic changes in phase were observed with depth; a gradual increase in delay was observed for ipsilateral afferents, and a decrease for contralateral afferents (Fig. 2). Outside the dorsal or ventral borders of the nucleus the phase shifts are less ordered, and the best frequency no longer constant. The conduction time through the nucleus reflects the time difference between the two ears (about 180 μsec) independent of frequency. Thus these afferents act as delay lines to create maps of interaural temporal disparities.



410.6 TUNING FOR INTERAURAL DIFFERENCE CUES VARIES WITH FREQUENCY FOR SPACE-SPECIFIC NEURONS IN THE OWL'S OPTIC TECTUM. S.D. Esterly and E.I. Knudsen. Department of Neurobiology, Stanford Univ. School of Medicine, Stanford, CA 94305.

The auditory system depends upon differences in the intensity and timing of a sound at the two ears to determine the location of the sound's source. The values of these interaural difference cues vary as a function of the frequency at which the cue values are measured. To exploit this information the auditory system must interpret interaural difference cues in a frequency-specific manner. The response properties of space-specific units in the optic tectum of the barn owl demonstrate that this is the case.

Interaural cue values were measured by placing probe tube microphones in the ear canals and presenting a wide-band noise stimulus from a moveable free-field speaker. The transfer function between the two microphone signals was calculated by a spectrum analyzer and provided values for interaural time differences (ITD's) and interaural intensity differences (ITD's). As expected, IID varied markedly with frequency for a given source location. In front of the owl, IID's were typically larger for high frequencies than for low frequencies. In contrast, for peripheral locations IID's could be larger at low than at high frequencies. ITD showed much less variation. Even so, at many locations there were large changes in ITD with frequency.

Space-specific units in the optic tectum respond to broad-band sounds only when the source is within a restricted region of space. We compared the responses of these neurons to free-field and dichotic stimulation. FFT-based filtering allowed precise control of dichotic parameters, including frequency-dependent specification of cue values within broad-band stimuli. Tuning for interaural cue values, within the frequency range of a unit, were well-matched to the probe tube data from the source location corresponding to the unit's receptive field. For example, units responding to frontal locations were typically found to be tuned to larger IID's at high frequencies and to smaller IID's at low frequencies. It is not yet clear whether tuning for ITD can also vary with frequency.

These results indicate that the frequency-specific interpretation of interaural difference cues is an important component of the auditory system's computation of the location of a sound source.

Supported by grants from March of Dimes, the Sloan Foundation, the NIH, a Neuroscience Development Award from the McKnight Foundation, and NIMH.

410.7 AZIMUTH AND INTENSITY SELECTIVITY OF SINGLE NEURONS IN FIELD AI OF CAT AUDITORY CORTEX. T.J. Imig, W.A. Irons, and F.R. Samson. Dept. of Physiology, Univ. Kansas Medical Center, Kansas City, KS 66103.

Single units (BFs 3-31 kHz) were studied using free-field stimulation. Barbituate anesthetized cats were placed in a support frame in a sound-isolated, quasi-anechoic chamber. Sounds were delivered from various locations (azimuths) in the frontal hemifield. Sound production, acoustic calibration, data collection and analysis were under computer control. Most units were studied in response to white noise bursts which varied between 0 and 80 dB SPL and in azimuth throughout the 180° frontal hemifield. Frequency selectivity and azimuthal selectivity to tone bursts were studied if time permitted. Isorate contours were computed and plotted in SPL-azimuth coordinates. Of a total of 51 well studied units, 10 exhibited labile or multipeaked functions and 12 were relatively insensitive to azimuth (omnidirectional). Many omnidirectional units were selective for intensity often exhibiting nonmonotonic intensity rate functions. Some exhibited similar best intensities for most or all azimuths. The remaining 29 units were azimuth selective, showing at least a 50% change in discharge rate with a change of <30° of azimuth. Sharper tuning was evident in 8/29 units in which a 50% change in discharge rate occurred over <10° of azimuth. Many azimuth selective units were completely unresponsive at certain azimuths. Many showed a well defined peak in discharge rate at a particular azimuth. Five units had peaks centered on the midline and did not respond at lateral locations. The remaining units had lateral receptive fields centered on the contralateral (18) or ipsilateral (6) sides of the head (hemifield and axial units, Middlebrooks and Pettigrew, J. Neurosci., 1:107-120, 1981). Within these receptive fields, there was a well defined peak of activity in some units and multiple peaks in others. Both the locations of the peaks and the boundaries of receptive fields varied among units. Most units were selective for stimulus intensity exhibiting nonmonotonic intensity rate functions. Best intensities ranged between 0 and 80 dB SPL. For many azimuth selective units, regardless of whether they were selective for a narrow or broad range of SPLs, azimuthal selectivity was relatively invariant with changes in SPL. At optimal or near optimal SPLs, azimuthal locations of receptive field peaks and one or both borders remained relatively invariant in spite of changes in SPL. This feature is appropriate for representing sound direction. Most units showed less (sometimes dramatically less) azimuthal selectivity in response to tones than to noise. In one case, a unit had a well defined midline receptive field when stimulated with noise, but was omnidirectional when stimulated with tones of various frequencies within its response area. Azimuthal selectivity of AI neurons is a product of interaural intensity differences, intensity selectivity and spectral complexity. (Supported by NINCDS Grant NS17720).

410.8 FREQUENCY CONTRAST FOR PURE AND COMPLEX TONES IN THE ALERT AUDITORY CORTEX. R.W.W. Tomlinson and D.W.F Schwarz. Lab. of Otoneurology, Div. Otolaryngology and Dept. Physiology, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

In search for a neuronal pitch mechanism we recorded from neurons in the auditory cortex of monkeys which had previously been shown to perceive the pitch of a missing fundamental. While recording carefully isolated extracellular spikes, responses to pure tone stimuli were contrasted with thoses to harmonic complexes, with and without the fundamental and other low components. We have previously shown that "filter neurons" respond to pure or complex tones and noises whenever sound energy falls within their v-shaped response area. In contrast, "Fo neurons" are as sharply tuned to physically present complex tone fundamental frequencies, as they are to pure tones. Sharp tuning in these cell is independent of intensity and is associated with side band inhibition with a broad inhibitory band below the best (excitatory) frequency region. We now report on a new response class, the "contrast neurons". These cells exhibit adjacent excitatory and inhibitory frequency bands with pure tone stimuli, the inhibitory band being located above the excitatory band with a more or less sharp border between the two. Excitation and inhibition occur with identical or similar pure tone and Fo frequencies when complex tone stimuli are used. The frequency borderline (contrast) between excitation and inhibition is identical for complex tone fundamentals and pure tones, however, it drops by about one octave when the fundamental is removed from the complex. The contrast is stronger with complex tones and the border is also stable with respect to intensity. Excitation or inhibition can dominate the response to noise at different intensities in the same cell. It is conceivable (although uncertain) that contrast neurons represent an inhibitory input to Fo neurons, moulding their common sharply tuned excitatory band for both complex and pure tones.

Supported by MRC and Pacific Otolaryng. Fdn.

410.9 ON-OFF CELLS IN THE INFERIOR COLLICULUS OF THE MUSTACHED BAT: ACOUSTIC GLINT DETECTORS? H.D. Lesser, R.D. Frisina, and W.E. O'Neill. Dept. of Physiology, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

Amplitude modulation is a dynamic feature common to communication sounds of many different species and plays a particularly important role in the case of echolocating bats pursuing flying insects. The mustached bat (Pteronotus parnelli) has a long constant-frequency component in its echolocation signal which, when reflected off of the beating wings of insect prey returns as an amplitude- (and frequency-) modulated echo. Modulations are also generated by interference between outgoing vocalizations and returning Doppler-shifted echoes (Henson et al., Nat'l Geographic Res. 3(1):82, 1987). Sudden amplitude changes in echoes ("acoustic glint") produced by reflections from insect wings may be important in both detection and discrimination of prey against a noisy background.

We have recorded from 354 neurons in the central nucleus of the inferior colliculus. Neurons were classified by their PST histogram response patterns to pure tones. Sinusoidal amplitude-modulated (SAM) stimuli (30 ms dur., 80% modulation, 33-800 Hz, 10 dB above threshold) were chosen to model echoes which occur during the approach phase of predation. Most units (81%) with on-off responses to pure-tone stimuli (n=42) showed no increase in firing or phase-locking to SAM stimuli. Many on-off units showed a period of inhibition preceding both the on and off responses. In units where background activity permitted detecting inhibition, 60% showed this biphasic, on-off response. Other unit classes, including on and on-sustained cells preferred SAM to pure-tone stimuli and showed phase-locked responses.

In one on-off unit, results of two-pulse stimulation and modulation of a pure tone with continuous pseudorandom (white) noise demonstrated the implications of this response pattern. This unit was unable to phase-lock to SAM and fired fewer spikes to SAM than to pure tones. It had a leading-inhibitory response to pure tone burst onset and offset.

In two-tone experiments, as the interval between two 2 msec tone bursts was reduced below 10 msec, the response to the **first** pulse decreased while the response to the **second** was unchanged. Two-pulse predictions made from the white-noise responses predicted the reduction in the first pulse response seen in the "classical" two-pulse experiment. This unusual response resulted from the interaction of the leading inhibitory response to the second pulse with late excitation from the first pulse.

In conclusion, a population of such neurons, whose high-pass characteristic makes them sensitive to transient, but not ongoing, amplitude modulations could function as "glint detectors" for echolocating bats hunting prey. [Support: PHS 5-T32-GM07356 to HDL, NIH-NRSA NS07343 to RDF, BNS 8311627 to WEO]

410.10 AUDITORY INPUTS AND RESPONSES OF SUPERIOR COLLICULAR NEURONS OF THE BIG BROWN BAT, EPTESICUS FUSCUS TO STATIONARY AND MOVING ACOUSTIC STIMULI. P.H.-S. Jen, W.P. Zhang*, X.D. Sun* and S.Q. Zhang*. Division of Biological Sciences, The University of Missouri, Columbia, MO 65212.

An echolocating bat has two well developed superior colliculi (SC) although its other visual centers are generally poorly developed. In order to study the functional role of the bat's SC during echolocation, we examined the responses of SC neurons of Eptesicus fuscus to stationary and moving pure tone pulses and/or frequency-modulated (FM) stimuli (4 ms duration, 0.5 ms rise-decay times) under free field stimulation conditions. Acoustic stimuli were broadcast either from a loudspeaker placed at several chosen azimuthal and elevational angles around the bat's head or from a moving loudspeaker broadcasting from the same azimuthal angles. Three angular velocities (36°/S, 90°/S, and 180°/S) were chosen and more than three stimulus intensities above the minimum threshold (MT) of each neuron were used. The MT and discharge rate of each neuron as a function of loudspeaker position was systematically examined. HRP solution was then iontophoretically ejected from micropipette glass electrodes onto the recording sites in order to study the auditory inputs to the SC. SC neurons generally discharged only a few action potentials to acoustic stimuli. Their response latencies were between 3.6 and 20 msec, but most were below 12.5 msec. All neurons had triangular threshold curves. They were more sensitive to downward-sweep FM stimuli than to pure tone pulses. They displayed their lowest MT and maximal discharge rate to acoustic stimuli delivered from the contralateral hemifield of the bat's frontal auditory space. The angle of maximal discharge rate of each neuron did or did not change as a function of stimulus intensity. The maximal discharge rate of SC neurons generally changed as a function of moving stimulus velocity. While some neurons discharged maximally at a specific angle regardless of direction of stimulus movement, other neurons only discharged maximally when the stimulus was moved at one direction or was moved away from the center line. The SC of Eptesicus fuscus can be classified into superficial, intermediate and deep layers according to the principal cell type of each layer. Neurons isolated from the superficial layer generally had poorer signal-to-noise ratio than those isolated from the deep layer. Histological examination showed that the SC receives auditory inputs from the inferior colliculus and nucleus of the lateral lemniscus. Labelled neurons were also found in visual cortex, medial vestibular nucleus, central gray, substantia nigra, reticular formation, lateral pontine nucleus, interpeduncular nucleus, fastigial and dentate nuclei. (Supported by NIH grant NS 20527 to P. Jen).

410.11 INTERCONNECTIONS BETWEEN THE MEDIAL GENICULATE BODY AND THE AUDITORY CORTEX IN AN FM BAT. Sharon Shannon* and Donald Wong. Department of Anatomy, Indiana University School of Medicine, Indianapolis, IN 46223.

The echolocating bat Myotis lucifugus contains delay-sensitive neurons that exhibit facilitative responses to FM sound pairs at specific echo delays (Sullivan, J. Neurophysiol. 48:1011, 1982). These delay-sensitive cortical neurons provide the bat with information about target distance. To explore the anatomical basis of how these physiological response properties are generated, the interconnections of this functional subregion in the auditory cortex with the medial geniculate body (MGB) were traced with horseradish peroxidase (HRP). HRP was iontophoretically injected into a zone containing delay-sensitive neurons mapped from extracellular single-unit recording. Cell and fiber labeling was found in the dorsal part of the MGB. Although larger injections (about 200 um dia.) resulted in more extensive labeling in the dorsal MBG, discrete labeling was also found in the ventral and dorsomedial parts. Injections made into the tonotopic central nucleus of the inferior colliculus resulted in a distinctive laminated pattern of fiber labeling. The differing patterns of geniculate labeling suggest that different parts of the MGB generate projections to functionally distinct delay-sensitive and tonotopic zones. These results are consistent with findings of separate FM-FM and tonotopic parts of the MGB as demonstrated in neurophysiological and neuroanatomical tracing studies in the mustached bat, Pteronotus parnellii (Olsen, Neurosci. Abstr. 8:349, 1982).

This work was supported by BRSF from Indiana University (PHS S01 RR 5371-H 46-821-01).

410.12 PERIODICITY OF SOUND WAVE ENVELOPES IS MAPPED IN THE FOREBRAIN OF THE MYNAH BIRD B. Hose, G. Langner, and H. Scheich (SPON: ENA) Zoological Institute, Technical University, 6100 Darmstadt, FRG

Coding of periodic acoustic signals was studied within field L, the avian analogue of the mammalian auditory cortex. Field L is trilayered and tonotopically organized. Isofrequency planes are oriented across the three layers L_1, L_2, and L_3 in a roughly orthogonal fashion. The central lamina L_2 receives most of the afferents from the thalamus while L_1 and L_3 are postsynaptic to L_2.

The synchronization of unit responses to envelopes of sinusoidally amplitude modulated tones (SAM) and repetetive narrow band noise bursts (RNB) were determined as a function of envelope frequency (EF). The degree of synchronization was expressed in terms of vectorlength of circular statistics.

From 428 acoustic field L units 77 % showed a synchronized response to RNB or SAM. 66% of these were tuned to a best envelope frequency (BEF). BEFs varied from 0.3 to 380 Hz and showed an orderly representation within isofrequency planes (Hose, B., Neurosci.Lett.Suppl., 22:170, 1985). BEFs were more or less symmetrically distributed above and below the input layer L_2. In L_2 units preferred significantly higher EFs than in the postsynaptic layers. A limen between overlapping ranges of EF representation could be drawn at about 20 Hz. 65 % L_2 units preferred EFs above 20 Hz, while in the postsynaptic layers L_1 and L_3 86 % respectively 83 % units had BEFs below 20 Hz. Sharpness of temporal tuning in terms of Q_{3dB} was significantly higher in L_1 and L_3 than in the input layer L_2.

The present results give a first idea of the organization of auditory forebrain fields: They demonstrate a spatial relationship between lamination, spectral (tonotopy), and temporal (EF representation) signal parameters. Along the ascending auditory pathway within the forebrain field the lamination separates coding of simple from specialized spectral (Langner, G., Exp. Brain Res., 43: 11, 1981) and temporal properties of sounds and high from low EFs. The latter finding may contribute to a discussion about a key issue of psychophysics and behavioural physiology of SAM signals: SAM signals with EFs below about 20 Hz in humans psychophysically elicit the perception of loudness fluctuations or rhythms. For higher EFs the sensation cannot follow the loudness fluctuations and, beside other sensations, one may perceive periodicity pitch. Temporal resolving power in birds was described to be only slightly better when compared to humans (Dooling, R.J., J.Comp.Physiol., 143: 383, 1981). Thus, following the comparison, the majority of postsynaptic L_1 and L_3 units were tuned to rhythms typical for animal communication sounds and speech, while units of the input layer L_2 covered at least four of five octaves of the range of periodicity pitch sensation. Supp. by DFG-SFB 45.

410.13 INTERHEMISPHERIC DIFFERENCES IN MID-LATENCY AUDITORY MAGNETIC SOURCES IN NORMAL SUBJECTS. M. Reite, P. Teale, J. Whalen, J.E. Zimmerman, and K. Davis. University of Colorado Health Sciences Center, Denver, CO 80262.

We recorded magnetic evoked fields (EF) from left and right hemispheres of 6 normal adult subjects ages 28-50, using a second order gradiometer with 20mm coil diameter, 6cm baseline, and DC SQUID. Recordings were obtained inside an aluminum room providing 50 dB attenuation at 60 Hz. We constructed a recording grid over each hemisphere referenced to the tragus-vertex (T-V) line and a line 2 cm above and parallel to the nasion-inion (N-I) line called the N-I reference line. Stimuli were 15 msec long 1 KHz 75 dB SPL tone pips delivered to the ear by a series of plastic tubing, with a repetition rate random within 800-1200 msec. Averages were obtained from 128 stimulus presentations. Averages were digitally filtered using a pass band of 4-40 Hz, resulting waveforms plotted, and the approximately 50 msec latency component (M50) identified. M50 amplitudes (in fT) were measured from a 200 msec pre-stimulus baseline and were used to construct isofield topographical contour plots, which were used in the calculation of subsequent source location estimates. Orientation of the source dipole (θ) was expressed in terms of positive degrees (forward tilt) or negative degrees (backward tilt) from the T-V line. Dipole strength (Q) in nA-m was estimated using the amplitude of the strongest extrema, and the calculated depth (d in cm). A prominent EF component with a latency of approximately 50 msec was clearly apparent over both left and right hemispheres in all subjects. The field was outgoing anteriorly (positive extrema anterior) and ingoing posteriorly (negative extrema posterior) over the left hemisphere and vice versa over the right hemisphere. Sources were significantly higher with reference to the N-I reference line over the left hemisphere (29.1$\pm$9.5mm) as compared to the right hemisphere (16.2$\pm$8.5mm) using a t-test (t= 2.48; df=10; p=.033, 2-tailed). This difference was not related to handedness. There was a trend for sources to be more anterior with respect to the T-V line over the right hemisphere (7.1$\pm$12mm) compared to the left hemisphere (-2.0$\pm$10.9 mm) using a t-test (t= 1.38; df=10; p=.10, 1 tailed). No significant differences were noted between left and right hemispheres for either θ, Q, or d, nor were these variables related to handedness. Magnetic resonance imaging in 5 of the 6 subjects demonstrated that the estimated sources were in the planum temporale bilaterally. Our findings are compatible with those of Geschwind and Levitsky (Science, 161:186, 1968), who demonstrated in a series of 100 brains examined postmortem that the planum temporale is larger over the left hemisphere and somewhat more anterior over the right hemisphere. Supported by USPHS MH41396 and MH46335.

NEUROTRANSMITTERS AND RECEPTORS: HISTAMINE

411.1 CIRCADIAN VARIATION OF HISTAMINE (Hm) RELEASE IN THE EXTRACELLULAR SPACE OF THE RAT BRAIN IN VIVO. W.L. Russell*, L.A. Phebus*, J.A. Clemens and D.P.Henry*. (Spon: L. Lemberger), Dept. of Pharmacology, Indiana Univ. Sch. of Medicine, 46202. Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285.

Our in vivo release studies suggest that Hm functions as a neurotransmitter in the rat (Russell et al., Soc. Neurosc. Abst. 12:431, 1986). However, the physiological function of Hm in the CNS has not been established. All H_1 Hm antagonists that permeate the blood brain barrier are sedating, therefore, Hm may be involved in the regulation of sleep-wake cycles. To test this hypothesis, we have correlated Hm content in the extracellular space of the brain with the sleep-wake cycle of the rat. Using in vivo brain microdialysis coupled with a sensitive radioenzymatic assay (REA) (Verburg, K.M. et al., Life Sci., 32: 2855, 1983), we find that Hm in the brain extracellular fluid of conscious rats demonstrates a diurnal cycle. Male Sprague-Dawley rats were anesthetized and microdialysis probes were placed stereotaxically into the posterior hypothalami or striata. Three days after surgery, sterile ringer solution was pumped through the probe (1 microliter per minute) and dialysate samples were collected at 3-hour intervals. With this flow rate, in vitro recovery of Hm was 40%. Hm level in each sample was measured by REA. Lights were turned on at 4am and off at 6pm.

Hm Dialysate Level (pg/ml) in Rat Brain Areas

	Striatum (n=8) mean ± SEM		Hypothalamus (n=5) mean ± SEM	
11am- 2pm	147	113	760	255
2pm- 5pm	128	83	739	292
5pm- 8pm	187	76	1167	412
8pm-11pm	251	73	1298	571
11pm- 2am	306	81	1429	422
2am- 5am	282	82	1162	430
5am- 8am	123	85	530	146
8am-11am	141	44	677	300

In the hypothalamus and corpus striatum, Hm levels were lowest at 5am-8am and peaked at 11pm-2am. In both areas, Hm levels were higher during the dark portion of the light-dark cycle ($p<0.0001$). The hypothalamic Hm levels were 4 times higher than in the corpus striatum. These results are in agreement with immunocytochemical studies, demonstrating histaminergic neuronal cell bodies in the posterior hypothalamus (Pollard & Schwartz, Trends in Neurosci. Feb. 10, 1987). Lesion experiments in monkeys and rats also suggested that a posterior hypothalamic area was necessary for the maintenance of wakefulness. In conclusion, the 24-hour diurnal rhythms that we have demonstrated support a physiological role for Hm neurons in modulating sleep-wake cycles.

411.2 EVIDENCE FOR RELEASE OF HISTAMINE BY IDENTIFIED *APLYSIA* NEURON C2 *IN VITRO*. H. J. Chiel, W. L. Russell*, K. Revesz*, and D.P.Henry*. Mol. Biophys. Res. Dept., AT&T Bell Labs, Murray Hill, NJ 07974 and Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, IN 46285

Histamine appears to be a neurotransmitter in vertebrates and invertebrates. Its presence, synthesis, and postsynaptic actions have been demonstrated in the mammalian central nervous system (Pollard et al., TINS, 10(2):86-89, 1987). Recent work suggests that histamine may be released from neuronal cells (Russell et al., Soc. Neurosci. Abstr., Vol. 12, part 1, p.431, 1986). In the marine mollusc *Aplysia* , the identified neuron C2 has been shown to contain and synthesize histamine, and C2's postsynaptic actions can be mimicked by histamine application (Weinreich, Nature, 267:854-6,1977). Release of histamine from C2 has been difficult to demonstrate *in vivo* because of a ubiquitous metabolizing enzyme, gamma-glutamyl histamine synthetase (Stein et al., J. Neurochem. 38(1):204-214,1982). Recent advances, however, have made it possible to study this problem: (1) Improvement of techniques for culturing identified *Aplysia* neurons and (2) Development of a sensitive histamine radioenzymatic assay (Verburg et al., Life Sci., 32:2855-67,1983).

C2 neurons were identified morphologically, extracted from the cerebral ganglia of juvenile *Aplysia* (3-5 gms), and plated in Sylgard microwells whose volume was 100ul, and which had been prepared for cell culture using the techniques of Schacher et al. (J. Neurosci 3:2403-13,1983). Experiments were done on freshly extracted cells, and on cells that had been plated for 24-36 hours, at which time the cells were attached to the substrate and showed normal resting and action potentials. C2s had 1.2 $\pm$ 0.2 pmoles histamine per cell (n = 4; all values are mean $\pm$ s.d.), levels comparable to those reported by Weinreich et al. (Br. Res., 84:341-5, 1975). Metacerebral cells, which are known to contain serotonin and not histamine, had levels of histamine equal to those measured in cell-free *Aplysia* saline (88 $\pm$ 5.6 pg/ml, i.e., $<$ 0.1 pmole/cell, n = 2). Levels of histamine in microwells containing 4 C2s showed no significant change when washed with a 5x normal potassium saline (20mM) containing 0.1x normal calcium (1mM), going from 47 $\pm$ 1.4 pg/ml before wash to 52.5 $\pm$ 23.3 pg/ml after (n = 2), but showed very significant increases when washed with a 5x potassium saline containing normal calcium (10mM), going from 53 $\pm$ 17 pg/ml before wash to 159 $\pm$ 7.1 pg/ml after (peak values, n = 2, $p<.05$). Levels of histamine in microwells containing single C2s rose from a resting level of 60.5 $\pm$ 36.4 pg/ml to 149 $\pm$ 61.5 pg/ml after washing with 5x normal potassium *Aplysia* saline, and these levels fell back to control after two washes with regular *Aplysia* saline (n = 4). In another experiment, when a single C2 was bathed with 0.1x calcium saline, and the cell was impaled with a microelectrode, levels of histamine in the microwell showed no increase after the cell had been fired steadily for 1 minute (223 pg/ml before firing; 205 pg/ml after firing). In contrast, after several washes with 5x normal calcium saline, the levels increased from 137 pg/ml to 217 pg/ml after C2 was fired steadily for 1 minute, and declined to 169 pg/ml after two more washes with the same saline.

These results support the hypothesis that, *in vitro*, C2 releases histamine in a calcium dependent manner when it is physiologically activated. (H.J.C. was supported by a grant from AFOSR under contract F49620-85-C-0009.)

411.3 ACTION OF HISTAMINE AND HISTAMINE ANTAGONISTS ON ACTIVITY OF AFFERENT NERVE FIBERS IN THE XENOPUS LATERAL LINE. S. C. Bledsoe, Jr. and R. Sinard*. Kresge Hearing Research Institute, Univ. of Michigan Medical School, Ann Arbor, MI 48109.

Guth et al. (ARO Abstracts 10:107, 1987) reported that histamine, at micromolar concentrations, excites afferent nerve fibers in the frog semicircular canal. We examined the effects of histamine and histamine receptor antagonists on the lateral line of Xenopus laevis to explore further the mechanisms of action of histamine at a hair cell-afferent nerve synapse.

The in vitro methods for isolating and recording afferent nerve responses from a single lateral-line organ and applying drugs dissolved in frog Ringer solution to the serosal surface of the skin were as previously described (Bledsoe and Bobbin, Neurosci. Letters 32: 315, 1982).

Histamine at concentrations between 2 uM and 2 mM had no effect on lateral line afferent nerve activity. In contrast, pyrilamine, an H_1 receptor antagonist, beginning at 20-50 uM, suppressed spontaneous activity. At 250-500 uM, pyrilamine abolished spontaneous activity and suppressed water-motion induced excitation. The effects on spontaneous activity were not fully reversible. Cimetidine, an H_2 receptor antagonist, had similar actions but was about one-half as potent as pyrilamine. Pyrilamine (50-200 uM) suppressed responses to a number of excitatory amino acids, including L-glutamate and L-aspartate (0.5-2.0 mM), kainate (5-10 uM), quisqualate (2-5 uM) and N-methyl-D-aspartate (0.5-1.0 mM). Histamine (1.0-2.0 mM) failed to block the suppressive effects of pyrilamine and cimetidine suggesting their actions are mediated through a mechanism other than blockade of histamine receptors. The effects of tetrodotoxin (0.005-0.1 uM) on spontaneous activity and water motion- and amino acid-induced excitation were similar to those of the histamine antagonists.

Our results are inconsistent with histamine being an excitatory transmitter in the Xenopus lateral line. Moreover, they reveal that the actions of histamine antagonists in this hair-cell system are nonspecific and possibly mediated by blockade of voltage-sensitive Na^+ channels. (Supported by NIH Program Project Grant NS-05785.)

411.4 DISTRIBUTION OF HISTAMINE-IMMUNOREACTIVE NERVE FIBERS IN THE RAT AND GUINEA PIG BRAIN AFTER FIXATION WITH CARBODIIMIDE. P. Panula, M.S. Airaksinen*, S. Auvinen*, A. Virkamäki*, U. Pirvola* and L. Kivipelto. Dept. Anatomy, Univ. Helsinki, 00170 Helsinki, Finland.

Histamine (HA) is a putative neurotransmitter in mammalian brain and HA-immunoreactive neurons are located in the posterior basal hypothalamus in the rat brain (Panula et al., Proc. Natl. Acad. Sci. USA 81:2572-2576, 1984). Although nerve fibers immunoreactive for HA have been localized in different parts of the rat brain, a complete mapping of these fibers has not been done, and little is known about HA-containing neuronal systems in other species. In this study, rats and guinea pigs were studied with an antiserum against HA conjugated to hemocyanin with 1-ethyl-3(3-dimethylamino-propyl)carbodiimide (EDCDI) to reveal the distribution of HA-immunoreactive neurons and nerve fibers in the brain.

Adult Sprague-Dawley rats and guinea pigs were perfused through the left heart ventricle with saline followed by 50-150 ml of ice-cold 4% EDCDI in 0.1 M sodium phosphate buffer. The brains were immersed in the same fixative for 2 h and washed overnight in phosphate buffer containing 20% sucrose. 10-60 µm thick cryostat sections were incubated with the HA antiserum HA11C diluted 1:1000-1:2000 overnight, washed and processed according to the indirect immunofluorescence or PAP procedure. The specificity of the antiserum was established with blocking controls with histamine and related substances, model experiments on nitrocellulose filters and affinity-purified antibodies.

Widespread distribution of HA-immunoreactive nerve fibers was revealed in both species. Fibers were detected in all parts of the cerebral cortex, especially in the piriform cortex. Moderate densities of immunoreactive fibers were seen in the olfactory tubercle, lateral septal nucleus, nucleus of the diagonal band, stria terminalis, amygdala, substantia nigra and dorsal medullary nuclei. Many fibers were observed in the immediate vicinity of the cerebral ventricles, and fibers were also seen among myelinated fiber bundles including corpus callosum, tractus opticus and commissura posterior. Many nerve terminal-like structures but few fibers were seen in the nucleus accumbens and neostriatum. Dense networks of immunoreactive nerve fibers were found in several hypothalamic nuclei, especially in the peri- and paraventricular nuclei, in the vicinity of the supraoptic nucleus and chiasma opticum and in the posterior basal hypothalamic nuclei. Only occasional immunoreactive nerve fibers were seen in the rat cerebellum, while many fibers were found in the guinea pig cerebellum, mostly in the granular layer but also in the molecular layer. The results provide detailed information on the distribution of HA-containing fibers in the brain and offer an opportunity to study the connections of HA-containing neurons with their target cells.

411.5 ENDOGENOUS HISTAMINE EXCITES POSTGANGLIONIC NEURONS IN THE GUINEA PIG SUPERIOR CERVICAL GANGLION. E.P. Christian[1,*], D. Weinreich[1] and B.J. Undem[2,*]. [1]Dept. of Pharmacology, Univ. of Maryland Sch. of Med., Baltimore, MD. 21201 and [2]Dept. of Medicine, Johns Hopkins University, Baltimore, MD 21239.

Substantial evidence has accumulated that exogenously applied histamine can affect peripheral autonomic neurons and synapses. In addition, histamine has been shown to be present in mast cells which reside in autonomic ganglia. Nonetheless, it remains unsettled, whether histamine contained in peripheral autonomic ganglia is released under physiological (or pathophysiological) conditions in sufficient amounts to affect autonomic neurons. We have obtained evidence that endogenous histamine can be released by an appropriate immunological stimulus in the superior cervical ganglion (SCG), and has specific electrophysiological effects on postganglionic neurons.

Adult guinea pigs were actively sensitized to the antigen, ovalbumin. Approximately three weeks later the SCG were isolated and maintained in vitro in a perfusion chamber for intracellular recording studies. When tissue was challenged with ovalbumin (10 µg/ml), approximately 30% of the endogenous histamine stores were released. Ovalbumin-induced histamine release reached a peak within one min. of antigen exposure, and declined to below measureable levels within ten min. Coincident with the time course of this release, intracellular current clamp recordings in 24 of 32 neurons (32 preparations) revealed a transient depolarization of membrane potential 6.1 ± 3.91 mV (mean ± S.D.; range, 2-16 mV, n = 24). The depolarization was usually accompanied by an increase in total input resistance of the neuron. These effects could be mimicked by exogenously applied histamine (10 µM) and blocked by pretreatment with the H-1 histamine receptor antagonist, pyrilamine (1 µM).

Histamine can therefore be liberated within the SCG by a immunological stimulus in sufficient amounts to affect the passive membrane properties of postganglionic neurons in a manner that presumably increases cell excitability. These data support a hypothesis that endogenous histamine can influence the functional integrative properties in autonomic ganglia.

Supported by NIH grants NS07865 (EPC) and NS22069 (DW).

411.6 SPECIFIC BINDING OF [^{3}H]HISTAMINE IN GUINEA-PIG CEREBRAL CORTEX. W.G. Sinkins*, D.L. Cybulsky*, M. Kandel*, S.I. Kandel*, W. Schunack* and J.W. Wells (SPON: J.W. Goh). Faculty of Pharmacy, University of Toronto, Toronto, ON M5S 1A1, Canada, and Institut für Pharmazie, Freie Universität Berlin, D-1000 Berlin 33, FRG.

Saturable binding of [^{3}H]histamine at equilibrium in homogenates of washed tissue from guinea-pig cerebral cortex appears to reveal two classes of sites. Simultaneous analysis of data acquired by isotopic dilution of 1.4 nM [^{3}H]histamine and by varying the concentration of the radioligand yields parametric values as follows: log K_{P1} = -8.46 ± 0.02, log K_{P2} = -6.36 ± 0.18, $[R_1]_t$ = 77-120 pmol/g of protein, $[R_2]_t$ = 750-1200 pmol/g of protein. The inclusion of 0.1 mM GMP-PNP is accompanied by a 50% reduction in $[R_1]_t$ and an increase in log K_{P1} to -8.38 ± 0.03; there is no discernible change in either K_{P2} or $[R_2]_t$. The data thus suggest that the nucleotide promotes the conversion of sites from a state of high affinity to a state in which binding is not measurable. Values of K_{P1} and K_{P2} agree well with those reported for H_2 receptors labelled by [^{3}H]histamine in rat cerebral cortex (Steinberg et al., Biochemistry, 24:6095-6125, 1985).

The inhibitory behaviour of 20 histaminic drugs is well described by a model with either one or two classes of sites depending on the drug. There is no correlation among H_2 antagonists between inhibitory potency versus [^{3}H]histamine (IC_{50}) and pA_2 for blockade of the H_2 response of guinea-pig right atrium; the pattern thus differs from the H_2 pharmacological specificity observed previously in rat cortex. In contrast, there is good numerical agreement and an excellent correlation between IC_{50} and apparent affinity at the sites labelled by [^{3}H]histamine in digitonin-solubilised preparations from rat cortex ($r = 0.985$, $P < 0.00001$). Comparisons of rank order suggest that IC_{50} may correlate with the reported potency for agonists ($P < 0.025$) but not for antagonists ($P > 0.1$) at H_3 receptors. The identity of the sites labelled in guinea-pig thus remains unclear.

Two lines of evidence suggest that inhibition of [^{3}H]histamine by at least some unlabelled drugs is non-competitive. Firstly, 10 compounds, including the H_2/H_3 antagonist S(+)sopromidine, require two classes of sites for a competitive model to describe the data; the apparent distribution of sites varies significantly from drug to drug. Secondly, simultaneous analysis of binding patterns obtained for S(+)sopromidine at 1.2 nM and 10 nM [^{3}H]histamine yield values of log K_{P1} and log K_{P2} for [^{3}H]histamine of -8.59 ± 0.10 and -8.12 ± 0.05, respectively; the latter value differs from that measured with histamine alone (log K_{P2} = -6.36, P = 0.0043). The inconsistency is resolved, however, if the data are analysed in terms of three classes of sites; apparently non-competitive behaviour thus may reflect the limits of resolution in single experiments. (Supported by the Medical Research Council of Canada)

411.7 REGIONAL BRAIN HISTAMINE LEVELS IN RATS FOLLOWING PREOPTIC RECESS PERIVENTRICULAR TISSUE LESIONS. V. Schaumloffel*, J. Van Huysse*, and S. L. Bealer. Department of Physiology and Biophysics, University of Tennessee, Memphis, TN 38163.

Electrolytic ablation of the periventricular tissue surrounding the anteroventral portion of the third ventricle (AV3V-X) results in both acute and chronic attenuation of vasopressin (AVP) release in response to hyperosmolarity. Histamine (Hm) has been implicated as a mediator of AVP secretion. Intracerebroventricular injection of Hm produces dose-related increases in AVP secretion (Dogterom *et al., Experientia* 32: 659, 1976), and an excitatory action of Hm on supraoptic neurons has been demonstrated *in vitro* (Armstrong and Sladek, *Neuroscience* 16: 307, 1985). Furthermore, levels of Hm and HNMT, the Hm-metabolizing enzyme, in the posterior pituitary indicate increased Hm turnover following chronic hypertonic saline administration (Verburg *et al., Neurosci. Abstr.* 9: 705, 1983). To determine if Hm could be a potential mediator of AV3V-X-induced changes in AVP secretion, Hm concentrations in several brain areas following AV3V ablation were measured.

Rats were either electrolytically lesioned in the AV3V region or underwent control surgery (Con). Animals were decapitated 1-5 hours postoperatively. The brain was removed, immediately frozen, subsequently blocked, and homogenized in 1 M perchloric acid. Hm concentrations in six brain regions were determined by radioenzymatic assay.

	Con (ng/g; n = 5)	AV3V-X (ng/g; n = 6)
Spinal cord	93.2 ± 28.2	65.8 ± 7.7
N. tractus solitarius	25.3 ± 1.4	26.7 ± 3.1
Midbrain	23.4 ± 2.4	26.2 ± 3.7
Forebrain	40.6 ± 4.8	53.5 ± 5.4
Hypothalamus	305.8 ± 44.9	424.3 ± 29.6*
Pituitary	213.4 ± 18.9	299.5 ± 40.3

*$p < 0.05$.

Hm concentrations in the hypothalamus of AV3V-X rats were significantly elevated (424.3 ± 29.6) compared to control-operated animals (305.8 ± 44.9). However, there were no differences in Hm levels in other regions.

Since measured levels of Hm obtained in these experiments reflect total intracellular and extracellular Hm, increased levels can indicate increased synthesis and/or release, or decreased metabolism. Therefore, the precise relationship of the AV3V region, vasopressin secretion, and histamine cannot be established. However, these data are consistent with the hypothesis that histamine may act as a mediator of altered vasopressin release in AV3V-lesioned rats. (Sponsor: P.K. Law)

411.8 ONTOGENY OF HISTAMINERGIC TUBEROMAMMILLARY NEURONS IN THE RAT. P.B. Reiner, K. Semba, H.C. Fibiger and E.G. McGeer, Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, BC, Canada V6T 1W5.

The tuberomammillary (TM) nucleus is populated by a group of neurons which are immunoreactive to both histamine and its synthetic enzyme, L-histidine decarboxylase (HDC). These neurons have received increased interest of late with the realization that their somata stain positively for a large number of putative transmitters and related enzymes. The present study was designed to determine the stage of fetal development at which TM histaminergic neurons undergo their final mitotic division.

Tritiated thymidine autoradiography was combined with HDC immunohistochemistry, using a polyclonal antibody whose specificity has been previously documented (Watanabe et al.,'84), and counts of single (HDC immunoreactivity only) and double (HDC immunoreactivity and tritiated thymidine) labelled neurons were carried out. For the purposes of the present analysis, the TM nucleus was parcelled into five subdivisions as defined by Staines et al. (in press): caudal, ventral, lateral, tuberal and interstitial. Percentages of double labelled neurons were plotted for days 13-18 of fetal life (conception counted as day 0). Only immunopositive neurons in which the nucleus was clearly identifiable, and double labelled cells with heavy grain counts were included in the analyses. The data showed that TM neurons undergo a peak of final mitotic activity on day 16 of fetal life. There was no apparent difference between subdivisions of the TM nucleus. These data are consistent with the results of Altman and Bayer ('86) using tritiated thymidine autoradiography alone. Adenosine deaminase, a reliable marker of TM neurons, has been detected immunohistochemically on embryonic day 18 (Senba et al., '87) and our preliminary data indicate that HDC immunoreactivity is detectable at day 19. Thus, TM neurons appear to begin expression of their transmitter phenotype soon after becoming post-mitotic.

Parallels between histaminergic TM neurons and brainstem aminergic neurons have been drawn on the basis of their strikingly similar anatomical, biochemical and physiological properties. However, brainstem aminergic neurons become post-mitotic much earlier in fetal life, around embryonic day 11-13. Thus, while histaminergic neurons share a number of properties with brainstem aminergic neurons, their ontogenesis occurs much later.

(Supported by the Medical Research Council.)

411.9 DISTRIBUTION OF MAST CELLS IN THE LEPTOMENINGES AND ALONG BLOOD VESSELS ON THE SURFACE OF THE MOUSE BRAIN. E.L. Orr. Dept. of Anatomy, Texas Coll. of Osteopathic Med., Ft. Worth, TX 76107.

Since the leptomeninges and velum interpositum of many vertebrate species have been reported to contain large numbers of mast cells, we have determined the relative distribution of mast cells in the leptomeninges and along surface blood vessels of the mouse brain using toluidine blue to specifically stain the mast cells of formalin-fixed whole mouse brains. The presence and distribution of mast cells in the velum interpositum and along the blood vessels of the velum interpositum were assessed in the same mouse brains after removal of the cerebral cortex covering the velum interpositum.

Except for some small concentrations of mast cells at the ventral junction between the olfactory bulbs and frontal lobes, and in the leptomeninges of the cruciate sulcus, we found that mast cells on the surface of the mouse brain are essentially confined to the area of the velum interpositum of the transverse fissure, primarily in association with the blood vessels which enter, traverse or exit the brain via the velum interpositum. Only a very few mast cells were found associated with the remainder of the vessels and leptomeninges of the mouse brain surface.

In the area of the velum interpositum, most of the mast cells were found associated with the following blood vessels: Arteries: posterior cerebral, longitudinal hippocampal, transverse collicular, and posterior choroidal; Veins: thalamostriate, internal cerebral, and basal. Thus, mast cells are distributed along the blood vessels which perfuse or drain the thalamus, hippocampal formation, corpus striatum, corpora quadrigemini, and the choroid plexi of the lateral ventricles. Such a distribution suggests that mast cells and their products (e.g., histamine, serotonin, leucotrienes) may be involved in regulating the regional permeability of, or the blood flow through the vessels supplying or draining these important brain regions.

The results of this study demonstrate that mast cells are differentially distributed on the surface of the mouse brain, and cannot be considered to be general component of the leptomeninges. Moreover, since the mouse brain contains very few, if any, intracerebral mast cells, it is likely that mast cells are not a major source of non-neuronal histamine or serotonin in the mouse brain. (Supported by an Organized Research Grant from T.C.O.M.)

411.10 HISTAMINE METABOLITES IN CEREBROSPINAL FLUID OF THE RHESUS MONKEY: CISTERNAL-LUMBAR CONCENTRATION GRADIENTS. G.D. Prell, J.K. Khandelwal*, R. S. Burns and J.P. Green. Dept. of Pharmacology, Mount Sinai School of Medicine of the City University of New York, New York City 10029 and Section on Experimental Therapeutics, NIMH, Bethesda, MD 20205

Similar to metabolites of other aminergic transmitters, the histamine metabolites of brain, tele-methylhistamine (t-MH) and tele-methylimidazoleacetic acid (t-MIAA), could have a concentration gradient between rostral and caudal sites of CSF. To test this hypothesis, cisternal and lumbar CSF samples were collected in pairs from 8 monkeys (Macaca mulatta), and levels of t-MH and t-MIAA were measured by gas chromatography-mass spectrometry. pros-Methylimidazoleacetic acid (p-MIAA), an endogenous isomer of t-MIAA that is not a histamine metabolite, was also measured as a control. Cisternal levels (pmol/ml, mean ± S.E.M.) of t-MH (9.9 ± 1.4) and t-MIAA (40.8 ± 7.6), but not p-MIAA (9.7 ± 1.2), exceeded those in lumbar CSF (t-MH, 1.8 ± 0.3; t-MIAA, 6.8 ± 0.9; p-MIAA, 8.6 ± 0.6) in every monkey. The magnitudes of the mean cisternal-lumbar concentration gradients for t-MH (6.6 ± 1.1) and t-MIAA (6.5 ± 1.3) were indistinguishable. There was no gradient for the levels of p-MIAA (mean cisternal-lumbar ratio 1.1 ± 0.1). The cisternal, but not lumbar, levels of t-MH and t-MIAA were correlated. There was no significant difference between the means of the metabolite concentration ratios (t-MIAA/t-MH) in cisternal (4.0 ± 0.4) and lumbar (4.4 ± 0.9) CSF. The steepness of these gradients, which exceed the gradients of the metabolites of most other transmitters, suggest that the levels of t-MH and t-MIAA in lumbar CSF might be useful to probe histaminergic metabolism in brain. (Research supported by NIMH grant 31805).

412.1 **SEROTONIN (5-HT_2) RECOGNITION SITES AND ^{3}H-IMIPRAMINE BINDING (IB) SITES IN THE BRAINS OF SUICIDE VICTIMS.** R.C. Arora and H.Y. Meltzer. Department of Psychiatry, School of Medicine, Case Western Reserve University, Cleveland, OH 44106.

IB sites and 5-HT_2 recognition sites were studied in the brains of suicides and normal controls who died due to heart diseases. Previous studies have indicated decreased number of IB sites and increase number of 5-HT_2 sites in suicides than normals but there are conflicting reports. Frontal cortex (Broadman's areas 8 and 9) from eighteen suicides and normal controls were obtained at autopsy. The medical examiner had determined the cause of death in suicides and normal controls. The mean interval between death and collection of the sample was 13.05 ± 6.82 hrs and 12.95 ± 7.1 hrs for suicides and normals respectively. The mean age of the two groups was 43.6 ± 17.9 yrs and 46.2 ± 21.4 yrs. 5-HT_2 binding was studied using ^{3}H-spiroperidol as the binding ligand. IB was determined with ^{3}H-imipramine.

5-HT_2 binding (B_{max}) was significantly increased in the brains of suicides compared to controls. Mean B_{max} for suicides and normal controls was 271.4 ± 128.4 and 194.6 ± 106.1 fmoles/mg protein. ANOVA with age, sex and post-mortem delay as covariates indicates that there was a significant sex effect on binding ($F=5.52$, $p < 0.025$). There was no effect of age and post-mortem delay on the ^{3}H-spiroperidol binding. Further analysis of suicides indicated that increase in B_{max} was more pronounced in suicides who used violent means, i.e. gunshot wound and hanging than non-violent suicides. No difference in K_d was observed between suicides and normals. There was no difference in K_d and B_{max} of IB in the frontal cortex of suicides. There was no correlation between the number of IB and 5-HT_2 recognition sites. The observed increase in 5-HT_2 recognition sites may be related to diminished biogenic amine turnover. (Supported in parts by USPHS MH 30,059 and MH 41684).

412.2 CEREBROSPINAL FLUID CORRELATES OF DEPRESSION IN HUNTINGTON'S DISEASE. R. Kurlan*, I. Shoulson, E. Caine, A. Rubin*, Univ. of Rochester Sch. of Med., Rochester, NY 14642. C. Nemeroff, G. Bissette, Duke Univ. Sch. of Med., Durham, NC, R. Zaczek*, J. Coyle, Johns Hopkins, Univ. Sch. of Med., Baltimore, MD. F.J. Spielman*, Univ. North Carolina Sch. of Med., Chapel Hill, NC.

Depression occurs commonly in Huntington's disease (HD). Elevated cerebrospinal fluid (CSF) concentrations of corticotropin-releasing factor (CRF) have been associated with depression in psychiatric populations (Science 1984; 226:1342), and reduced CSF levels of 5-HIAA have correlated with depression in Parkinson's disease (Neurology 1984; 34:642). We obtained CSF by lumbar puncture from 56 non-medicated HD patients in the earliest stages (I and II) of illness and from 21 control subjects who underwent spinal anesthesia. HD subjects underwent standardized assessments to determine psychiatric diagnoses and severity of symptoms by DSMIII, Research Diagnostic Criteria and Life Psychiatric Status Ratings (J Nerv Ment Dis 1981;169:764). Investigators unaware of clinical status measured CSF concentrations of CRF by radioimmunoassay and 5-HIAA by HPLC. Twenty-four (46%) HD patients were judged to be depressed, including major depression (N=19) and dysthymia (N=5). CSF concentrations of CRF(107.2 ± 35.3 pg/ml, mean ± SD) and 5-HIAA (185 ± 97 pmol/ml) in the depressed HD patients were not statistically different from the non-depressed HD subjects (CRF = 95.8 ± 38.9, 5-HIAA = 201 ± 176). No significant correlations between Life Ratings and CRF or 5-HIAA levels were found. As a group, however, the 56 HD subjects had a significantly ($p < 0.01$) higher level of CRF than the control subjects (74.2 ± 27.1). Our findings indicate that CRF concentrations are elevated in the CSF of non-medicated early HD patients; however, neither CRF nor 5-HIAA levels correlate with the presence or severity of depression. (Supported by USPHS grants NS17978, NIMH 40524, NIMH 39415, NIA AG05128 and the U. of Rochester CRC USPHS RR-0044)

412.3 PLATELET ADRENERGIC RECEPTOR BINDING STUDIES IN DEPRESSIVE ILLNESS. J.E. Piletz, and A. Halaris. Dept. of Psychiatry, Case Western Reserve Univ. and Cleveland Metropolitan General Hospital, Cleveland, Ohio 44109.

At least 10 laboratories have reported radioligand binding to platelet alpha$_2$ adrenoceptors from depressed patients. The results have shown no differences using radio-labelled antagonists, but mixed conclusions of increased or decreased receptor density in depressed patients using agonists or broadly specific ergot derivatives. Based on the K_D values reported in those studies, we hypothesized that the previous data may be consistent with a redistribution of agonist-receptor states from high (~3nM) to lower (~6nM) affinity states in patients. In order to test this we developed an assay using sucrose gradient purified platelet plasma membranes which allowed detection of super-high affinity (K_D ~35pM) and high affinity (~1nM) binding sites for ^{3}H-p-aminoclonidine (^{3}H-PAC). This technique resulted in a high percentage of specific binding (avg = 69%). By contrast, ^{3}H-PAC binding to washed intact platelets displayed <10% specific binding and was non-saturable. ^{3}H-PAC binding to washed membrane lysates showed K_D >10nM, representing only about 40% specific binding, and did not display the super-high affinity site. By using purified plasma membranes we and others (Neubig and Szamraj, Biochim et Biophys. Acta 854, 1986 p.67) appear to have removed an inhibitor which probably confounded previous binding studies using platelet lysates. Results will be presented from Scatchard binding analyses with depressed patients versus normal volunteers and from depressed patients before and after a six week treatment with desipramine.

412.4 **SEASONAL RHYTHM OF [3H]-IMIPRAMINE BINDING TO HUMAN PLATELETS.** E. DeMet, R. Gerner*, C. Kaufmann*, K. Bell*, A. Chicz-DeMet*. Dept. of Psychiatry & Human Behavior, Univ. of California, Irvine, CA 92717.

Platelet [3H]-imipramine binding was studied in 20 normal controls (11 males, 9 females) at 6 week intervals for one year. The maximum number of binding sites (Bmax) and the binding affinity (Kd) were determined using Scatchard plots, linear, and non-linear regression. No significant differences were found in the Kd values between groups or across time. In contrast, a pronounced seasonal rhythm of Bmax values was found with a maximum variation (amplitude) of ± 35% of the yearly mean (mesor). Peak binding was found in March to April and minimum binding was found in July to September.

Platelet binding was also studied in 20 patients meeting DSM-III and RDC criteria for unipolar depression. The Bmax values of these patients were expressed as a percentage of the seasonally matched control mean. The results were then normalized to the control mesor in order to permit seasonally neutral statistical comparisons between the groups. As a group, depressed patients showed less binding (62%; $p<0.005$) than controls. However, the degree to which patients and controls differed was related to the date of sampling. Maximum differences were noted at the time of the normal seasonal peak whereas values obtained from patients sampled during the fall were indistinguishable from their matched controls.

412.5 A RE-EXAMINATION OF BLOOD ELEMENT ADRENERGIC RECEPTOR REGULATION IN PSYCHIATRIC DISORDERS. B.D. Perry[1], S.M. Southwick*[2], and E.L. Giller, Jr.,[2]. [1]Division of Child and Adolescent Psychiatry and Harris Center Developmental Studies, The University of Chicago, Chicago, IL 60637. [2]Dept. of Psychiatry, Yale University and West Haven VAMC, West Haven, CT 06516.

Preclinical and clinical studies have suggested that CNS adrenergic receptor dysregulation plays a role in the pathophysiology of a variety of psychiatric disorders. Parallel distribution of α_2- and β-adrenergic receptors (R) in brain and blood elements (platelet - PLT, α_2: monocyte-M - β) has motivated study of PLT α_2 and M-βR in psychiatric disorders using radioligand binding or other functional assays. The results have been variable and difficult to interpret. The current studies were performed to clarify and extend previous reports by 1) examining the multiple affinity states of a subject's PLTα_2- and M-β-R (drawn at same time) using extended saturation and competition assays, 2) using many subjects (total n> 250), 3) comparing "state" vs. "trait" binding values, 4) comparing binding parameters with symptom/s (as well as diagnostic category) and 5) by employing a dynamic, 'in vitro' assay of changes in R following incubation of intact PLT or M in agonist (Soc. Neurosci. Abstr. Vol. 12, 414, 1986).

Subjects were in good physical health and had no concurrent substance abuse (>2 wks). SADS/RDC diagnostic criteria were used. A variety of clinical rating tools were administered, including the DIB and DEQ. PLT and M were isolated from whole blood using standard techniques and membranes were prepared for binding assays using ^{3}H-rauwolscine (RAUW) for PLTα_2- and ^{125}Iodocyanopindolol (ICYP) for M-β-R. In vitro incubations of intact cells were performed as described previously (see above). Extended saturation (12 point) and epinephrine competition (12 point) studies were performed in both systems. Computer curve fitting techniques resolved two components of interaction for both RAUW and ICYP. No group differences in K_D (at either site) were demonstrated for either EPI, RAUW or ICYP. Significant findings include differences in the ratio of affinity states in PTSD and major depression (MDD) (e.g., PLTα_2-ratio α_2(L)/α_2(H)x100 + SEM: CONTROL = 13.1 + 1.33, (N=25); PTSD = 23.5 + 1.97, (N=25); MDD = 31.9 + 3.7, (N=15)). More interesting was the demonstration of population differences in rate of R "internalization" after EPI incubation (e.g., PLTα_2-R; PTSD 4 times more rapid than Control; Bipolar depressed 1/2 as rapid). The results demonstrate that standard radioligand binding assays using limited ligand concentrations can result in misleading results (e.g., inferred changes in either total R # or ligand affinity) and that dynamic assays of R-regulation may be more suitable for testing dysregulation hypotheses in psychiatric populations.

Supported by VA funds.

412.6 A COMPARISON OF BLOOD ELEMENT ADRENERGIC RECEPTOR BINDING SITES IN BORDERLINE PERSONALITY DISORDER AND MAJOR DEPRESSION. S.M. Southwick*[2], E.L. Giller, Jr.,[2], and B.D. Perry[1]. Child & Adolescent Psychiatry, Harris Center for Developmental Studies, The University of Chicago, Chicago IL 60637. [2]Dept. of Psychiatry, Yale University and West Haven VAMC, West Haven, CT 06516.

Borderline Personality Disorder (BPD) is a psychiatric diagnosis characterized by chronic impulsivity, affect lability, brief intermittent psychoses, and unstable interpersonal relationships. In DSM III it is classified as a character disorder on Axis II. An association between BPD and DSM III Axis I affective disorders (primarily major depressive disorder--MDD) has been suggested. Studies of putative biological markers for affective disorders (e.g., DST, REM latency) in BPD support this association. Other putative markers for affective disorders are the platelet (PLT)α_2 receptor (R) and monocyte (M)β-R. The present studies were performed to compare PLTα-2 and Mβ-adrenergic R in BPD and MDD relative to "controls" (C). These studies represent, to our knowledge, the first investigation of adrenergic R in BPD.

PLT and M were isolated from normal volunteers and from subjects meeting SADS/RDC criteria for MDD and DIB criteria for BPD. Subjects were in good physical health and drug free (>2 wks). Membranes were prepared, washed extensively with EDTA containing buffers, and radioligand binding assays were performed in PLT using ^{3}H-rauwolscine (RAUW) (Eur. J. Pharm. 84:79, 1982) and in M using ^{125}Iodocyanopindolol (ICYP) (N.S.A.Pharm. 317:277, 1981). In both systems extended saturation and competition studies (12 concentrations of (-)epinephrine--EPI) were performed to assess high and low affinity states of the R. Data were analyzed with LIGAND.

Under the assay conditions used, two sites of binding (high and low affinity) were demonstrated for PLTα_2 and MβR.

No population differences in the affinity of RAUW or EPI for the two PLTα2-R sites were seen (e.g. for RAUW, group: K_{D1}, K_{D2} (nM ± S.D.), N; C = 0.4±0.2, 27.3±8.8, N=30; BPD = 0.45±0.2, 24.6±10, N=20; MDD = 0.4±0.1, 22.8±9.9, N=15). The total number of PLTα_2 sites (SITES/PLT) in BPD was approximately equal to the total number in C (C = 229±92, BPD = 191±40, MDD = 228±90). Significant differences included altered ratios of high to low affinity states (α_2(L)/α_2(H) x 100±SEM: C = 13.1±1.3; BPD = 31.9±3.7; MDD = 28.1± 3.5) with a significantly higher number of α_2 low affinity states in the BPD and MDD groups (C = 24.4±10; BPD = 49±20; MDD = 47.4±18).

The findings suggest that BPD and MDD share certain pathophysiological features. Further studies investigating 'in vitro' regulation of blood element adrenergic R support this conclusion (see Perry et al., Neurosci. Abstr., 1987). Supported by VA funds.

412.7 IN VITRO INHIBITION OF ARYLSULFATASE C BY DOPAMINE AND RELATED COMPOUNDS. D.E. Enyedy* and T.J. Shickley. Department of Pharmacology and Toxicology, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104.

There are three types of arylsulfatases known (EC 3.1.6.1): types A and B which are lysosomal sulfohydrolases necessary for the maintainance of myelin, and type C (ARS-C) which has been shown to be microsomal in liver but whose function in the CNS is unknown. The deficiency or absence of one or more of the arylsulfatases results in metachromatic leucodystrophy or multiple sulfatase deficiency, which are progressive demyelinating diseases. In the adult form of the diseases, a schizophreniform behavior has been observed, the onset of which can precede CNS demyelination by decades (Manowitz, P., Kling, A., Kohn, H. J. Nervous Mental Disease 166: pp500, 1978.). A possible arylsulfatase variant in the blood of schizophrenic patients has also been reported (Manowitz, P., Goldstein, L., Nora, R. Biol. Psychiatry 16: 1107-1113, 1981).

The potential involvement of arylsulfatases in schizophreniform behaviors and the lack of functional information concerning ARS-C, compelled us to explore the interaction of dopamine (DA) and ARS-C.

Purified ARS-C (Sigma, St. Louis, MO: S-1629) was assayed at 37°C (pH 7.1) with p-nitrophenol sulfate as substrate using a spectrophotometric technique modified from that of Fowler and Rammler (Biochemistry 3: pp 230, 1964.) which measures the absorption at 400 nanometers of enzymatically liberated p-nitrophenol. DA and other compounds were included in the incubation at concentrations up to 80 mM.

DA produced 50% inhibition of ARS-C activity at a concentration of 100 uM. Other catecholamines at equimolar concentration with the IC-50 of DA produced the following % inhibition: L-DOPA 34.6; Norepinephrine 30.8; Octopamine 29.2; Epinephrine 20.5. Related compounds produced lower inhibition: Catechol 19.4; Tyrosine 10.1; B-Phenethylamine 5.0. No inhibition was found with tyramine. Interestingly, the greatest inhibition of ARS-C was from 5-OH-DA and 6-OH-DA producing 87.6% and 77.6% inhibition at 100 uM, respectively.

These results suggest: (1) that a high electron density at the 2-position on the phenolic ring is necessary both for binding of arylsulfates and for strong inhibition of the ARS-C enzyme by dopamine and related compounds; (2) there may be an important link between ARS-C and the dopaminergic system which is relevant to schizophreniform behaviors.

412.8 NEUROCHEMICAL ANALYSIS OF COMPLEX BIOLOGICAL MATRICES USING MULTIPLE ELECTRODE LIQUID CHROMATOGRAPHY. C.N. Svendsen*, C.C. Hrbek* and E.D. Bird. SPON: (V. Shashoua). Brain Tissue Resource Center, McLean Hospital/Harvard Medical School, 115 Mill St., Belmont, MA 02178.

Using a newly developed neurochemical analyzer (Neurochem™, developed by ESA Inc., Bedford, Mass.), a wide range of biogenic amines and related metabolites, peptides, purines and pyrimidines, in addition to certain neuroleptic drugs, have been simultaneously quantitated in crude extracts of human and rat brain tissue and cerebral spinal fluid.

The equipment consists of a 16 electrode coulometric electrochemical detector, preceded by a high performance liquid chromatography system, which together are capable of separating and quantitating 150 compounds in a single sample within an hour. This inherent separating power has been interfaced with an enhanced IBM AT computer capable of saving real time data from each electrode and allowing extensive post-run analysis.

Preliminary data will be presented from two studies. The first uses this method to study a hypothesized, but as yet unproven, neurochemical pathology in schizophrenia, by screening tissues for the majority of the tyrosine and tryptophan neurotransmitters, precursors, metabolites and cofactors. The second shows the effects of administering the two isomers of thioridazine, a neuroleptic drug, on rat brain neurochemistry.

The method has been applied for determining 115 compounds including the following: ascorbic acid, biopterin, bufotenine caffeic acid, catechol, chlorpromazine and metabolites, dihydroxyphenyl glycol, dihydroxyphenylacetic acid, 4,8-dihydroxyquinoline, dopamine, dopamine sulfate, epinephrine, fluphenazine, formylanthranilic acid, homogentisic acid, homovanillic acid, 3-hydroxyanthranilic acid, 5-hydroxyindoleacetic acid, 5-hydroxytryptamine, kynurenine, l-dopa, leu/met enkephalin, melatonin, metanephrine, 3-methoxy-4-hydroxyphenyl glycol, n-methyl-5-hydroxytryptamine, neurotensin, norepinephrine, octopamine, pyrodoxamine, thioridazine & metabolites, tryptamine, tryptophan, ureic acid, vasopressin, vanillymandelic acid, and xanthine.

The compounds assayed will be subject to classical intersample multivariate statistical comparisons, in addition to newer statistical methods which can be used when large numbers of intrasample data points are available. This approach allows the investigation of disease or drug induced alterations in a number of neurochemical pathways simultaneously. By eliminating the use of multiple methods for different compounds, more consistent results can be attained in a shorter period of time.

412.9 EXPERIMENTAL DIABETES: DEGENERATIVE ATROPHY OF SUBSTANCE P PRIMARY AFFERENTS AND MET-ENKEPHALIN INTERNEURONS IN THE RAT SPINAL CORD. A. Gorio, °A.M. Di Giulio, °A. Mannavola*, °B. Tenconi* and °P. Mantegazza*. Inst. of Pharmacological Sciences and °Dept. of Medical Pharmacology, Univ. of Milano, 20133 Milano, Italy.

Well known consequences of diabetes include profound functional and morphological alterations of central and peripheral nervous system structures. It has been shown that experimentally induced diabetes is characterized by a decrease in slow axonal transport and by a reduction in the conduction velocity in both sensory and motor axons. These effects are accompanied by a progressive axonal atrophy and, at a later stage of the disease, by distal axonal degeneration.

In this report we show that alloxan-induced experimental diabetes causes a loss of the sensory input to the dorsal horn of the spinal cord, as indicated by the significant decrease in substance P innervation detected in the spinal cord of diabetic rats. In addition, we have found that such a change is correlated with a loss of radioimmunoassayable met-enkephalin, the opiate peptide contained in a selective population of second order neurons present in laminae I and II of the dorsal horn spinal cord. These results indicate that diabetes may cause profound alterations in peripheral as well as central sensory pathways.

It is well known that diabetic neuropathy is often accompanied by pain sensations and sensory perception modifications. We would like to suggest that these dramatic consequences of diabetes are related to the findigs here reported, showing significant alterations in substance P and met-enkephalin containing neurons in the spinal cord.

412.10 DIFFERENTIAL EFFECTS OF EXPERIMENTAL DIABETES ON THE CATECHOLAMINERGIC INNERVATION OF THE MAJOR CEREBRAL ARTERIES AND ON CENTRAL CATECHOLAMINE CONCENTRATIONS IN THE RAT. U. Wesselmann+, R. J. Konkol*, G. L. Leo* and D. R. Harder*. +Dept. of Physiology, Northwestern Univ. Med. Sch., Chicago, Ill. 60611 and Res. Serv. 151, Neurology, VAMC, Milwaukee, WI.53295.

Studies in experimental animals and man indicate that the activity of the sympathetic nervous system is altered during diabetes. The brain vascular bed receives an ample supply of sympathetic adrenergic fibres originating in the superior cervical ganglion. The purpose of the present study was to investigate the effect of diabetes on the sympathetic innervation of the major cerebral arteries. Ten weeks after induction of experimental diabetes by alloxan (50 mg/kg) the adrenergic innervation density (grading scale: 0-3) of the internal carotid, anterior cerebral, middle cerebral, posterior cerebral and basilar arteries in control and diabetic rats was studied by glyoxylic-acid-fluorescence-histochemistry. The density of adrenergic fibres was markedly decreased in all cerebral arteries of diabetic rats compared to age-matched controls. In contrast to the decrease of noradrenergic innervation in the brain arteries, central norepinephrine levels (LCEC-Assay) in diabetic rats were significantly ($P<0.05$) increased compared to controls: spinal cord (+109%), cerebellum (+17%), pons/medulla (+67%), mesencephalon/diencephalon (+39%), parietal/occipital (+33%). The pathophysiological mechanisms underlying these differential responses need to be clarified. Since one of the postulated functions of the adrenergic innervation of cerebral arteries is to maintain blood-brain-barrier (BBB) integrity (Edvinsson & MacKenzie, Pharmacol. Rev., 28:275, 1977), our hypothesis is that the observed decrease in adrenergic innervation of cerebral arteries in diabetic rats might be one of the reasons for the increase in BBB permeability described in diabetes (Stauber et. al., Diabetes, 30:500,1981; Lorenzi et. al., Diabetologia, 29:58, 1986). Such a disruption of BBB integrity could cause leakage of circulating catecholamines into brain tissue.

412.11 BLOCKADE OF N-METHYLASPARTATE-INDUCED ARCUATE NUCLEUS DAMAGE BY PCP, MK-801 AND RELATED COMPOUNDS. T.A. Fuller, J.J. Lawrence and J.W. Olney. Dept. of Psychiatry, Washington Univ. School of Medicine, St. Louis, MO 63110.

Systemic administration of the excitotoxic glutamate analog, N-methylaspartate (NMA), destroys neurons in certain brain regions that lie outside blood brain barriers (BBB), such as the arcuate hypothalamic (AH) nucleus. Competitive NMA antagonists such as D-2-amino-5-phosphonopentanoate, effectively block the toxic action of NMA on AH neurons, but non-competitive NMA antagonists, such as phencyclidine(PCP), MK-801 and related compounds, have not been tested in this in vivo model. Since these agents are potentially of value as BBB-permeable neuroprotective agents in the treatment of neurodegenerative conditions, we tested them for efficacy in protecting AH neurons in vivo against NMA toxicity.

Sprague Dawley rats (26-28 days old) were injected ip with saline or a test compound 15 min prior to NMA (50 mg/kg sc). Four hours after NMA treatment, the animals were sacrificed under deep anesthesia by perfusion fixation and their brains prepared for histopathological examination. Saline pretreated rats consistently had a circumscribed easily quantifiable neuron necrotizing lesion in AH. Pretreatment with PCP, m-amino-PCP or MK-801 provided complete protection against NMA toxicity at doses of 10, 5 or 1 mg/kg respectively; smaller doses of these agents provided partial protection in a dose related manner. Ketamine substantially protected (77% reduction in lesion size)at 20 mg/kg, but repeated doses were required. The prototypic sigma opiate agonist (+)-SKF 10,047 (which binds at both PCP and sigma receptor sites) provided 55 and 80% protection at 20 and 40 mg/kg respectively. Haloperidol, which reportedly blocks sigma but not PCP receptors, was administered either alone or together with MK-801 to NMA treated rats and did not protect against NMA toxicity or interfere with the NMA blocking activity of MK-801. Diazepam, an agent which has no NMA blocking activity in other systems, was administered at 20 mg/kg and failed to block NMA toxicity in this system.

Here we confirm, in an in vivo system, the in vitro observation that agents which have PCP receptor binding properties block the neurotoxicity of NMA, and we show that their order of potencies for blocking NMA toxicity is the same in vivo as in vitro. MK-801, which has been described as the most potent in vitro NMA antagonist known (Price et al., this meeting) is also the most potent known antagonist of NMA neurotoxicity in the in vivo AH.

Supported by RSA MH 38894 (JWO) and a grant from the Washington Univ/Monsanto Biomed. Res. Fund. MK-801 was generously provided by Merk Sharp & Dohme.

412.12 RELATIONSHIP OF VIP, A LIGAND FOR TWO DISTINCT RECEPTORS IN RAT BRAIN, TO THE AIDS VIRUS RECEPTOR. J.M. Hill*, N.E. Jelesoff, P.L. Hallberg, B.M. Martin, M.R. Ruff and C.B. Pert. Section on Brain Biochemistry and Section on Pre-Clinical Studies, Clinical Neuroscience Branch, National Institute of Mental Health, ADAMHA, Bethesda, Maryland 20892.

The pentapeptide sequence, TDNYT, occupying positions 7 to 11 within vasoactive intestinal peptide (VIP), shares homology with the proposed attachment sequence, peptide T, of gp120, the envelope protein of the AIDS virus HIV. Direct high affinity binding of peptide T to rat brain and human T-cells has been demonstrated. This binding is potently displaced by native gp120 as well as VIP. These data indicate that both VIP and peptide T are ligands for the same recognition molecule utilized by the HIV envelope protein gp120. This suggests that VIP or a VIP fragment may be an endogenous ligand for T4 (or CD4), the HIV receptor, present on brain cells, T lymphocytes and other cells. Autoradiographic studies of VIP birding in rat brain from different laboratories report dissimilar distribution patterns which may be due to the use of ligands iodinated on different tyrosines; for example, we have shown that iodination of the tyrosine at position 10 will prevent binding to T4 since this tyrosine is within the bioactive pentapeptide sequence. In the present study, autoradiography of the binding patterns of various isomers of mono- and diiodo ^{125}I-VIP are displaced with "cold" VIP, various VIP fragments and peptide T. By careful comparison with adjacent sections on which the brain distribution pattern of T4, localized with the antibody OKT4 and radioimmunocytochemistry was visualized, we have reached several conclusions.

The binding patterns achieved with the various VIP's differ from each other or from the T4 pattern depending on which tyrosine is iodinated and permit the discrimination of the differences in the regional distribution of the two receptors. The ^{125}I-Tyr^{10}-VIP pattern is the least like T4 distribution pattern since the iodination of Tyr^{10} apparently destroys the ability of VIP to bind to T4. With ^{125}I-Tyr^{10}-VIP the highest binding occurs in the thalamus, including the medial geniculate, with low binding in the ventral hippocampus. T4 is abundant in the hippocampus but is negligible in the thalamus.

The results of this study suggest that VIP functions as a ligand for two distinct receptor systems which have different regional distribution patterns in brain. One pattern visualizes T4 and thereby defines the recognition molecule for the HIV virion and peptide T related sequences such as TDNYT of VIP. The other pattern is likely associated with the classical VIP receptor which coupled to adenylate cyclase.

412.13 REDUCED LENGTH OF PRIMARY CLEFT IN DYSTROPHIC MICE; A SCANNING ELECTRON MICROSCOPIC STUDY. C. Labrecque*, J.P. Tremblay and M.A. Fahim. Lab. of Neurobiology, Fac. of Med., Laval Univ., Québec, Canada and Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA 90089.

The observation of smaller endplate potentials in the muscle fibers of dystrophic mice have led Law et al. (Exp. Neurol. 51, 434-443, 1976) to suggest that the muscle was functionally denervated. Our scanning electron microscopic study of the endplate regions of normal (C57BL/6J +/+) and dystrophic (C57BL/6J dy^{2J}/dy^{2J}) three month old mice supports this concept. The gastrocnemius was exposed and fixed in situ with 2.5% glutaraldehyde. The regions containing NMJs were identified by their cholinesterase activity (Tennyson et al., J. Neuropath. Exp. Neurol. 36, 245-275, 977) and isolated by microdissection. The presynaptic terminals were removed by treatment with 8N hydrochloric acid. The digestion was stopped by filtering the muscle fibers through a glass filter (Nucleopore 8 um pore-size). The fibers were rinsed with water, dehydrated, critical point dried and coated with palladium-gold on the filter. A sample of 40 normal and 53 dystrophic endplates were photographed at x2000 or x4500. The following parameters were measured: 1) muscle fiber diameter, 2) endplate area, 3) number of primary cleft, 4) length of primary cleft branches, 5) number of branch points. The appearance of the endplate is extremely variable in dystrophic animals. Some endplates are rather normal in appearance. Other endplates are smaller, elongated or fragmented or have wider and more shallow primary clefts. Quantitative analysis revealed a positive correlation between the endplate area and the muscle fiber diameter in both the normal and dystrophic animals. However, the motor endplate area is significantly lower (by 29%) in the dystrophic mice. The total length of primary cleft of an endplate correlates positively with the endplate area and with the muscle fiber diameter in both normal and dystrophic mice. However, the total length of the primary cleft is significantly lower (by 34%) in dystrophic mice, especially in large diameter muscle fibers. Finally, the endplate of dystrophic mice is characterized by shorter primary clefts with less branching points. The reduced length of primary cleft in dystrophic endplate is probably a consequence of the reduced presynaptic terminal length. Such a reduction of the terminal length could be responsible for a lower transmitter release capacity and for the smaller endplate potentials previously observed in dystrophic muscles.

412.14 SUBSTANCE P RECEPTORS MAY BE INVOLVED IN THE PATHOPHYSIOLOGY OF INFLAMMATORY BOWEL DISEASE. C.R. Mantyh*, S.R. Vigna*, P. Popper, J.E. Maggio, M.L. Welton*, E.P. Passaro Jr.*, and P.W. Mantyh. Center for Ulcer Research and Education, Department of Surgery, VA Wadsworth 90073, Brain Research Institute, UCLA, Los Angeles Ca 90024 and Harvard Medical School, Boston Ma 02115

Recent advances in understanding a variety of inflammatory diseases reveals a complex interaction between the nervous and the immune systems. One group of neurotransmitters which we have hypothesized form a critical link in communicating between the nervous and immune systems consists of two members of the tachykinin family, substance P (SP) and substance K (SK), which are expressed by sensory neurons. In preliminary experiments we have shown that in experimental animals SP and SK are involved in regulating several gastrointestinal activities including gastric motility, digestive enzyme secretion, mucosal ion transport, hemodynamics, neuronal excitability, inflammation, and the immune response. In the present report we have explored whether tachykinin receptor binding sites are abnormally expressed in inflammatory bowel disease and whether this expression is correlated with the extent of these diseases. To test this hypothesis we obtained fresh surgical specimens from normal patients during resection from malignancy (n=10), ulcerative colitis patients (n=7) and patients with Crohn's disease (n=3). In all cases the specimens were obtained less than 5 minutes after removal which greatly diminished the problems associated with degradation artifacts. The tissue was immediately placed in Tissue-Tek, frozen on dry ice and processed for quantitative receptor autoradiography using Bolton-Hunter labeled NK, SK and SP. In normal colon a high concentration of SP receptors is present in circular muscle, a moderate concentration in the myenteric plexus and submucosal blood vessels, and low concentrations in the mucosa. In the normal colon very high concentrations of SK receptors are present in the circular muscle while moderate concentrations are expressed by myenteric neurons. In colon tissue obtained from the ulcerative colitis and Crohn's patients, very high concentrations of SP receptors are seen in blood vessels in both the submucosa and all muscular layers, and in lymph nodes, whereas both SP and SK binding sites are greatly reduced in the circular muscle. Little change in SP receptor distribution is observed in the plexus or mucosa in the diseased tissue when compared to normal tissue. These results demonstrate that SP receptors are upregulated in the diseased tissues in the submucosal blood vessels and is ectopically expressed by blood vessels in both circular and longitudinal muscles which normally do not express detectable SP binding sites. In addition both SP and SK binding sites are downregulated in the circular muscle which may account for the changes seen in the motility of the gut in these diseases. These results suggest that tachykinins are involved in the pathophysiology of IBD and for the first time suggests a rational approach to designing neuropeptide analogues which may be efficacious in treating these and other inflammatory diseases. Supported by NIH 23970.

412.15 BIOCHEMICAL ACTIVATION OF LOCALIZED EEG ACTIVITY IN TOURETTE SYNDROME AND CONTROLS K.A.Bonnet, R.A.Primas, C.J.Walsh. Department of Psychiatry, New York University Medical Center New York, NY 10016

Tourette syndrome and control subjects males on no medication were studied with baseline brain electrical activity and during three successive hours following biochemical challenge with amino acid precursors of neurotransmitters. Each subject was challenged with L-tryptophan, choline chloride or L-DOPA each on separate days of study.

Brain electrical activity was recorded through 28 monopolar electrodes using ear reference electrodes. The computer-based EEG system permitted storage of all 28 active channels and four movement artifact channels. Age-matched comparisons between baseline records showed slight but significant increased slowing in the midline frontal and central regions of Tourette subjects, and decreased total power in left frontotemporal and right asymmetric alpha in the right parietooccipital area.

The comparison to controls challenged with the three amino acid substances showed Tourette syndrome (TS) subjects to show greater reduction in beta2 and reduction in slowing in the central region. L-DOPA reduced Tourette subjects' occipital alpha and betal activity, and increased midline frontal and central slowing. Each of these occurred in a time-dependent and reversible manner.

The localization of the effects are consistent with previous assumptions made from localization of effects reflected in neuropsychological testing of Tourette and control subjects (Bonnet, 1981). These findings correlate well with pharmacological history in the Tourette subjects in regard to benefits, and to history of side effects.

The patterns of "serotonergic", "cholinergic" and "dopaminergic" activation are empirically derived in this study with higher resolution based upon the use of computer-based EEG and use of 28 monopolar electrodes for better resolution.

412.16 TRH RECEPTORS ARE DECREASED AND 5-HT1A RECEPTORS ARE INCREASED IN ALS SPINAL CORD. S. Manaker, S.B. Caine* and A. Winokur. Depts. of Pharmacology and Psychiatry, Univ. of Penna., Philadelphia, PA 19104.

Numerous studies have demonstrated that thyrotropin releasing hormone (TRH) is highly concentrated in the ventral horn of the spinal cord and that it exerts potent effects on motoneuron activity. Recent evidence indicates that TRH is colocalized in the spinal cord with two other putative neurotransmitters, serotonin (5-HT) and substance P. Additionally, TRH appears to participate with 5-HT and substance P in a co-modulatory fashion in regulating motor function. In parallel with these preclinical observations, several findings link TRH to the severe neurodegenerative disorder amyotrophic lateral sclerosis (ALS). Thus, infusions of TRH to patients with ALS have been reported to produce transient improvement in motor strength and coordination. Additionally, reductions in TRH levels in the ventral horn of ALS spinal cord have been reported.

We have utilized the technique of quantitative autoradiography to evaluate changes in TRH receptors, 5-HT1A receptors, muscarinic cholinergic receptors, beta-adrenergic receptors, choline uptake sites, and norepinephrine uptake sites in discrete regions of spinal cord from patients with ALS and control subjects. Additionally, we have used saturation (Scatchard) analyses to evaluate both the binding affinity (Kd) and the total number of receptors (Bmax) in each region.

Our current findings confirm and extend our previous report of reductions in the concentration of TRH receptors in lamina IX of ALS spinal cord, the region containing the motor neurons. Reductions in binding are a result of reductions in the Bmax and not changes in Kd, and represent decreases of 55% to 75% in cervical, thoracic, and lumbar levels of ALS spinal cord (n= 8-10). Similar reductions occur in the same region for muscarinic cholinergic receptors. Significant increases in 5-HT1A receptors were observed in the ventral horn of ALS spinal cord utilizing the ligand [3H]-8-OHDPAT. The increases in Bmax ranged from 60% to 145% in the ventral grey and lamina IX regions, across all levels of spinal cord (cervical, thoracic, lumbar, and sacral) and were accompanied by similar increases in binding affinity (n= 6). No statistically significant changes were observed for beta-adrenergic receptors, choline uptake sites, or norepinephrine uptake sites. These findings suggest the involvement of TRH and 5-HT neurochemical systems in the pathophysiology of ALS. Alterations in TRH receptors and 5-HT1A receptors in ALS spinal cord might reflect compensatory changes between two neurochemical systems that are involved, through a comodulatory relationship, in regulating motor function.

412.17 ANTIDEPRESSANTS BIND TO P_2 PROTEIN OF PERIPHERAL MYELIN AND MAY INDUCE AUTOIMMUNITY. D.S. Dwyer and E. Grant*. Neuropsychiatry Research Program, University of Alabama at Birmingham, Birmingham, AL 35294.

The P_2 protein of peripheral myelin has been well-characterized, however, its function is not completely understood. P_2 is a highly basic protein with a molecular weight of about 15,000 daltons. Its amino acid sequence is known, and based on the sequence, P_2 has been assigned to a family of lipid-binding proteins which includes β-lactoglobulin, retinol binding protein and fatty acid binding protein. Immunological studies have shown that challenge of animals with P_2 protein elicits an autoimmune response and demyelination which is similar to the Guillain-Barre syndrome (GBS). GBS has also been observed in humans as a consequence of treatment with the antidepressant zimeldine. We have been investigating linkage between these two observations and the initiation of autoimmunity.

During these studies, we discovered that the P_2 protein binds antidepressants such as imipramine and zimeldine. Binding was demonstrated by equilibrium dialysis. Competitive inhibition experiments indicated that retinoic acid also binds to P_2. We suggest that the binding site for these substances is located in a region at the N-terminal which is highly conserved among lipid-binding proteins.

In related studies, monoclonal antibodies (Mabs) were raised against zimeldine. One Mab, ZMD 13, was purified for further characterization. In a second fusion, we obtained another Mab, from mice immunized with zimeldine, which reacts with P_2 protein. This Mab, ZP 3, also binds strongly to ZMD 13. These data suggest that P_2 protein and ZMD 13 are antigenically similar (they are both recognized by ZP 3) which is not surprising in view of the fact that both proteins bind antidepressants. We propose that antibodies like ZMD 13 (anti-zimeldine) may arise in susceptible individuals during treatment with antidepressants. In addition, antibodies like ZP 3, which is an anti-idiotype against ZMD 13, may be produced to regulate the anti-drug response. However, ZP 3 also binds to P_2 protein and is therefore an autoantibody. The existence of such autoantibodies could explain how zimeldine triggers GBS in certain patients. Alternatively, antidepressant bound to P_2 protein could render the P_2 immunogenic and directly initiate an autoimmune response.

412.18 CNS BETA-ENDORPHIN AND CHOLECYSTOKININ IN SEVERE LIVER DISEASE: PEPTIDE CONCENTRATIONS AND PRECURSORS' mRNA. A.Brini*, C.De Giuli Morghen*, P.Custode*, M.Bianchi*, A.E.Panerai, Dept. Pharmacology, University of Milano, Milano, Italy.

Severe liver disease is often accompanied in the human by signs of the involvement of CNS such as impairment in the accomplishment of simple tasks and derangement of neuroendocrine responses. This observation is consistent with the finding both in the experimental animal and in the human of modifications in the CNS patterns of neurotransmitters and aminoacids. We previously reported on the decrease of beta-endorphin and cholecystokinin concentrations in brain areas of rats suffering for experimental severe liver disease and of humans who died for esophageal bleeding consequent to liver cirrhosis (Salerno F., Life Sci., 33: 377, 1983; Panerai A.E., Brain Res., 247: 188, 1982). The changes observed were specific for the two peptides since neither met-enkephalin nor substance P or somatostatin concentrations were affected. The decrease in the concentrations of cholecystokinin and beta-endorphin can suggest both an increased and a decreased turnover of the two peptides and is therefore ambiguous. In order to obtain a more straightful insight in the involvement of CNS neuropeptides in severe liver disease, we repeated our previous study in the experimental animal, and paralleled the measurement of the concentrations of beta-endorphin, met-enkephalin and cholecystokinin with the evaluation of the mRNA for the respective precursors. We confirmed that both the concentrations of beta-endorphin and cholecystokinin and the mRNA for POMC and preprocholecystokinin decrease in the hypothalamus and the cortex of rats chronically treated with CCl_4, while preproenkephalin-A was only marginally affected. These data indicate that the synthesis of the two peptides is reduced and therefore the decreased concentrations are index of a decreased beta-endorphin and cholecystokinin mediated neurotransmission.

It is interesting also to observe that POMC mRNA is also present in the cortex and follows the modifications induced by severe liver disease in the hypothalamus, although it does not seem to be expressed, since the beta-endorphin immunoreactivity in this region was not detected both in normal or CCl_4 treated rats.

PROCESS OUTGROWTH V

413.1 EFFECTS OF DIVALENT IONS ON NEURONAL SURVIVAL AFTER LASER LESION IN CULTURE. X.-Y. Xie*, B. Brass* and J.N. Barrett. Dept. of Physiology and Biophysics, Univ. of Miami Med. Sch., P.O. Box 016430, Miami, Fla. 33101.

Neurons from the septum of E15 fetal rats were grown in cell culture to study the mechanisms involved in neurite repair and regeneration. Neurite processes were severed with a nitrogen laser using methods described by Higgins, Smith and Gross (J. Neurosci. Meth. 3:83, 1980). Survival of the lesioned neurons and regeneration of the processes were greater for younger neurons (in culture for less than 7 days) than for older neurons (14 to 28 days in vitro), and for lesion sites farther from the cell body (more than 100 μm) compared to lesions nearer the cell body (about 50 μm). Calcium influx is thought to have a role in the resealing of neuronal processes, possibly because of Ca-dependent activation of phospholipase A2 (Yawo and Kuno, Science, 222:1351, 1983). Lucas and Gross (Neurosci. Soc. Abstr. 12: 265, 1986) found reduced resealing and survival of neurons lesioned in low [Ca] solutions, although they found that some neurons did reseal and survive for at least 2 hours in low [Ca]. In our study more neurons (60 - 80%) survived when lesions were made in the presence of 2 mM Ca than in lower [Ca]. However, 20 - 40% of the neurons still survived and regrew processes when lesions were made in low [Ca] solutions (0.01 - 1 μM, buffered with EGTA); similar survival rates were seen when the lesion was made in solutions in which Ca was replaced with 1 mM Mn. These results indicate that membrane resealing in small neuronal processes probably occurs in extracellular solutions containing low [Ca] or Mn. Thus Ca-dependent activation of phospholipase A2 by Ca influx may not be absolutely necessary for resealing of small neuronal processes after neuronal injury. However, it does appear that Ca-dependent mechanisms do contribute to resealing and repair of neuronal processes. Supported by NIH grant NS 12207.

413.2 FLUORESCENCE IMAGING OF ENDOPLASMIC RETICULUM IN GROWTH CONES OF CULTURED RAT SUPERIOR CERVICAL GANGLION NEURONS. M.E. Dailey and P.C. Bridgman. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The endoplasmic reticulum (ER) is believed to play a central role in lipid metabolism and regulation of intracellular calcium. At present, the exact role of the ER in neurite outgrowth and/or growth cone (GC) guidance is not well understood. We have used the fluorescent dye $DiOC_6(3)$ (Terasaki et al., Cell 38: 101-108, 1984) to localize the ER in living and fixed neuronal GCs in vitro. Explants from 21d rat superior cervical ganglia (SCG) were grown on laminin-coated glass coverslips. We found the best visualization of ER to be 12-30 hr after explantation, when GCs are large and before neurites have fasciculated. Cultures fixed with glutaraldehyde (0.25%, 2 min) and stained with $DiOC_6$ (2.5 μg/ml, 10 sec) were viewed with fluorescein fluorescence microscopy. The ER has a characteristic, polarized appearance. In general, the ER is concentrated at the base of the GC, and thin processes of ER often radiate from the base toward the GC margin. $DiOC_6$-stained growth cones were photographed, permeabilized (0.1% Triton X-100, 10 min) and immunofluorescently labelled with anti-tubulin antibodies (rhodamine secondary) to localize microtubules (MTs) and ER within single GCs. Superimposition of ER and MT images demonstrate that these structures are highly colocalized within GCs, especially along processes of ER that extend toward the GC margin. However, the colocalization is not absolute. For example, MTs occasionally extend beyond ER processes in the GC periphery, and the abundance of ER at the GC base precludes strict colocalization with MTs. Living cultures were stained with a reduced concentration (0.3 μg/ml) of $DiOC_6$ for 10 min, then grown in fresh, dye-free media. Control cultures not exposed to dye were otherwise treated identically. Measurements of maximum neurite outgrowth 14 hr after staining were not significantly different for stained and control cultures, suggesting that neurite outgrowth is unimpaired by $DiOC_6$ staining. Hence, we used fluorescence microscopy coupled with a SIT video camera and image processor to obtain still time-lapse images of the ER in living, motile GCs. As in fixed cells, finger-like processes extend from the intensely fluorescent GC base, suggesting that images of ER in fixed GCs correspond to the living condition. In addition, sequences of still images of single GCs reveal changes in the distribution of fluorescence, consistent with the possibility that processes of ER extend and retract during GC motility. These results demonstrate that: (1) ER is a polarized structure within GCs, concentrated at the base, and may extend to the GC margin; (2) finger-like extensions of ER are highly colocalized with MTs; and (3) ER is a dynamic structure in living GCs.

413.3 CHARACTERIZATION OF GROWTH CONE CYTOSKELETONS AND IDENTIFICATION OF AN ASSOCIATED TUBULIN TYROSINE KINASE. N. Cheng*, N. Sahyoun* (Spon: Susan K. Burgess) Dept. of Molecular Biology, Wellcome Research Lab., Research Triangle Park, NC 27709.

Lysed growth cone particles from one day old rats were resolved into detergent-soluble and cytoskeletal components. These two components could be distinguished by their polypeptide and glycoprotein composition, ^{125}I-calmodulin binding, and endogenous phosphorylation.

The cytoskeletal fraction was of relatively low density, sedimenting at the 0.4/0.8 M sucrose interface. Thin-section transmission electron microscopy revealed a homogeneous matrix composed of clusters of punctate structures. SDS-PAGE documented the prominence of tubulin in the cytoskeletal fraction; electrophoretic transfer of the separated polypeptides followed by overlay with enzyme-linked concanavalin A or wheat-germ agglutinin confirmed the presence of numerous high- and low-molecular weight glycoproteins. ^{125}I-Calmodulin gel overlay showed the presence of three high molecular weight (Mr: 140K, 230K and >230K) calmodulin-binding polypeptides.

The phosphorylation of several substrates by endogenous protein kinase(s) was also examined. Endogenous as well as exogenous tubulin proved to be the most heavily phosphorylated substrate. The phosphorylation reaction was greatly enhanced in cytoskeletal preparations compared to lysed growth cone particles, suggesting the presence of a phosphotubulin phosphatase or a tubulin kinase inhibitor. The phosphorylation reaction was not influenced by Ca^{++}, calmodulin, phorbol esters, phosphatidylserine, or cyclic AMP. Growth cones from fetal brains showed less tubulin phosphorylation, although isolated cytoskeletons had significant activity; in contrast, postsynaptic densities of mature rat brain had little tubulin kinase activity. Phosphorylated tubulin was partially resistant to alkaline hydrolysis, and upon partial acid hydrolysis phosphotyrosine as well as phosphoserine were released, indicating that at least one cytoskeletal tubulin kinase is a tyrosine kinase. These observations may prove relevant to critical growth cone functions including shape, motility and target recognition.

413.4 PROTEIN FATTY ACID ACYLATION IN RAT NEURONAL CULTURES. N.I. Perrone-Bizzozero, P.J. Apostolides*, S.P. Finklestein, L.I. Benowitz and O. Bizzozero*. Mailman Res. Ctr., McLean Hosp., Belmont, MA 02178; Dept. of Neurology, Massachusetts General Hosp.; Harvard Med. Sch.; and Eunice Kennedy Shriver Ctr., Waltham, MA 02254.

The modification of polypeptides by covalent attachment of fatty acids is a post-translational process that affects a number of cellular proteins. To determine which neuronal proteins are acylated during process outgrowth, primary cultures of E17 rat cortical neurons were incubated for 16-18 hrs with either [^{3}H]-fatty acids or [^{3}H]-amino acids. Proteins were analyzed by 2-D gel electrophoresis and radioactivity detected by fluorography. Of all the proteins synthesized, as visualized by amino acid incorporation, only four of these showed extensive labeling with myristic acid. The apparent molecular weight and pI of these were 80K, 4.2; 63K, 4.4; 40K, 4.4 and 20K, 5.3, respectively. All of these proteins were associated with the particulate fraction, and were enriched in a growth cone preparation. In all cases, the radioactivity was shown to be bound covalently and could only be removed by hydroxylamine treatment, indicating an ester linkage. Most of the radioactivity associated with these proteins was identified as myristic acid by reversed phase TLC. When [^{3}H]-palmitic acid was used as a precursor, the same group of proteins was labeled, though to a lesser degree.

By molecular weight and pI, the 80K and 40K proteins coincide with the identified growth cone phosphoproteins pp80 and pp40 (Katz et al, J. Neurosci. 5:1402-1411, 1985). However, the growth-associated protein GAP-43 (growth cone phosphoprotein pp46, F1, B-50) did not show acylation under the same conditions. The fact that the acylated proteins described here are membrane-bound and enriched in the growth cone suggests that this post-translational modification may play a role in the intracellular transport and attachment of these proteins to the membrane.

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413.5 INDUCTION OF NEUROFILAMENT PROTEIN AND THY-1 ANTIGEN BY NGF IN PC12 CELLS IS MODULATED BY CELL-CELL INTERACTIONS. P. Doherty, D.A. Mann and F.S. Walsh. Institute of Neurology, Queen Square, London WC1N 3BG, U.K.

Nerve growth factor induced differentiation of PC12 cells is associated with up to a 30-fold increase in the level of a 155Kd neurofilament protein antigen (Doherty, Mann, Walsh, in review) and a 2-3-fold increase in the cell surface expression of the Thy-1 glycoprotein (Doherty and Walsh, 1987, J. Neurochem, in press). The former response can be taken as an index of morphological differentiation, whereas the latter is an early response that can be dissociated from morphological differentiation (see Walsh and Doherty, this volume). We have grown both naive and primed PC12 cells on monolayer cultures of non-neuronal cells to determine if a) the microenvironment can modulate both morphological dependent and independent responses to NGF, and b) if PC12 cell responsiveness to the cues in the microenvironment is in itself subject to regulation by NGF.

Over a 2-3 day period, and in the absence of NGF, both naive and primed PC12 cells failed to differentiate on any tested monolayer. NGF dependent increases in the neurofilament protein antigen and Thy-1 were greatly suppressed when naive cells were grown on fibroblast monolayers as compared to C2 and G8 myotubes and C6 glioma. This suppression was associated with an inhibition of neurite outgrowth. In contrast, there was no difference in the neurofilament protein response from PC12 cells that had been pretreated with NGF prior to co-culture and the now primed cells readily extended axons over fibroblast monolayers. There was no evidence that fibroblasts secrete soluble molecules that directly inhibited the neurofilament and Thy-1 responses.

The reported results suggest that 1) neuronal responsiveness to NGF may be critically dependent on the nature of the local microenvironment, 2) responsiveness is not immutable and may in itself be modified by pre-exposure to NGF, 3) responses other than overt morphological differentiation are also influenced by the microenvironment. It appears probable that control is mediated via direct cell-cell and/or cell matrix interactions.

413.6 INSULIN INDUCED NEURITOGENESIS AND TUBULIN MESSAGE STABILIZATION IN A CLONED HUMAN NEUROBLASTOMA CELL LINE. J.F. Mill*and D.N. Ishii (SPON: E. Freese). Lab. Molec. Biology, NINCDS, NIH, Bethesda, MD 20892 and Dept. Physiology, Colorado State Univ., Fort Collins, CO 80523.

Microtubules are important components of the axonal cytoskeleton. They are assembled from heterodimers comprised of alpha- and beta-tubulins. We have previously shown in a cloned human neuroblastoma cell line, SKN-SH-SY5Y (SY5Y), that physiological concentrations of insulin can increase tubulin mRNA levels as a prelude to neurite outgrowth. Both responses follow the same dependency on insulin dose. Here we explore whether the increase in transcript levels is due to an increase in the transcription rate or an increase in message stability. To address the first possibility, nuclear run-off experiments were performed. SY5Y can survive with no loss in cell number in serum-free medium for at least a week and resume growth on the addition of serum. After 2 days in serum-free medium some cultures were exposed to 0.1 uM insulin for 24 hours. Equal numbers of nuclei isolated from treated and control cultures were incubated in 100 ul of transcription buffer containing 500 uCi of ^{32}P-UTP at 29°C for 30 min. RNA samples containing equal numbers of precipitable counts were hybridized to nitrocellulose discs with 1 ug of plasmid DNA containing either no insert, alpha-, or beta-tubulin coding region cDNA inserts. Tritiated cRNA to either alpha- or beta-tubulin was included to determine the extent of hybridization, and nonspecific background counts were subtracted using the control disc with no insert. Results from these experiments showed no significant differences between treated and untreated control cultures.

To study tubulin message stabilization, cells were grown in serum-free medium without or with insulin for 2 hours to increase tubulin messages. Actinomycin D (2 ug/ml) was used to inhibit further RNA synthesis. Samples were collected every hour for 5 hours in guanidinium isothiocyanate, and the RNA was purified by CsCl step gradient centrifugation. Equal quantities of RNA were electrophoresed in denaturing formaldehyde gels and transferred to nitrocellulose. The blots were sequentially hybridized to the labeled nick-translated alpha- and beta-tubulin cDNAs. The blots were also hybridized to labeled oligo dT. Insulin was found to decrease the rate of decay of tubulin messages, showing that it stabilized these transcripts. In contrast, the oligo dT binding to total poly A mRNA indicated that the total pool of mRNA was not stabilized. We infer that insulin can increase the tubulin mRNA levels by specifically stabilizing these transcripts, and not through enhanced transcription. (Supported by NIADDK grant)

413.7 CHANGES IN THE PATTERN OF MAP2 EXPRESSION ARE CONSERVED IN AVIAN AND MAMMALIAN BRAIN. C.C. Garner, R. Tucker and A. Matus. Friedrich Miescher-Institut, P.O.Box, 4002 Basel, Switzerland.

The pattern of expression of microtubule-associated protein 2 (MAP2) in the neonatal rat brain changes abruptly between postnatal day 10 (P10) and P20. Prior to this time there are two MAP2 species, MAP2b (M_r 280,000) and MAP2c (M_r 70,000). By P20 the MAP2c levels have dropped more than 10-fold and a second high molecular weight species, MAP2a, has appeared and is equi-abundant with MAP2b. Earlier still, at embryonic day 14 (E14) MAP2c is the most abundant isoform suggesting that it is the first MAP2 species to appear in the differentiating neurons. In the developing quail brain a similar set of changes are seen. Again two major MAP2 forms are present, a very high (M_r 260,000) form and a smaller species (M_r c. 65,000). At embryonic day 10 (E10) the smaller MAP2c homologous form is the most prominent whereas by E14 both a large MAP2b-homologous form and the MAP2c species are present. By P2 the MAP2c form levels are many times lower and the pattern of expression resembles that of the adult rat brain. Given the differing rates of maturation in the two species these observations suggest that essentially the same changes in MAP2 gene expression are linked to the same set of events in brain development, namely the termination of axon and dendrite growth and the achieving of adult neuronal form. Then fact that the molecular changes are conserved in both avian and mammalian brain development suggests that these molecular mechanisms are of fundamental importance in neuronal differentiation.

413.8 CYTOSKELETAL ELEMENTS IN MOTOR NERVE OUTGROWTH IN VIVO: CHANGES DURING AXONAL REGENERATION AND TERMINAL SPROUTING. W.C. Yee* and A. Pestronk. Neuromuscular Unit, Johns Hopkins School of Medicine, Baltimore, MD. 21205.

Cytoskeletal elements play a variety of roles in the growth, maturation and maintenance of axons. In this study we have examined the distribution and forms of cytoskeletal elements in normal and growing rat motor axons in vivo. Cytoskeletal elements in the terminal portions of axons were visualized with immunocytochemical methods (peroxidase-antiperoxidase) in frozen longitudinal sections of muscle that were also stained for cholinesterase to localize neuromuscular junctions (NMJs). We compared the cytoskeletal composition of normal axons and their terminal branches to axonal processes forming during two types of outgrowth: 1) axonal regeneration - studied following a crush injury to the nerve at its point of entry into muscle, and 2) ultraterminal sprouting - induced by blockade of neuromuscular transmission with botulinum toxin. At times from 5 to 30 days after the onset of sprouting we stained axons and sprouts for neurofilaments, tubulin, and actin.

Our results show significant variations between in the distribution of different cytoskeletal elements, both in normal axons and in sprouts. In normal nerves neurofilaments are abundant in both preterminal axons and their terminal arborizations at NMJs. In contrast, tubulin and actin staining, while abundant in preterminal axons, decreases abruptly as axons reach NMJs. Tubulin and actin staining is minimal in terminal axonal arborizations. In regenerating axons 2 weeks after nerve crush, reinnervation of most NMJs can be demonstrated with neurofilament antibodies. The entire length of the regenerated axons is visualized. Tubulin staining of axons tends to end in intramuscular nerve bundles or in individual axons at least 100-200 μm before they reach NMJs. Staining of neurofilaments also precedes that of tubulin and actin during the formation of ultraterminal sprouts. By 15 days after botulinum treatment neurofilament antibodies often demonstrate multiple ultraterminal sprouts arising from NMJs. At the same time tubulin is apparent mainly extending in a single column from preterminal axons into the NMJ area and, occasionally, out into a single ultraterminal sprout.

These results show that the distribution of neurofilaments and tubulin in terminal axons is different. In general, neurofilament staining is abundant in terminal aspects of normal and growing axons while tubulin and actin are difficult to demonstrate. Actin and tubulin appear to be quantitatively or qualitatively different in preterminal axons than in terminal axons and sprouts.

413.9 PHYLOGENETIC CONSERVATION OF GAP-43, A GROWTH ASSOCIATED PROTEIN. E. Nedivi* and J. H. Pate Skene. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

GAP-43 is a protein induced in neurons during axonal outgrowth, in the course of both neuronal development and axon regeneration. If this protein performs a function fundamental to neural growth, then we would expect it to be highly conserved during evolution. We were therefore interested in studying GAP-43's evolutionary conservation as an indicator of the protein's functional importance.

We employed GAP-43 cDNA recently cloned from developing rat brain (Basi et. al., Cell, in press, 1987) to probe the genomes of a variety of vertebrate and invertebrate species, using genomic Southern blot hybridization. Genomic DNA isolated from human, rat, chicken, toad, turtle, goldfish, Botryllus(a tunicate), Aplysia and Drosophila was prepared and hybridized by the method of Southern with a cDNA probe containing the entire coding sequence for rat GAP-43. Under stringent hybridization conditions that allow reannealing of the probe with only closely related sequences, homologous DNA fragments were detected in all of the DNAs tested. The homologous sequences were confined to one or two restriction fragments in the pattern produced by an Eco RI digest. Our Southern blot screen of the genomes of members of the animal kingdom indicates that the GAP-43 gene is widely and highly conserved in metazoans. Whether the homologous genes function in axonal outgrowth is still unknown, but phylogenetic conservation of the GAP-43 coding sequence indicates that the gene is important for some fundamental neuronal process.

Of all the phyla we examined, Arthropods are phylogenetically most distant from rat. We have isolated several clones from a Drosophila genomic library that will permit detailed analysis of a GAP-43 gene separated from the mammalian gene by a wide evolutionary distance. Comparison of the rat and Drosophila Gap-43 sequences should disclose the most highly conserved structural aspects of the protein and its gene, possibly yielding insight into the functionally important parts of this molecule.

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413.10 IMMUNOHISTOCHEMICAL LOCALIZATION OF A MAJOR GROWTH-ASSOCIATED PROTEIN IN THE DEVELOPING HAMSTER VISUAL SYSTEM. K.L. Moya, S. Jhaveri*, L.I. Benowitz, and G.E. Schneider. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139 and Dept. of Psychiatry, Harvard Med. Sch., McLean Hosp., Belmont, MA 02178.

In previous studies that examined the transport of radiolabeled proteins we showed that the growth-associated protein GAP 48 (GAP 43/B-50) is synthesized in the retina at high levels during development and rapidly transported to maturing retinal axon terminals. In the present study, using a polyclonal antibody against GAP/B-50, we examined developmental changes in localization of this protein in the optic tract (OT), dorsal lateral geniculate nucleus (LGBd) and superior colliculus (SC) of postnatal hamsters. Postnatal day 2 (P2; P0= day of birth), P5, P8, P12, P19 and adult hamsters were perfused with 4% paraformaldehyde and the brains sectioned in the coronal or parasagittal plane at 40um. Sections were incubated with the antibody and processed using the ABC/HRP technique to visualize the protein.

In general, GAP/B-50 is preferentially localized in fiber tracts and in axon fascicles in the younger animals, whereas in more mature brains the staining shifts to the target neuropil. Thus, the optic tract is darkly stained on P2 and P5, is somewhat lighter by P8 and exhibits virtually no staining by P19. In the LGBd on P2 and P5, the predominant pattern is the dark staining of dorsoventrally coursing axon bundles, corresponding in position to the deeper-running retinal axons. By P12, the staining of fiber fascicles is no longer evident, but the antigen is found throughout the neuropil of the LGBd resulting in a dark background mottled by the presence of unstained perikarya. On P8, the staining is intermediate between the immature and the more mature patterns. In the SC, GAP/B-50 is localized in longitudinal axon fascicles throughout the superficial layers on P2, resembling the distribution of retinotectal axons at this age. By P5, stained axons are concentrated in the optic fiber layer (SO), where retinal as well as the later arriving cortical axons reside. Especially noteworthy at this age is a mediolateral gradient of staining in the SO with medial axons exhibiting more intense staining. By P8, this gradient is significantly diminished, as is the distribution of the antigen in all SO axons. The adult SC exhibits very little GAP/B-50; a light staining of the superficial tectal neuropil begins to resemble the mature pattern by P12.

Our results demonstrate that GAP/B-50 shows striking changes in location within developing axons during maturation. The protein is present in OT fibers when retinal axons invade their targets and initiate arbor formation, but appears to concentrate in terminal neuropil as arbors mature. Studies with prenatal hamsters are currently underway to determine if GAP/B-50 is also present in axons during the time of initial outgrowth from the eye and early elongation along the diencephalic surface. The mediolateral gradient of fiber development in the SC has not been reported previously, and may represent a gradient in maturation of retinal or cortical afferents to this structure. (Supported by an NSF graduate fellowship and NIH grants EY 00126, EY/NS 05504, EY 05690 and EY 02621.)

413.11 GAP-43 IMMUNOREACTIVITY IS CORRELATED WITH RATE OF GROWTH CONE TRANSLOCATION AND WITH INITIATION OF NEURITE OUTGROWTH IN CULTURED SYMPATHETIC NEURONS. M.I. Johnson+, N. Kleitman, M.B. Willard and K.F. Meiri*, Dept. Anat. & Neurobiology (+also Pediatrics), Washington Univ. Sch. Med., St. Louis, MO 63110.

GAP-43 is a neuronal protein synthesized at elevated levels during both developmental and regenerative axonal growth. In cultured rat sympathetic neurons, GAP-43 immunoreactivity is concentrated at the growth cone. On both collagen and laminin, neurites of these neurons from embryonic animals extend more rapidly than those of neurons from postnatal animals. Here we report experiments to investigate the relationship between GAP-43 immunoreactivity and the initiation and rate of neurite elongation.

Explants or dissociated neurons from the superior cervical ganglia (SCG) of embryonic (E21) or postnatal (P30) rats were cultured either separately or on the same laminin-coated coverslip. After 1-3 days, when E21 neurites are extending 2-3 times more rapidly than P30 neurites, the cells were fixed in paraformaldehyde, made permeable with digitonin and incubated with an affinity purified polyclonal antibody against GAP-43. Immunoreactivity was detected with a fluorescein-conjugated secondary antibody. Under phase microscopy, growth cones of E21 and P30 neurites with similar size and shape were paired, and each pair photographed under identical conditions in a fluorescence microscope. When 22 such morphologically matched pairs were evaluated by 12 observers unaware of their age, the E21 growth cones were considered to be unambiguously more fluorescent in 20 out of 22 of the pairs. These results are supported by a preliminary computer analysis of digitized images of growth cones; the average fluorescence per area of the P30 growth cones was 65% of that of E21 growth cones.

Over time in culture, E21 SCG neurons will extend neurites at a greatly reduced rate, but will re-initiate growth if they are re-dissociated and plated onto fresh coverslips. We assessed GAP-43 immunoreactivity in E21 SCG neurons that had been in culture for three to six months, both at the time of and after re-dissociation. Prior to re-dissociation, anti-GAP-43 immunoreactivity was much less than when the neurons were first cultured. Following re-dissociation, the number of cells with augmented immunoreactivity increased with time: at 1 day, 8% were more reactive, by 2 days, 19%, and by 9 days, 78%. At nine days, cells which had begun to extend neurites were intensely fluorescent. We conclude that GAP-43 immunoreactivity correlates with the initiation and the rate of neurite extension in SCG cultures. GAP-43 immunoreactivity increases in the somata of mature neurons when they have been stimulated to re-extend neurites by dissociation. [Supported by NSF grant BNS8508148 and NIH grant NS21771 (MIJ), NIH grant EY06082 (KM and MW), and a McKnight Foundation grant (KM and MW).]

413.12 INDUCTION AND LOCALIZATION OF THE GROWTH-ASSOCIATED PROTEIN, GAP-43, IN NGF- STIMULATED PC12 CELLS. Brian Costello*, Jeanette J. Norden and John A. Freeman. (SPON: D. M. Buxbaum), Dept. of Cell Biology, Vanderbilt University Medical School, Nashville, TN 37232.

Expression of GAP-43, a fast-transported neuronal protein, is elevated during periods of axonal growth. We have utilized the PC12 line of rat pheochromocytoma cells as an in vitro model for studying the role of GAP-43 in neurite outgrowth. Previous work from this laboratory has demonstrated induction of GAP-43 in PC12 cells upon stimulation of neurite outgrowth by treatment with nerve growth factor (NGF). In the present studies, quantitative immunoblotting procedures were used to measure GAP-43 levels. Coincident with NGF-stimulated neurite outgrowth, levels of GAP-43 increase by an order of magnitude. Turnover rate studies employing [35S]-methionine pulse labeling show that this increase is attributable mostly to a corresponding increase in the GAP-43 synthesis rate. These results are consistent with a role for GAP-43 in neurite outgrowth in PC12 cells. In order to gain some insight into its precise function, electron microscopic immunolocalization of GAP-43 was performed using affinity purified antibody and the avidin/biotin/peroxidase method. In NGF-stimulated PC12 cells, GAP-43 is seen associated with vesicle clusters within varicosities along neurites and in growth cones. Such vesicle clusters are reminiscent of those present in cholinergic nerve endings and suggest that GAP-43 plays a role in synaptic vesicle function, possibly related to vesicle fusion and membrane addition. Supported by NIH Grants EY01117 and NS18103 to JAF.

413.13 THE GROWTH-ASSOCIATED PROTEIN, GAP-43, IS ASSOCIATED WITH SYNAPTIC VESICLES AND WITH PLASMA MEMBRANE IN PRESYNAPTIC TERMINALS. Jeanette J. Norden, Brian Costello*, and John A. Freeman. Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232.

GAP-43 is a 43 KDa fast axonally transported neuronal protein which shows a 20- to 100-fold increase in synthesis and transport during nerve growth and regeneration. Recently we have shown that GAP-43 is identical to the synaptic plasticity-associated PKC substrate F1 (Snipes et al, J. Neurosci, in press), which has been shown to undergo an increased phosphorylation during long-term potentiation in the hippocampus. In an effort to elucidate the possible role this protein might play in nerve growth and synaptic plasticity, we have raised specific antibodies to purified GAP-43. Using light microscopic immunocytochemistry, GAP-43 appears to be localized to growing processes throughout the rat brain during development. In the mature brain, GAP-43 immunoreactivity is localized to synaptic areas and is particularly intense in the molecular layers of the neocortex, cerebellum, and hippocampus. Using affinity purified antibodies, we have used immunoperoxidase methods to localize GAP-43 at the EM level in the adult rat brain. GAP-43 immunoreactivity is specifically localized at synapses to the synaptic vesicle membrane and to the presynaptic plasma membrane. Synapses in control sections in which affinity purified antibody was blocked with an excess of GAP-43 were completely negative. The localization of GAP-43 immunoreactivity to synaptic vesicles and plasma membrane suggests that GAP-43 might play a role in vesicle fusion and release of neurotransmitter. This also suggests that the PKC phosphorylation of GAP-43 is linked to the increased release of neurotransmitter which has been shown to occur during long-term potentiation. Supported by NIH Grants EY01117 and NS18103 to JAF.

413.14 POST-TRANSLATIONAL MODIFICATION OF A GROWTH CONE PROTEIN, GAP-43: AN ANALYSIS USING MONOCLONAL ANTIBODIES. D.J. Schreyer and J.H.P. Skene. Dept. Neurobiology, Stanford University School of Medicine, Stanford, CA 94305

GAP-43 is an acidic protein component of growth cones and synaptosomes whose expression is elevated during axon growth. It is the product of a single gene in the rat, and its primary structure is known. GAP-43 can be phosphorylated in vivo, and such modification is likely to be important to its function within growth cones and synaptic terminals. Isoelectric focusing reveals that native GAP 43 may be resolved into at least four species, representing isoforms differing in pI. In order to further investigate the nature of the post-translational modifications that produce heterogeneity of a protein translated from a single mRNA species, we have produced a panel of monoclonal antibodies against GAP-43.

Mice were immunized with a crude GAP-43 preparation produced by preparative isoelectric focusing. Antibody producing hybridoma cell lines were produced and cloned using standard methods, and screened for reactivity with the antigen preparation in a dot-blotting assay. Positive clones were further characterized using Western blot analysis of SDS-PAGE of solubilized membrane fractions from neonatal rat brain. Eight antibodies were identified which reacted with single bands possessing electrophoretic mobilities identical to purified GAP-43.

The monoclonal antibodies were then used to analyze "prints" of isoelctric focusing gels, using a technique analogous to Western blotting. Of the eight anti-GAP-43 monoclonal antibodies, six recognized all four isoelectric isoforms, one recognized only the three most basic isoforms, and one recognized only the three most acidic isoforms. The latter two antibodies thus recognize sites which are sensitive to post-translational modification. Preliminary Western blot analysis of GAP-43 from growth cone and synaptosome subcellular fractions in SDS-PAGE suggests that growth cones contain a slightly less mobile form of GAP-43 not found in synaptosomes. Investigations are underway to determine which of the isoforms of GAP-43 are present in growth cone and synaptosome subcellular fractions.

Supported by NIH grant NS-20178, the Isabelle Niemela Fund, and a Searle Scholars Award.

413.15 IMMUNOCHEMICAL EVIDENCE THAT A GROWTH-ASSOCIATED PROTEIN (GAP-43) IS LOCALIZED SPECIFICALLY TO DEVELOPING NEURONAL PROCESSES AND MATURE PRESYNAPTIC TERMINALS. C. Brian McGuire*, G. Jack Snipes* and Jeanette J. Norden (SPON: A.M. Burt). Dept. of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232.

A growth-associated protein designated GAP-43 is a developmentally regulated neuronal protein which is synthesized and rapidly transported to axonal endings in high amounts during periods of axon growth and synaptic terminal formation in both the peripheral and central nervous systems and is enriched in isolated growth cone particles, as well as in synaptic plasma membranes. Much evidence exists suggesting it is a phosphorylation substrate of protein kinase C (PKC), and may be identical to the B-50 protein associated with synaptic transmission and the F1 protein, which is involved in neuronal plasticity. We have raised a specific antiserum against GAP-43, and have used this to characterize the tissue distribution of this protein. Western blot analysis of proteins from a variety of rat organs, together with immunocytochemical data, provide evidence that the presence of GAP-43 is limited to neurons of the peripheral and central nervous systems. Careful analysis of GAP-43 immunolocalizations in paraffin sections of developing rat brain suggests that GAP-43 is limited to growing neuronal processes. In sections of mature brain, the distribution of punctate immunoreactive sites suggests a subcellular localization exclusively to presynaptic terminals. Its neuronal specificity and subcellular localization, together with biochemical data concerning the dynamics of its synthesis and probable role in the phosphatidylinositol cycle, are consistent with hypothesized functions of GAP-43 in axon growth or synaptic terminal differentiation.

413.16 DISTRIBUTION OF GAP-43 WITHIN ISOLATED NEURONAL GROWTH CONES. K.F. Meiri and P.R. Gordon-Weeks (SPON: M. Willard). Dept. of Pharmacology, SUNY Health Science Center, Syracuse, NY 13210 and Dept. of Anatomy and Human Biology, Kings College, London U.K.

Embryonic or neonatal rat forebrain can be fractionated to yield a population of particles which, by morphological criteria, appear to be derived from neuronal growth cones (GCPs). These GCPs have ultrastructural characteristics of growth cones, including filopodia, which many of them retain through the purification procedure. The neuronal phosphoprotein GAP-43, a polypeptide whose synthesis is induced in neurons when they grow axons during development or regeneration, is highly enriched in a particulate fraction derived from GCPs, but is probably not an integral membrane protein. We have used an affinity purified antibody against GAP-43 to determine its distribution within these growth cone particles.

Isolated GCPs were prepared from forebrains of neonatal rat pups and allowed to settle onto polyornithine-coated coverslips. The particles were then fixed, made permeable with digitonin and incubated with anti-GAP-43 antibody. Immunoreactivity was detected with a fluorescein-conjugated secondary antibody.

Upon attachment to the coverslips the spherical particles spread out (the average size of the particles was 2-5 μ), became phase bright and extended fine processes. All the particles that had attached reacted with the GAP-43 antibody, indicating their neuronal origin. GAP-43 immunoreactivity was present throughout the extent of every fine process. The punctate nature of the staining resembled that seen when growth cones from cultured neurons were reacted with anti-GAP-43.

The particulate fraction of GCPs probably contains both plasmalemma and intracellular membranes, as well as cytoskeletal proteins. To clarify which of these subcellular structures GAP-43 is associated with, the GCPs were further fractionated by extraction in non-ionic detergent followed by density gradient centrifugation of the detergent insoluble material. This yields a 'membrane skeleton' which is believed to comprise the plasma membrane which is closely associated with the cytoskeleton. From Western blotting GAP-43 appeared enriched in this membrane skeleton by a factor of about 5 compared with whole GCP homogenate. However, GAP-43 immunoreactivity was not present in a different detergent-insoluble fraction which is believed to contain only cytoskeleton. We conclude that the punctate staining with anti-GAP-43 antibody seen in both cultured growth cones and GCPs is likely due to the presence of GAP-43 in membrane which is closely associated with the cytoskeleton, but that under these preparation conditions GAP-43 is not directly associated with the cytoskeleton itself.

413.17 DEVELOPMENT OF QUANTITATIVE IMAGE PROCESSING METHODS TO QUANTITATIVELY ANALYZE mRNA REGULATION USING IN SITU HYBRIDIZATION. John A. Freeman, Jeanette J. Norden, Afshin Meymandi*, and Phillip Samson*. Department of Cell Biology, and Vanderbilt University Medical School, Nashville, TN 37232

We are interested in studying the regulation and function of GAP-43 (identical to protein F1 and B50), a 43 KDa acidic phosphoprotein whose expression is selectively enhanced in neurons that are in an active state of growth, and which undergoes a dramatic increase in phosphorylation during the changes in synaptic function that accompany long term potentiation. As a first step in studying the regulation of this protein, we constructed a specific GAP-43 [35S]-cDNA probe, using the base sequence previously obtained for the protein (Karnes et al, Science, in press), and have used *in situ* hybridization to probe for GAP-43 mRNA levels in different neural systems in the rat CNS and PNS during development and after nerve injury. To quantitate binding, we have developed several powerful digital image processing methods. Microscopic images are digitized by a 12-bit linear photodiode array and displayed on a high-resolution video monitor. The specific probe signal is isolated from non-specific binding by a new referred mapping technique that spatially transforms and subtracts from each section the optical densities of control sections brought into precise registration with experimental sections by a non-linear gradient search algorithm. The specific binding signal is further enhanced by a specially designed digital convolution filter. Images from serial LM sections are then reconstructed in 3 dimensions, and quantitative morphometric measurements are obtained of specific binding per unit neuronal area and volume in order to reveal quantitatively the amount and cellular localization of labeled mRNA. Combined quantitative densitometry of autoradiographic standards, in which OD measurements are calibrated in terms of DPM, allows specific binding to be expressed in terms of number of molecules of hybridized mRNA per neuron. These methods, which are implemented on a laboratory IBM AT computer, promise to be of general utility in quantitating gene expression in a variety of different systems. Supported by NIH Grants EY01117 and NS18103 to JAF.

414.1 DEVELOPMENT OF AUDITORY AND VESTIBULAR NEURONS IN CULTURE: MIGRATION AND FIBER OUTGROWTH. K. Book* and D.K. Morest (SPON: S.J. Potashner). Univ. Connecticut Health Center, Farmington, CT 06032

Neurons have been shown to migrate from the matrix zone of the rhombic lip to the presumptive cochlear and vestibular nuclei in the mantle zone of the E.4.5-7.5 chicken embryo (Hamburger-Hamilton staged) (Book et al, SN Abstr 1985). In the first stage of migration nucleus magnocellularis neurons extend leading processes which form axonal branches that enter the crossed cochlear tract at the border between the matrix and mantle zones. In the next stage of migration the cell bodies travel through their leading processes into the mantle zone by perikaryal translocation. Finally the young cell bodies lose the processes connecting with the matrix zone and start to differentiate. We now report our direct obervations of this process in living medullas in vitro.

At E5.5-7.5 nucleus magnocellularis neurons are the only ones known to send axonal processes across the midline within the crossed cochlear tract, projecting laterally beyond the medial longitudinal fasciculus (MLF). This circumstance allows one to label the migratory magnocellular neurons by retrograde transport through their crossed axons. Medullas (E5.5-7.5) were dissected, rostral to the Vth, and caudal to the Xth, cranial nerves, and embedded in collagen gel. Using a lipid soluble fluorescent dye, diI, as a retrograde marker (Honig & Hume, J Cell Biol 103, 1986), we made in vitro injections on one side of the medulla into the crossed tract between the rhombic lip and the MLF. In all ages examined, brightly labeled crossed tract fibers extended across the midline contralateral to the injection into the region occupied by nucleus magnocellularis during the first day in vitro (1 DIV). Labeled cell bodies could be seen in this region until at least 4 DIV. Observations of transverse slices of the explants confirmed that these labeled cells were in the matrix zone of the rhombic lip and in the position of nucleus magnocellularis at this stage of development. The morphology of the cells corresponded to each particular stage of the migration previously seen in situ.

In separate experiments unlabeled cell clusters from the rhombic lip (E5-7) were grown in cultures on various substrates. Individual cells migrating from the clusters were followed sequentially and photographed at intervals along their migration routes, thus providing direct visualization of the migratory process for a large number of neurons. Migration was effected by elongation of a leading process, neurite outgrowth, and perikaryal translocation. No locomotory cells were seen.

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414.2 TEMPORAL AND SPATIAL DISTRIBUTION OF FIBRONECTIN DURING INNERVATION OF THE AUDITORY RECEPTOR IN THE CHICKEN EMBRYO. S.G. Hemond* and D.K. Morest. Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032.

The developing chicken auditory receptor, the basilar papilla (BP), elongates greatly from base to apex during St 20-30 (Hamburger-Hamilton), while auditory nerve fibers penetrate its basal lamina (BL) and contact hair cell precursors. We have previously described a distinct temporo-spatial pattern of fiber ingrowth (Soc Neurosci Abstr, '86). The present study examines fibronectin (FN) distribution in the BL and in the matrix surrounding the BP during innervation.

Fifteen white Leghorn chick embryos were fixed in buffered paraformaldehyde and prepared for cryotomy. Immunocytochemistry was performed on 10-20 um thick sections with 3 different antisera raised against either human or chicken plasma FN, followed by a biotinylated secondary antibody, avidin-biotin-HRP (Vectastain), and a DAB reaction. Control sections were treated either with buffer, secondary antibody, normal serum or pre-immune serum.

In the stages studied immunoreactivity is not observed in the auditory ganglion and BP, but it is conspicuous in the surrounding matrix, which is rich in FN fibrils. The distribution of FN in the BL changes during innervation. At early stages (St 20-25) only the base of the BP is infiltrated by fiber bundles. The fiber bundles themselves are not immunostained, but the BL exhibits a patchy distribution of FN. A patchy distribution of FN also appears in the basal and apical areas at St 26-29, when these regions receive fiber ingrowth. However, the mid-basal region at St 26-29, still to receive fiber ingrowth, exhibits an uninterrupted FN distribution in the BL. The mesenchyme separating the ganglion from this region of the BP is densely stained; FN fibrils are oriented parallel to the long axis of the BP. The mid-basal region of the BP is innervated after St 29, and at this time a patchy FN distribution of the BL can be observed in this area.

The present results suggest that 1) fiber ingrowth in the BP correlates temporally and spatially with disruption of FN distribution in the BL; 2) in the region of patchy immunostaining, where fiber bundles are penetrating, fibronectin is lodged between the unstained cochlear nerve fascicles; 3) the areas of the BP yet to be innervated in subsequent stages have an uninterrupted FN distribution. Perhaps the fibers themselves affect the distribution of FN in the BL.

(Supported by NIH grant NS14354)

414.3 IDENTIFICATION AND PURIFICATION OF A HIGH AFFINITY LAMININ RECEPTOR FROM EMBRYONIC CHICK BRAIN: EVIDENCE FOR DEVELOPMENTAL REGULATION IN THE CNS P. Douville*, W. Harvey and S. Carbonetto Neuroscience Unit, Montreal General Hospital Research Institute, McGill University, Montreal, Quebec, Canada H3G 1A4

Laminin, a glycoprotein of the extracellular matrix (ECM), is normally found in the PNS of both adult mammalian and avian species but is absent from adult CNS. Laminin is also found in developing CNS (Liesi, EMBO J.,4, 1163, 1985). Laminin mediates nerve fiber growth in culture through specific cell surface receptors and may be involved in nerve fiber growth during development and regeneration.

In earlier studies we have shown that a variety of neural cells grown in culture bind ^{125}I-laminin specifically with a single Kd of 10^{-9}M (Harvey and Carbonetto, Soc. Neurosci. abstr., 12, 1109, 1986). We have now identified and purified a high affinity laminin receptor from embryonic chick brain that is distinct from the low affinity fibronectin/laminin receptor integrin. ^{125}I-labelled membranes isolated from chick brains were passed over laminin-Affigel affinity columns and eluted with 0.2M glycine buffer pH3.5. Autoradiography of SDS polyacrylamide gels of the eluant reveal a single band with a M_r of 67,000 daltons. Peptide mapping with trypsin have shown this protein to be distinct from serum albumin (M_r=68,000) but homologous to the high affinity laminin receptor (M_r=67,000) found in skeletal muscle (Lesot et al, EMBO J., 2, 861, 1983). Moreover, both the skeletal muscle and brain receptors focus isoelectrically at a pI of 6.2 versus 5.7 for serum albumin.

We have begun to investigate the developmental regulation of high affinity laminin receptors in embryonic chick brain using ^{125}I-laminin binding to purified plasma membranes and to optic lobe cells in culture. ^{125}I-laminin binding to both membranes and cultures is constant from day 8 to day 12 and then falls to 25-30% of the day 8 value at days 14-16. This drop correlates with the decreased ability of optic lobe neurons to extend nerve fibers on laminin-coated substrata. Chick brain membranes (11d) or laminin-affinity purified proteins electroblotted with ^{125}I-laminin (10^{-10}M) reveal two distinct bands, one at 67kD and another at approximately 140kD. This 140kD polypeptide, which is present in trace amounts, may represent an additional high affinity laminin receptor which is not seen on stained gels nor after autoradiography of iodinated purified material. Thus, developing CNS neural cells have multiple high affinity laminin receptors in addition to the low affinity receptor (integrin). We are currently in the process of raising antibodies against these putative receptors to further characterize them structurally and functionally in nerve fiber growth.

414.4 ROLE OF LAMININ AND THE CYTOSKELETON IN CELL FLATTENING AND NEURITE OUTGROWTH IN NG108-15 CELLS. L. Luckenbill-Edds Dept. Zoological and Biomedical Sciences and College of Osteopathic Medicine, Ohio University, Athens, OH 45701

Laminin, a glycoprotein found in basement membranes, has several separate domains that mediate different functions, e.g., collagen binding, heparin binding, cell attachment, cell flattening, and neurite outgrowth. To learn more about how laminin promotes cell flattening and neurite outgrowth, we have investigated the response of a neuroblastoma x glioma cell line (NG108-15) to cytoskeletal inhibitors when cultured on a laminin substrate in the absence of serum. We used vinblastine (1 μg/ml) to depolymerize microtubules, and cytochalasin D (CD) at both high (1.0, 2.0, 5.0 μM) and low (0.01, 0.1 μM) concentrations to depolymerize microfilaments. On tissue culture plastic, vinblastine inhibits both flattening and neurite outgrowth. On a laminin substrate, vinblastine causes withdrawal of established processes, indicating depolymerization of microtubules, but has no effect on flattening. High CD inhibits flattening, and low CD permits flattening, regardless of laminin or plastic substrates. However, a laminin substrate alters the cells' response to CD with respect to process outgrowth. Cells on a laminin substrate treated with low CD extend morphologically normal processes, whereas on plastic, cells with low CD extend aberrant thin, curved, highly branched processes. Aberrant processes also form on a laminin substrate in high CD after a delay. Thus the effect of CD on cell flattening is independent of laminin, whereas the effect of CD on neurite outgrowth depends on the presence or absence of laminin. These results suggest that laminin-mediated flattening of the cell body and laminin-mediated outgrowth of processes involve different mechanisms in these cells.

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414.5 INTERACTIONS BETWEEN GLIAL CELLS AND AXONS IN VITRO.
J.W. Fawcett*, I. Bakst* and I.Rokos* (SPON: R. Keynes) Physiological Laboratory, Downing Street, Cambridge, England, and Salk Institute, La Jolla, California.

Cultures of 7 day old rat optic nerve contain predominantly type 1 and 2 astrocytes and oligodendrocytes. When newborn rat dorsal root ganglia (DRG's) were added to mature optic nerve cultures in the presence of NGF, there was extensive neurite outgrowth. The neurites grew profusely on type 1 and 2 astrocytes, but avoided mature oligodendrocytes. The effect was very local, since DRG neurites grew right up to the oligodendrocyte processes. However, when growing neurites encountered an oligodendrocyte sitting on an astrocyte, they were able to grow on the surface of the astrocyte underneath the oligodendrocyte. Oligodendrocytes prepared in different ways (Percoll density gradient centrifugation, shaking of mixed glial cultures) have the same effects on DRG neurite growth. We conclude that oligodendrocytes are not adhesive to growing DRG neurites, but do not actually inhibit their growth.

Astrocyte cultures made in the usual way from neonatal rat forebrain soon become covered with a layer of oligodendrocytes, type 2 astrocytes and their precursors. We noted that in regions where there are few of these cells, the underlying type 1 astrocytes are generally flat and fibroblastic in appearance, whereas where there are many the type 1 astrocytes tend to be more numerous, have fine processes, and stain more strongly for GFAP. If all cells of the oligodendrocyte lineage are killed with tetanus toxin, anti-tetanus toxin, A2B5 monoclonal and complement, the culture contains only type 1 astrocytes, and these will maintain the flat fibroblastic morphology for much longer than usual, or indefinitely. However, if oligodendrocytes from an adult rat, obtained by Percoll density centrifugation are added, the astrocytes rapidly become process bearing and more strongly GFAP+ve. It appears that oligodendrocytes have a trophic effect on astrocytes, although this must be a very local effect, and cannot be transmitted with conditioned medium.

Dorsal root ganglia were plated onto forebrain astrocyte cultures. Neurites grew rapidly over the astrocytes, even when the type 1 astrocytes were covered with a layer of oligodendrocyte lineage cells; the axons could be seen in SEM pictures to be growing on type 1 astrocytes underneath the oligodendrocytes. Some cultures contained areas of leptominingeal cells; DRG neurites grew well on these also. However, at the boundary between astrocytes and meningeal cells there was often a "barrier" of piled up reactive looking astrocytes, and these could form an obstacle to neurite growth. Axons growing from chick retina grew well on astrocytes; however on meningeal cells they either did not grow at all, or grew sparsely in fascicles.

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414.6 NEURITE-OUTGROWTH ON LAMININ BY CULTURED RETINAL GANGLION CELLS IS REGULATED BY THE TECTUM. J.Cohen* (SPON: K.Caddy) Dept. of Zoology, University College London, London WC1E.

Chick retinal ganglion cells (RGCs) send axons to their major target, the optic tectum, during the first two weeks of embryonic development. We have shown that between embryonic day 6 (E6) and E11, Thy-1-positive, cultured retinal ganglion cells lose the ability to extend neurites on laminin whilst retaining the surface laminin/fibronectin receptor complex, integrin, recognised by monoclonal antibodies CSAT and JG22 (Cohen et al., Nature 322, 465, 1986; Cohen et al., Dev.Biol. 1987 in press). One possible explanation for the loss of responsiveness to laminin by RGCs is that it is regulated by the target. To test this possibility optic tecta were removed in ovo at E6, prior to tectal innervation and the loss of responsiveness to laminin. Embryos were allowed to survive until E11, by which time most RGC axons would normally have reached the tectum. Retinae were dissociated and cultured for 48 hr on a laminin substrate. Compared with RGCs from unoperated E11 control retinae there was a three-fold increase (14% vs 44%) in the number of RGCs with neurites from tectum-less embryos. Our findings suggest that the down-regulation of response to laminin by RGCs may be controlled by tectal innervation and raise the possibility that the binding specificity of the integrin receptor complex may be modified during the transition from a target-independent to a target-dependent phase of RGC development.

414.7 **PROCESS OUTGROWTH ON CONTINUOUS AND DISCONTINUOUS GRADIENTS OF LAMININ.** **L.R. Dawes* and R.N. Pittman, Dept. of Pharm. Univ. of PA, Phila., PA 19104 (Spon: C. Romano).**

A challenging problem confronting neurobiologists is the elucidation of the mechanisms endowing the growth cone with the capacity for directed process outgrowth and pattern formation. A number of signals have been proposed to influence these phenomena. Among them are the various distribution patterns of extracellular matrix molecules which may mediate adhesive interactions, and in concert with intrinsic differences in neuronal cell membranes, could act to guide axons along their divergent pathways.

Therefore, an apparatus was devised, similar to a casting stand for multiple acrylamide gels, to apply a gentle, substrate bound gradient to glass coverslips. When a gentle gradient of laminin (LM) was applied, it was observed that neither rat sympathetic nor sensory neurons show any tendency to orient towards higher concentrations of LM. We concluded that either the gradient was outside a critical concentration range, or that it was of insufficient steepness to be detected by growth cones.

A more fruitful approach might be to analyze the smallest concentration difference detected by growth cones across a step gradient. Step gradients are prepared by sandwiching trituum labeled LM between the glass substrate and a piece of hemocytometer coverslip. The well is flooded with saline and additional LM is added and allowed to bind uniformly over the remainder of the substrate. Cells are cultured, fixed, and processed for autoradiography. LM is then quantified by densitometry with the DUMAS image analysis system. Cultures are analyzed by determining for each condition the proportion of neurites reoriented at the gradient boundary.

414.8 ELONGATION OF EMBRYONIC CNS NEURONS ON SCHWANN CELL-DERIVED EXTRACELLULAR MATRIX. M. Schinstine and C.J. Cornbrooks. Dept. of Anat. and Neurobiol., Univ. of Vermont, Burlington, VT 05405.

In the developing CNS, axons require permissive substrates in order to arrive at their appropriate targets. The ability of various substrata to modulate neurite outgrowth in vivo can be readily examined in culture. In our study, the pattern and rate of neurite outgrowth was examined on the following substrata: Type I collagen (control), detergent-treated collagen (dColl), and Schwann cell-derived extracellular matrix (ECM). ECM and dColl substrata were prepared by detergent extraction of mature Schwann cell-neuron cultures maintained in complete medium for at least 6 weeks. CNS explants from rats were prepared from embryonic day (E) 15 parietal cortex (PC), septal-basal forebrain (SBF) and lumbosacral spinal cord (LS) and E18 PC and SBF.

Neurite growth on collagen (9-12 um/hr) and dColl (5-9 um/hr) was independent of age. Explants on these substrata displayed radial neurite outgrowth with some fasciculation. In contrast, growth on ECM channels varied with age. E15 explants placed on matrix substrates exhibited 2 types of neurite outgrowth. One type was characterized by neurites that grew without deference to ECM channels (non-orienting neurites). Most non-orienting neurites were not deflected from their initial, radial trajectory by interposing ECM channels. Preferential growth on ECM channels typified the other outgrowth pattern. These orienting neurites were less abundant and were hallmarked by faithful alignment with ECM channels. The growth rate of orienting neurites was similar to those calculated for collagen with the notable exception of E15 LS which grew 1.7X faster on ECM than on collagen. Growth for all orienting neurites was consistently ~2.0X faster than neurites on dColl. Neurites that demonstrated preferential growth on ECM channels were occasionally observed to abandon the ECM and grow onto areas of dColl. In contrast to E15 explants, E18 explants only extended orienting neurites. Although these neurites consistently aligned with ECM channels, they differed from E15 orienting neurites since: a) E18 orienting neurites were rarely observed to elongate from ECM channels onto dColl substrata and b) the growth of E18 SBF on ECM increased significantly ($p<0.05$), rising to 1.7X the growth on collagen. Orienting PC neurites did not demonstrate an increase in growth rate as compared to rates on collagen. Growth for all orienting neurites was 1.5-2.0X faster than growth on dColl.

These observations suggest that ECM derived from Schwann cells can promote neurite outgrowth from CNS tissue in vitro; however, not all neurites respond to the neurite-promoting signals present in ECM. The ability to recognize these signals appears to be related to the developmental age of the neurons and may indicate a switch in the requirement for neurite-promoting factors as development proceeds.

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414.9 RAT AMNION MEMBRANE IS A GROWTH-PROMOTING SURFACE FOR CULTURED PERIPHERAL AND CENTRAL NEURONS. M. Manthorpe, N. Danielsen*, H.L. Vahlsing*, B. Pettmann*, G.E. Davis*, E. Engvall* and S. Varon. Dept. of Biology, Univ. California San Diego, La Jolla, CA 92093; La Jolla Cancer Research Foundation, La Jolla, CA 92037.

We have recently reported (Dev. Brain Res. 1987, in press; Science 1987, in press) that the acellular basement membrane surface of human placental amnion membrane is a potent neurite-promoting culture substratum for developing peripheral and central neurons and that rolled up pieces of human amnion membrane implanted into the adult rat brain act to promote and guide regenerating adult cholinergic axons. Based on these results we attempted to use human amnion matrix grafts to promote and guide axonal growth in the adult rat peripheral nervous system using a silicone chamber model. In contrast to human amnion grafts in the rat brain, similar grafts were consistently rejected when placed in a silicone tube anchored between the transected stumps of an adult rat sciatic nerve. That the same type of human amnion graft was rejected from peripheral but not central neural tissues may be related to the relative isolation of the adult CNS from the immune system.

The same in vitro techniques used for human amnion membrane preparation have been used here to generate and characterize an analogous rat amnion membrane preparation. Fresh amnion membranes were dissected from 20 day old rat fetuses, the epithelial cell layer removed by incubation in dilute ammonium hydroxide, and the membranes washed extensively in sterile saline. Small pieces of membrane were anchored to nitrocellulose paper and used as a culture substratum for developing chick embryo parasympathetic, sympathetic, sensory and motor neurons, the latter three of which have adult counterparts contributing axons to the sciatic nerve. In all cases, neurons exhibited lavish neurite growth on the basement membrane surface but not on the opposing stromal surfaces or on the underlying nitrocellulose. Neurite growth on some pieces of membrane appeared strikingly oriented in parallel bundles, while on other pieces growth was randomly oriented. The underlying cause of these differences, if understood and controlled, could be used to increase directional guidance by the amnion grafts in vivo. Preliminary experiments have shown that the adult rat amnion membrane, unlike the human one, is not rejected when implanted between the stumps of the transected sciatic nerve.

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414.10 REGULATION OF NEURITE OUTGROWTH BY CALCIUM, CYCLIC AMP, AND PROTEIN KINASE C SECOND MESSENGER SYSTEMS IN A DIVERSE POPULATION OF IDENTIFIED AND UNIDENTIFIED HELISOMA NEURONS. A. Taylor-Hunter*, M. P. Mattson, and S. B. Kater, Program in Neuronal Growth & Devolpment, and Dept. of Anatomy, Colorado State Univ., Fort Collins, CO 80523.

Growth factors, neurotransmitters, and electrical activity are known to act as signals which affect neurite outgrowth (i.e., neurite elongation and growth cone motility) in identified Helisoma buccal neurons. The intracellular messengers which communicate the outgrowth-regulating signals are unknown, but we have recently provided evidence that calcium (Ca) plays an important role. Cyclic AMP (cAMP) and inositol phospholipid-protein kinase C (PKC) are additional second messengers which mediate many physiological effects exerted on neurons by neurotransmitters and growth factors. These second messengers often act and interact in different ways in different cell types, but it is not known to what extent second messengers are involved in the regulation of neurite outgrowth. Here we used cultures of a relatively small population of diverse neurons (< 300) which comprise the buccal ganglion of Helisoma to test the possible involvement and interactions of the Ca, cAMP, and PKC systems in the regulation of neurite outgrowth.

Calcium ionophore A23187 (1 - 100 nM) suppressed both neurite elongation and growth cone motility (GCM) in all buccal neurons tested in a dose-dependent manner. The cAMP-elevating agents forskolin (5 - 100 uM) and dibutyryl cAMP (5 - 10 mM) suppressed neurite elongation and GCM in identified neurons B5 and B19 and in approximately 50% of all buccal neurons. This suppressive effect of cAMP required Ca influx in neurons B5 and B19 and in 25% of all buccal neurons tested (i.e., both La^{3+} and a reduced Ca medium prevented the suppression). Phorbol activators of PKC (e.g., phorbol-12-myristate-13-acetate; PMA; 1 - 100 nM) suppressed neurite elongation and GCM in neurons B19 and B5 and in 90% of the population of buccal neurons. The suppressive effects of PKC activators on neurite outgrowth did not require calcium influx since La^{3+} did not prevent suppression by PMA. Taken together, these results demonstrate that Ca is a general regulator of neurite outgrowth in buccal neurons while cAMP and PKC systems affect outgrowth in a neuron-specific manner. In addition, the effects of cAMP may either be dependent upon or independent of Ca. These results reveal that **within even a small population of neurons there is considerable variability in how individual second messengers and their interactions regulate neurite outgrowth.**

Additional results of these studies provided insight into the respective contributions of neurite elongation and GCM to the generation of neuronal form. Exposure of neurons B19 and B5 to 10 uM La^{3+} or a medium with reduced Ca caused, simultaneously, an accelerated neurite elongation and reduced GCM. Such neurites elongating with reduced GCM displayed a marked reduction in branching. These results indicate that while neurite elongation can occur without prominent GCM, **motile growth cones can act to significantly alter both the rate of neurite elongation and the branching pattern.** (Supported by NIH grants NS08054 [MPM]; NS24683, NS2456, NS15350 [SBK]).

414.11 RESPONSE OF CHICK SENSORY GROWTH CONES TO LAMININ AND FIBRONECTIN: EXTERNAL MORPHOLOGY. R. W. Gundersen. Biological Sciences, University of Wisconsin-Parkside, Kenosha, WI. 53141.

Laminin and fibronectin have been shown to exert both tropic and trophic effects on chick sensory neurons. One effect is the ability of laminin, but not fibronectin, to provide a preferred substrate for neurite elongation. The substrate preference for laminin was correlated with a marked decrease in growth cone-substrate adhesion (R. Gundersen. 1987. Develop. Biol. 122: in press). In order to better understand the substrate preference phenomenon, in the absence of an obvious adhesion increase, studies of growth cone external morphology in response to substrates of type IV collagen (control), laminin, and fibronectin were conducted after 24 H in vitro. The following growth cone parameters were quantified: overall area, numbers of microspikes and lamellipodia, microspike length and kinetics of microspike extension.

Microspike number significantly increased ($p<.05$) 280% and 250% in response to laminin and fibronectin respectively. The number of lamellipodia significantly ($p<.05$) increased 190% in response to both laminin and fibronectin. Growth cone area significantly increased ($p<.05$) 180% and 140% in response to laminin and fibronectin respectively; includes increase in process number. Neither microspike length or rate of extension (calculated at 50% maximal extension) changed in response to laminin or fibronectin. These results indicate that laminin and fibronectin can affect growth cone external morphology without an associated change in the kinetics of microspike extension. Only the increase in process number may provide an insight into the substrate preference for laminin.

In order to determine if the number of growth cone processes play a role in the substrate preference response to laminin, the numbers of microspikes and lamellipodia were quantified for growth cones following a type IV collagen-laminin border. The number of microspikes on the laminin side of the growth cone significantly ($p<.05$) increased by 380% while the number of lamellipodia remained unchanged. These results suggest that the growth cone preference for laminin involves the ability of laminin to promote the formation of a greater number of growth cone processes. However, fibronectin also increases the number of growth cone processes, without eliciting a substrate preference response, laminin must be modulating other growth cone parameters which are more important to the substrate preference response.

414.12 NEURITE GROWTH PROMOTING FACTOR(S) OF ASTROCYTE EXTRACELLULAR MATRIX: IDENTITY AND DEVELOPMENTAL REGULATION. Jerome R. Wujek and Ernst Freese. Laboratory of Molecular Biology, NINCDS, NIH, Bethesda, MD 20892.

Extracellular matrix (ECM) derived from cultured astrocytes has been shown to stimulate de novo neurite outgrowth from pheochromocytoma (PC12) cells (Wujek and Akeson, Dev. Brain Res., in press). Experiments have been undertaken to determine the identity and the developmental regulation of the neurite growth promoting factor(s) within the astrocyte ECM.

Primary cultures of astrocytes were dissociated from the cerebral cortex of neonatal Sprague-Dawley rat pups and maintained in culture flasks for 5 days to 3 months. At different times, the astrocytes were trypsinized, passaged into 35 mm Primaria culture dishes at $1x10^6$ cells/dish and incubated for 24 hrs. Using the method of Vlodavsky et al. (Dev. Biol. 93:285, 1982), astrocytes were then removed non-enzymatically by incubation with 0.5% Triton X-100 in phosphate-buffered saline (PBS) for 30 minutes, followed by extensive rinsing with PBS. This treatment leaves a layer of ECM attached to the surface of the culture dish. PC12 cells were seeded at low density onto the astrocyte ECM in serum-free medium and the outgrowth of neurites was measured at 24 hr intervals.

We examined whether astrocytes change their capability for promoting neurite outgrowth during extended growth in culture. Astrocytes were grown in culture, passaged into 35 mm culture dishes and incubated for 1 day; ECM was obtained and tested for neurite promoting activity as described above. Surprisingly, it was found that ECM derived from astrocytes cultured for 2-12 weeks had approximately 2 times more neurite promoting activity than ECM derived from younger astrocytes (5 days in vitro).

We investigated the identity of the neurite growth promoting factor(s) in astrocyte ECM by using an antibody blocking paradigm in the PC12 cell neurite outgrowth assay. Rabbit antiserum against laminin (an ECM protein) or normal rabbit serum was applied to the astrocyte ECM prior to seeding PC12 cells into the test dishes. Neurite outgrowth was inhibited significantly more by anti-laminin than by normal rabbit serum.

414.13 CHARACTERIZATION OF A NEURONAL SERINE PROTEASE WHOSE INHIBITION ALTERS NEURITE OUTGROWTH. J.K. Ivins*, P. Midgette* and R.N. Pittman. (SPON: B. Wolfe) Dept. of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104

Sympathetic neurons from neonatal rats release a urokinase-like plasminogen activator (PA) from distal processes and growth cones. To determine possible functions of the neuronal PA, cultures of sympathetic neurons were exposed to inhibitors of PA. In serum-free medium, inhibitors of urokinase-like serine proteases increase neurite outgrowth ca. 2 fold over an 18 hr period.

An IgG fraction of antiserum against human urokinase (UK) was fractionated into antibodies which bound at the active site of UK and antibodies that bound to other parts of the molecule. Only the antibodies that bound at the active site increased neurite outgrowth. Because active sites of all trypsin-like serine proteases are very similar, the ability of the antibodies to block activity of other serine proteases was determined. The fraction of the antiserum that bound to the active site of UK was 30-200 fold more selective for inhibition of UK activity than for inhibition of acitivity of other serine proteases. These data still leave open the possibility that the protease whose inhibition alters neurite outgrowth may not be UK but rather a serine protease with an active site similar to UK. The recent acquisition of selective peptide inhibitors of thrombin-like, plasmin-like, and UK-like proteases should help define the nature of this neuronal serine protease.

In order to determine the specific aspect of neurite outgrowth that is being affected by inhibitors of UK-like serine proteases, a videomicroscopy and morphometry system has been assembled. In addition to time-lapse video microscopy of neurite outgrowth, the system will be used to micropipette proteases, inhibitors, and antibodies near the growth cones to more directly determine effects of these agents on growth cone dynamics.

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414.14 NEURITE PENETRATION INTO 3-DIMENSIONAL COLLAGEN MATRICES CORRELATES WITH CALCIUM-DEPENDENT METALLOPROTEASE ACTIVITY. R.N. Pittman, H. Buettner*, A. Williams*. Dept. of Pharmacology, Univ. of PA Medical School, Philadelphia, PA 19104.

Sympathetic neurons from neonatal rats release a Ca^{2+}-dependent metalloprotease from their distal processes and growth cones (Dev. Biol. 110:91, 1985). Of protein substrates tested, only native and denatured Type I collagen appear to be substrates. This suggests that a possible function for this protease is to enable growing neurites to penetrate collagen rich extracellular matrices. To test the possibility that neurites use the Ca^{2+}-dependent metalloprotease to penetrate collagen-rich matrices, two assay systems have been developed.

Sympathetic neurons are plated either onto native collagen gels or onto a collagen film inside a doughnut-shaped native collagen gel. The activity of the Ca^{2+}-dependent metalloprotease is inhibited by specific peptide inhibitors, or by chelating calcium or zinc (the protease appears to have zinc at its active site). The ability of neurites to penetrate and grow within the collagen gel is then correlated with the metalloprotease activity. A good correlation exists between the activity of the metalloprotease, the amount of ^3H-collagen released into the culture medium and the ability of neurites to penetrate into the collagen gels. A 5-10 fold decrease in the penetration of neurites into collagen gels occurs when metalloprotease activity is inhibited > 95%; however, no effect is seen on the growth of processes on 2-dimensional collagen films. These data suggest that sympathetic neurons use the Ca^{2+}-dependent metalloprotease to penetrate and grow within 3-dimensional matrices of collagen, but not to grow along a 2-dimensional collagen substrate.

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414.15 **ACTIVITY OF TISSUE PLASMINOGEN ACTIVATOR IN THE DEVELOPING CNS.** **N. Cocero* and R.N. Pittman (SPON:R.Orkand) Dept. of Pharmacology, School of Medicine, Philadelphia, PA 19104.**

Plasminogen activator (PA) activity is present in the developing as well as the adult central nervous system. PA activity has been localized to granule cells of the developing cerebellum, to areas of the rat cortex rich in cell bodies and to synaptosomes from bovine cortex. Although the function of PA in the CNS has not been determined, PA activity has been implicated in granule cell migration in the cerebellum and in glial function in the spinal cord

We have assayed PA dependent proteolysis in the rat brain (cortex,cerebellum, brainstem, midbrain) as a function of age (from E18 to P120). Both urokinase and tissue plasminogen activator activities are present in the developing CNS. The majority of the PA activity is tissue-type PA (t-PA); therefore, we have concentrated in determining the location, regulation, and function of t-PA in the CNS. Considerable differences in t-PA activity exist as a function of age and brain area, with a general trend of decreasing t-PA activity with increasing age. Changes in the level of t-PA activity is not only dependent on the number of molecules of t-PA, but also on the form of t-PA (one chain vs. two chain), as well as the presence of endogenous inhibitors (for which we have evidence). To deal with ambiguity between t-PA activity and the number of t-PA molecules, we generated a panel of monoclonal antibodies (mAbs) against t-PA. These mAbs will be used to study the regulation, location and function of t-PA in the developing rat CNS as well as for studying the levels and location of t-PA in normal and pathological human CNS tissue.

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414.16 IN VITRO STUDIES OF TRIGEMINAL GANGLION SENSORY NEURONS AND MERKEL CELLS. P.M.Vos* and R.N.Pittman (Spon: P. G. Conway) Dept. of Pharmacol., U. of PA, Sch. of Med., Phila. PA 19104

Establishment of specific connections between sensory neurons of the trigeminal ganglion and their peripheral targets (epidermally located Merkel cells) is believed to play a critical role in directing subsequent development of centrally located, somatotopically organized, sensory maps.

Neurons from the trigeminal ganglia of neonatal rats were plated at low density in serum free medium on one of the following substrates: Collagen **(C)**, polylysine **(PL)**, laminin and collagen **(LC)**, laminin and polylysine **(LP)**, or fibronectin and collagen **(FC)**. Cultures were grown overnight and then fixed in glutaraldehyde. Morphometric analysis was carried out on 543 cells using a computer assisted morphometry program.

Growth on different substrates influenced the length, branching, and complexity of neurites. Average lengths for primary processes (processes extending directly from the cell body) were observed in the following descending rank order: **LP > C = LC = PL > FC**. Average lengths of secondary, tertiary and quartenary branches followed a similar pattern. The observed rank order of average numbers of primary processes per cell was: **LP = C = LC = FC > PL**. The observed rank order of average numbers of secondary branches per cell was: **LP > C = LC > FC = PL**. The observed rank order of average numbers of tertiary branches per cell was: **LP = C > LC > FC = PL**.

In conclusion, sensory neurons grown on **PL** exhibit fewer neurites overall, but the neurites that do grow are not markedly shorter than those grown on most other substrates. Neurons grown on **LP** demonstrated larger average process lengths as well as a generally increased average number of process branchings per cell.

Since the sensory neurons in the trigeminal ganglion are known to specifically innervate Merkel cells in vivo, we are currently developing conditions for establishing co-cultures of neurons and Merkel cells in serum free medium.

414.17 PLASMINOGEN ACTIVATOR (PA) PRODUCTION DURING THE "IN VITRO" DEVELOPMENT OF EMBRYONIC MOUSE SPINAL CORD CULTURES. C.B.Kahler*, J.S. Rao*, K.D. Tennant*, B.W. Festoff (SPON: P.A. Singer). Dept. of Neurology, UKMC, Kansas City, KS. and Neurobiology Research Laboratory at VAMC, Kansas City, MO 64128.

PAs play an important role in fibrinolysis, modifying the extracellular environment during embryogenesis, inflammation, and neoplasia. They also appear to be involved in neuronal migration, secreted both by glial and Schwann cells, as well as by the neuronal soma and growth cones. Our present studies focused on the production of PAs by spinal cord cells in culture. Embryonic mouse spinal cords were cleaned free of meninges, enzymatically dissociated, and grown in culture. At time points during the ten day culture period, growth media was replaced with media without serum. Twenty four hours later both conditioned media and cell layers were collected. Samples were analyzed for the presence of PAs using the chromogenic tripeptide S-2251. Two distinct enzymes were detected and characterized by their ability to produce a color reaction in the presence or absence of fibrin monomer (fm). FM-dependent PA (tissue or tPA) was detected only in the conditioned media, while a FM-independent PA (urokinase-type or uPA) was present only in the cell homogenates. Both types of enzymatic activity increased over the developmental time-course, the uPA showing the greatest change. Preliminary experiments with serially-passaged glial cells from these cultures show a high level of both enzymes, with a time-in-culture profile very similar to that of the spinal cord cultures. This pattern was distinctly different in primary glial cell cultures.

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414.18 BOTH FIBRONECTIN AND LAMININ ARE NEURITE GROWTH INDUCING COMPONENTS OF RAT ASTROGLIAL CONDITIONED MEDIUM. C.Schmalenbach*, H.P. Matthiessen* and H.W.Müller. Molecular Neurobiology Laboratory, Department of Neurology, University of Düsseldorf, F.R.G.

The interaction of neurite growth stimulating proteins accumulating in serum-free conditioned media (CM) from highly enriched neonatal cerebral astrocytes (less than 5 % nonglial cells) with neurons from embryonic (E18) hippocampus was studied using a quantitative cell culture bioassay. CM were fractionated by FPLC (Pharmacia) on an anion exchange column (Mono Q) and by gel filtration (Superose 12). Column fractions were analyzed by SDS polyacrylamide gel electrophoresis, immunoblotting and ELISA using antibodies to laminin (LN) and fibronectin (FN). In addition, the neurite growth promoting activity was tested by incubating aliquots of the eluted fractions with poly-L-lysine precoated glass coverslips prior to addition of neurons suspended in chemically defined medium. The astroglial culture was characterized by immunofluorescence microscopy and expressed both LN and FN. Biological activity (percentage of viable neurons bearing processes > 2 cell diameters) was almost exclusively recovered in fractions containing either FN and/or LN. A separation of astroglial CM on Mono Q revealed two main peaks of biological activity. The highest activity (60 % neurite bearing cells) was eluted at 800-1000 mM NaCl and coincided with the highest anti-LN immunoreactivity. It possibly consists of a complex of LN with a heparan sulfate proteoglycan. The immunoreactivity for FN in these fractions was very low. A smaller neurite promoting activity (18 % neurite bearing cells) was eluted at 200-300 mM NaCl and corresponded to the peak fractions of anti-FN immunoreactivity for free FN. These fractions also contained small amounts of free LN (<1/20th compared to FN). By comparison with a dose response curve of pure LN in the bioassay, however, the concentration of free LN in these fractions was too low to account for the observed neurite promoting activity. We conclude that both LN and FN released from astrocytes express neurite promoting activities for hippocampal neurons in culture.

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414.19 GROWTH OF CNS AXONS ON A MODEL OF REACTIVE ASTROCYTES. D.G. Munoz* and A. Scaringi* (SPON: S.K. Ludwin). Department of Pathology (Neuropathology), University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

The formation by reactive astrocytes of glial scar following injury has been considered an obstacle to the regeneration of CNS axons. We have examined this hypothesis in an in vitro model of reactive astrocytosis. Treatment of astrocytic cultures with the cyclic adenosine monophosphate (cAMP) analogue dibutyryl cAMP (dBcAMP) results in marked morphological and biochemical alterations in these cells, including increased content of the astrocytic specific protein, GFAP, and extension of numerous processes. Fedoroff et al (J Neurosci Res 12: 15, 1984) have proposed that dBcAMP-treated astrocytes are the in vitro equivalent of reactive astrocytes. Furthermore, only type 1 astrocytes of Raff are responsive to dBcAMP and participate in the formation of the glial scar (Miller et al J Neurosci 6: 22, 1986). Normal astrocytes in culture are a preferred substrate for axonal growth. (Fallon, J Cell Biol 100: 198, 1985). We compared neurite extension on unmodified and dBcAMP-treated astrocytes. Glial monolayer cultures were prepared from newborn mouse neopallium by mechanical dissociation, plated at a density of 1000 cells/mm^2, and grown to confluence in medium containing 10% fetal calf serum for two weeks. During the third week some cultures were continuously treated with medium containing 0.25 mM dBcAMP and showed the expected morphological change; other cultures were maintained in standard conditions. Embryonic day 13 mouse retinal explants were placed on top of treated and untreated three-week-old glial cultures. At 48 hours post-explantation the cultures were fixed, and the profuse, radial neuritic outgrowth from the explant visualized by immunofluorescence using an antibody against neurofilaments. The average neuritic length of 55 explants on treated and of 36 explants on untreated cultures was identical (1.18 mm). Maintaining treated cultures on dBcAMP after explantation showed no effect on the length of neuritic outgrowth. Thus, the modifications of astrocytes induced by dBcAMP have no influence on the demonstrated high competence of the surfaces of these cells in supporting the growth of immature CNS axons.

415.1 RELEASE OF ENDOGENOUS ADENOSINE FROM RAT CORTICAL SLICES BY K^+ AND GLUTAMATE. K. Hoehn* and T.D. White, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7.

Extracellular adenosine acts at specific adenosine receptors in the CNS to inhibit the release of neurotransmitters and decrease firing of central neurons. In order to exert its CNS functions it must first be released. We have studied the release of endogenous adenosine from rat cortical slices following depolarization by K^+ or the excitatory neurotransmitter glutamate. 0.4 mm slices were incubated in 1 ml of oxygenated Krebs solution at 37°C. Medium surrounding the slices was exchanged at 10 min intervals. After an initial 130 min equilibration period, slices were exposed for 10 min to releasing medium. Samples were collected and reacted with chloracetaldehyde to form the etheno-derivative of adenosine, which was measured by HPLC with fluoresence detection. 30 mM K^+ released 800 pmoles adenosine/ g cortex/ 10 min. Release continued for a further 10 min after removal of the releasing medium. Similarly, 5 mM glutamate released approximately 2300 pmoles/ g cortex during exposure and release continued for 20 min after removal of the glutamate. Experiments were performed in which the specific NMDA antagonist, 2-amino-5-phosphonovaleric acid (APV), was present before, during, and after exposure to glutamate. APV had no effect on basal release of adenosine. It blocked adenosine release during exposure to glutamate, but failed to block the release of adenosine during the 20 min period following the removal of glutamate. It can be concluded that both K^+ and glutamate-evoked depolarization of cortical slices releases adenosine. The glutamate-evoked release is receptor-mediated and the NMDA receptor subtype appears to be involved. Adenosine may be released in the CNS following depolarization of central neurons to act in a negative feedback fashion, dampening the firing of neurons.

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415.2 AUTORADIOGRAPHIC LOCALIZATION AND PHARMACOLOGICAL CHARACTERIZATION OF ADENOSINE RECEPTORS IN RAT BRAIN USING THE XANTHINE CONGENER, [^{3}H]XCC. George A. Stone*, Kenneth A. Jacobson*, William J. Brooks*, Michael Williams and Michael F. Jarvis (SPON: A. Mellow). Drug Discovery Division, Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, NJ 07901 and Laboratory Of Chemistry NIDDK-NIH, Bethesda, MD 20892.

The use of alkylxanthines as radioligands for adenosine receptors has not been widespread due to their low potency at adenosine receptors as well as their high lipophilicity, low affinity and low specific activity. Recently, a xanthine carboxylic acid congener (XCC) of 1, 3-dipropyl-8-phenylxanthine has demonstrated favorable properties as a ligand for adenosine receptors. Autoradiographic studies have revealed that [^{3}H]XCC binds to rat brain sections with nanomolar affinity and in a heterogenous manner consistent with the labeling of adenosine A-1 receptors. The greatest density of [^{3}H]XCC binding sites occurs in the molecular level of the cerebellum followed by thalamus > cortex = striatum. In the present study, the activity of a number of adenosine agonists and antagonists was examined in various brain regions (hippocampus CA-1 region, cerebral cortex and striatum) using quantitative autoradiography. The pharmacological activity of these compounds in displacing [^{3}H]XCC was higly correlated ($r = 0.96$-0.99, $P < 0.01$) in the brain regions examined. For agonists, the rank order of activity was: R-PIA $\geq$ CPA > NECA $\geq$ 2-CADO > S-PIA. Thus the binding of [^{3}H]XCC was stereospecific, R-PIA being 20-fold more active (IC-50 = 0.5 nM) than S-PIA (IC-50 = 11 nM). For antagonists, in the various brain regions studied, the rank order of activity was: XCC > PACPX > DPX. Despite a close concordance in the pharmacological profile of both agonists and antagonists in the various brain regions, all ligands had greater affinity in striatum as compared to either hippocampus or cerebral cortex. The present data indicate that [^{3}H]XCC is a high affinity, selective ligand for adenosine A-1 receptors. However, [^{3}H]XCC may be active at striatal A-2 receptors since NECA, CPA and 2-CADO exhibited essentially equal activity in displacing XCC binding in this tissue.

415.3 CHARACTERIZATION OF A_1-ADENOSINE RECEPTORS IN PORCINE ATRIAL MEMBRANES. M. Leid, P.H. FRANKLIN AND T.F. MURRAY (SPON: M.I. SCHIMERLIK), College of Pharmacy, Oregon State University, Corvallis, OR 97331.

Adenosine and related compounds exert negative chronotropic and inotropic effects on the intact heart and in isolated atria. These inhibitory responses are thought to be mediated by an A_1-adenosine receptor (AR). Experiments were carried out to characterize the AR of porcine atrial membranes (PAMs) using the agonist radioligand [125-I]hydroxyphenylisopropyladenosine ([125-I]HPIA). Kinetic studies were undertaken to address the mechanism of [125-I]HPIA binding. The Kobs displayed a hyperbolic dependence on [125-I]HPIA concentration, consistent with a two-step sequential binding mechanism. [125-I]HPIA bound saturably, reversibly and with high affinity to an apparently homogeneous population of recognition sites with a Bmax of 51.8 $\pm$ 4.4 fmoles/mg protein and a K_D of 3.6 $\pm$ 0.6nM. Specific binding of [125-I]HPIA was enhanced by addition of 5mM Mg^{++} to the incubate. Moreover, guanine nucleotides were found to inhibit specific binding of [125-I]HPIA. These findings are consonant with an agonist-induced formation of a ternary complex consisting of the ligand, adenosine receptor and a guanine nucleotide binding protein. Competition binding experiments were used to determine the agonist pharmacological profile of the PAM AR. The rank order potency as inhibitors of the specific binding of [125-I]HPIA was as follows: R-phenylisopropyladenosine (R-PIA) > cyclopentyl-adenosine > hydroxyphenylisopropyladenosine > cyclohexyl-adenosine > N-ethylcarboxamidoadenosine = S-phenylisopropyl-adenosine > 2-chloroadenosine (2-CADO) >> CV-1808. Moreover, the potency difference between the diastereomers of PIA was approximately 22-fold. This rank order potency profile is consistent with the labeling of an A_1-AR.

Toward the goal of understanding transduction mechanisms associated with the AR of PAMs, the effects of R-PIA and 2-CADO on adenylate cyclase (AC) activity were studied. Both agonists dose-dependently decreased basal and isoproterenol- or forskolin-stimulated AC activity as measured by the conversion of [32-P]ATP to [32-P]cAMP. These findings provide functional evidence for the existence of A_1-adenosine receptors in PAMs. Thus, the results of both pharmacological competition binding and functional experiments suggest that the adenosine receptor of PAMs is of the A_1-subtype. (Supported by a grant from the Oregon Affiliate of the American Heart Association).

415.4 SEPARATION OF A_1 AND A_2 ADENOSINE RECEPTORS OF RAT BRAIN MEMBRANES BY HYDROXYLAPATITE CHROMATOGRAPHY. H. Nakata* (Sponsor: A. Laties), Laboratory of Clinical Science, NIMH, Bethesda, MD 20892.

Adenosine receptors have been divided into two major subclasses A_1 and A_2. A_1 adenosine receptors are linked to inhibition of adenylate cyclase whereas A_2 adenosine receptors are linked to activation of adenylate cyclase. Although it is important to characterize these subtypes biochemically and pharmacologically, poor selectivity of the agonist radioligands such as [^{3}H]phenylisopropyladenosine (PIA), [^{3}H]cyclohexyladenosine (CHA) or [^{3}H]N-ethylcarboxamidoadenosine (NECA) often makes such studies difficult. As a first step toward characterization of these adenosine receptor subtypes, we solublized these receptors from rat brain membranes where both A_1 and A_2 adenosine receptors are present and screened various chromatography systems which can resolve A_1 and A_2 adenosine receptors from the crude receptor solution. I chose to use hydroxylapatite columns.

Rat brain membranes were then prepared from whole rat brains and were solubilized using 1% digitonin. Typically 20-30% of [^{3}H]NECA binding sites were recovered in the supernatant after centrifugation at 100,000 g. The solubilized preparation was then applied to a hydroxylapatite column and the column was eluted with a gradient of potassium phosphate. Three major peaks which had adenosine binding activity were found (designated Peak A, Peak B and Peak C by the order of elution). [^{3}H]PIA (5 nM) binding activity was detected only in Peak A fraction and [^{3}H]NECA (16nM) binding activity was detected mainly in Peak B and Peak C. Displacement of [^{3}H]PIA bound to Peak A fraction by adenosine receptor agonist yielded the following rank order of potency: CPA>R-PIA>CHA>NECA>S-PIA. [^{3}H]NECA bound to Peak B fraction was displaced by NECA or NCCA. However CPA, R-PIA, S-PIA or 2CA did not displace the [^{3}H]NECA bound to Peak B fraction even at 200 µM. [^{3}H]NECA bound to Peak C fraction was displaced by the following order of potency: NECA>NCCA>CPA>R-PIA=S-PIA>IBMX. These displacement experiments suggest Peak A and Peak C correspond to A_1 and A_2 receptors, respectively. These results show hydroxylpatite chromatography appears to be useful for resolution of A_1 and A_2 adenosine receptors and particularly valuable as an initial step for the purification of these subtypes.

415.5 ADENOSINE RECEPTOR SUBTYPES: TOWARD AN UNDERSTANDING OF IN VIVO EFFECTS. R.M. Palmour* and F.R. Ervin. Dept Psychiatry and Centre for Human Genetics, McGill Univ., Montreal QUE H3A 1A1.

The relative contributions of A1 and A2 receptor actions to neuromodulation and to Ado analog-induced changes in behavior remain a topic of contention. In studying this paradox, we compared the actions of cyclopropylAdo (CPA), an A1 selective analog; N-ethylcarboxamideAdo, a potent but unselective A2 agonist and R-phenylisopropylAdo (PIA), a potent A1 agonist with pronounced A2 effects.

In cultured clonal neuroblastoma cells with both A1 and A2 receptors, CPA (1-1000 nM) reduces both basal and PGE1-stimulated cAMP accumulation, while nM concentrations of NECA, PIA and other analogs only reduce stimulated cAMP accumulation. Conversely, between 0.1 and 100 μM, NECA and PIA enhance cAMP accumulation. CPA only stimulates cAMP accumulation at concentrations above 10 μM. In addition, the maximum stimulation of cAMP accumulation varies inversely as a function of the potency of a given analog at the A1 receptor (NECA>PIA>Ado>CHA>CPA). Taken together with studies in cells possessing only A1 or only A2 receptors, these data suggest that maximal cAMP accumulation is the sum of opposing actions at A1 and A2 receptors.

In rat and monkey brain, and in neuroblastoma cell membranes, ^{3}H-NECA labels A1 or A2 receptors in the absence or presence, respectively, of 80 nM CPA. Caudate, hippocampus and cortex have both A1 and A2 receptors, as do neuroblastoma cells. Cerebellar receptors are mostly A1, while pituitary and amygdaloid regions primarily contain A2 receptors.

In the isolated male mouse, aggressive behavior (as measured by # attacks and duration of fighting) is enhanced at very low doses of PIA and NECA (narrow range) and over a much wider range for CPA. Aggressive behavior is dramatically diminished by doses of NECA which minimally reduce motor activity, and by doses of PIA which have a somewhat greater inhibitory effect. In grouped mice, effects of Ado analogs on motor activity follow these same patterns. All behavioral effects were shifted toward the basal state by pretreatment with 15-30 umol/kg caffeine, confirming Ado receptor mediation.

Taken together, these data suggest that in many brain regions, the net effect of Ado or Ado analog agonism is the sum of interactions with A1 and A2 receptors, and that the predominant inhibitory behavioral effects reflect actions at the A2 receptor, while stimulatory behavioral effects may involve A1 receptors. These observations are in concert with the hypothesis that Ado acts physiologically via a mechanism of balanced opposition to regulate levels of arousal in vivo .

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415.6 CARDIORESPIRATORY EFFECTS OF MICROINJECTIONS OF ADENOSINE AND CYCLIC AMP INTO THE NUCLEUS TRACTUS SOLITARIUS OF RATS. P.M. Polasek*, C.J. Janusz*, W.R. Campbell*, E.P. Schoener*, M. Parizon* and R.A. Barraco. Wayne State Univ. Sch. of Med., Detroit, MI 48201.

It is well established that the Nucleus Tractus Solitarius (NTS) is a major relay nucleus for afferent input from baroreceptors and cardiopulmonary receptors and thereby plays a critical role in brainstem mechanisms involved in central cardiovascular and respiratory control. The NTS has been shown to have a complex morphology in addition to possessing a site-specific topographical distribution of a variety of neurotransmitters and putative neuromodulators. Numerous studies have shown that adenosine (ADO) and its analogs depress the release of both excitatory and inhibitory neurotransmitters in the brain. Many of the physiological effects of ADO are accomplished by modulating adenylate cyclase via extracellular receptors which are negatively and positively coupled to the enzyme. In our laboratory, we have recently shown that injections of ADO analogs into the cerebral ventricles of rats produced pronounced bradycardia and hypotension. In separate experiments, it was shown that the fourth ventricle in the region of the area postrema and the NTS were the most sensitive sites for ADO-induced cardiovascular responses. The aims of the present study were to (1) examine the dose-response effects of microinjections of ADO itself into the NTS and (2) to examine the possible involvement of cyclic nucleotides in ADO-mediated responses. Adult male Sprague-Dawley rats were anesthetized with urethane, the femoral artery and vein cannulated for blood pressure (P_A) and heart rate (HR) recordings and systemic drug administration, respectively, and the trachea was intubated and tidal volume (V_t) and respiratory frequency (f) were measured via a Fleisch pneumotachograph. Following a limited craniotomy to expose the brainstem in the region of the obex, microinjections were made via glass micropipettes (60-70 μm) at 100 nl · 15 sec. Drugs were dissolved in artificial CSF. The results show that ADO produced potent effects on P_A and HR. In fact, at lower doses, ADO produced pressor responses while at higher doses, ADO caused hypotension and bradycardia. These findings may reflect an A_1/A_2 receptor-mediated response difference. To examine this, 8-bromocyclic AMP (BcAMP) was microinjected into the same site (AP: -0.2; ML: 0.3; DV: 0.3). BcAMP produced dose-related decreases in P_A and HR. However, BcAMP elicited even greater effects on f and V_t, producing periodic apnea. These findings support the notion that ADO may play a neuromodulatory role in the NTS. (Supported by NSF (R1186-04084), NIH (RR-08167-OX) and Am. Heart Assoc. of Mich.).

415.7 CHARACTERIZATION AND AMINO ACID SEQUENCE OF RAT STOMACH PHOSPHOLIPASE A_2: A PREVIOUSLY DESCRIBED MODULATOR OF THE PERIPHERAL BENZODIAZEPINE RECEPTOR AND VOLTAGE-DEPENDENT Ca^{2+} CHANNEL. A.R. Lingford-Hughes*, B.M. Martin*, R.E. Martenson*, E.I. Ginns*, P. Skolnick*[1], S.M. Paul* (Spon: R.H. Cagan). Clinical Neuroscience Branch, NIMH, and [1]Lab. of Bioorganic Chemistry, NIDDK, NIH, Bethesda, MD 20892.

Recent studies have demonstrated inhibition of [^{3}H]Ro5-4864 binding to the peripheral benzodiazepine receptor and of [^{3}H]nitrendipine to the dihydropyridine Ca^{2+}-channel (Mantione et al., Biochem. Pharm., in press, 1987) by acidified extracts of rat antral stomach. Subsequently termed "antralin", this endogenous material was found to be a protein and to possess phospholipase A_2 (PLA_2)-like activity. We now report that "antralin" is PLA_2 as judged by both its enzymatic activity and amino acid sequence. Acidified extracts of rat antral stomach were applied to octadecylsilyl-silica cartridges (Sep-pak) and eluted with 80% acetonitrile/0.1% trifluoroacetic acid (TFA). The eluates were pooled and applied to a hydroxyapatite column and the protein eluted with a linear NaCl gradient (0-1M). Fractions were tested for PLA_2 activity with [^{3}H]phosphatidylcholine (labelled in the 2 fatty acid position) as substrate followed by heptane extraction of the radiolabelled arachidonic acid product. Enzymatic activity was observed in fractions eluted from 0.5 - 0.08M NaCl. Reversed phase HPLC was used for subsequent purification yielding a specific activity of approximately 100 umoles/mg/min, which represents a 500-1000 fold purification relative to the crude acidified extract. The enzyme was rechromatographed using HPLC to obtain highly purified enzyme for amino acid sequencing, antibody production and characterization of its catalytic properties. The complete amino acid sequence was determined by automatic Edman degradation of tryptic peptides and will be presented. Ionic requirements and substrate kinetics of the highly purified enzyme were characterized. Enzymatic activity was reduced in the absence of Ca^{2+}, but was abolished in the presence of EGTA, suggesting that PLA_2 possesses very high affinity for Ca^{2+}, and that Ca^{2+}/enzyme binding occurs even during purification. We are presently purifying brain PLA_2 using similar methods and studying the effect(s) of PLA_2 on a variety of neurotransmitter receptor-gated and voltage dependent ion channels.

415.8 IDENTIFICATION OF A KYNURENATE-LIKE SUBSTANCE IN HUMAN BRAIN TISSUE. M. Nakamura, W.A. Turski, W.O. Whetsell, Jr.[1] and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228 and [1]Dept. Pathology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Kynurenic acid (KYNA), a tryptophan metabolite known to be present in urine and peripheral tissues, has been shown to possess neuroinhibitory activity (Brain Res., 247, 184, 1982). Moreover, a deficiency of KYNA in the brain has been speculatively linked to the pathogenesis of human neurodegenerative disorders (Neurosci. Lett., 48, 243, 1984). However, the presence of KYNA in human brain tissue has as yet not been examined. Using a novel isolation procedure (established with the use of ^{3}H-KYNA), we have now purified a KYNA-like substance from post mortem human brain material and tentatively established its identity by several chromatographic methods.

Brains from individuals who died without neurological disease were homogenized (1:2; w/v) in distilled water. The homogenate was then boiled (10 min) and the pellet separated by centrifugation. After application of the supernatant to a Dowex AG 50W cation-exchange resin, the KYNA-fraction was eluted with distilled water. The eluate was then applied to a Seppak® C_{18} column and the KYNA-fraction obtained by adding solution containing 100mM acetic acid:methanol (50:50). The resulting eluate was lyophilized and taken up in water to be assessed in 5 different TLC systems (using cellulose or silica gel plates) or by HPLC (mobile phase: 50 mM ammonium acetate/5% methanol) on a 3 μm C_{18} reverse phase column.

Human brain contained a substance which in all systems behaved identically to ^{3}H-KYNA or non-radioactive KYNA. The following regional distribution was found to exist (data are expressed as pmol/mg tissue and are the mean ± S.E.M. of seven brains):

Cerebellum	0.14 ± 0.02
Frontal cortex	0.29 ± 0.03
Parietal cortex	0.39 ± 0.12
Globus pallidus	0.98 ± 0.27
Hippocampus	0.42 ± 0.11
Nucleus caudatus	1.58 ± 0.43
Thalamus	1.11 ± 0.34

These data are in good accordance with the regional distribution in human brain of KYNA's bioprecursor kynurenine (Neurochem. Res. 5, 223, 1980). However, additional (physico-chemical) methods are needed for the unequivocal identification of the isolated substance.

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415.9 GAMMA-AMINOBUTYRIC ACID-INDUCED ENHANCEMENT OF 2-(^{125}I)-IODO-MELATONIN BINDING IN BRAIN MEMBRANES. L.P. Niles and D.S. Pickering*. Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Similarities in the psychopharmacological properties of the pineal hormone, melatonin, and the benzodiazepines suggest an interaction between this hormone and central GABAergic systems as previously reported for these drugs. This view is supported by our earlier findings that melatonin enhances gamma-aminobutyric acid (GABA) binding in vivo (1) and in vitro (2).

A reciprocal and functional interaction exists between the central binding sites for GABA and those for benzodiazepines. Thus, benzodiazepine agonists such as diazepam, enhance GABA binding while GABA, in turn, increases diazepam binding in synaptic membranes. In order to determine whether a similar reciprocal interaction occurs between the binding sites for GABA and those for melatonin, we have now examined the in vitro effects of GABA on the binding of the melatonin analog, 2-(^{125}I)-iodomelatonin ((^{125}I)MEL) in synaptosomal membranes from hamster brain. (^{125}I)MEL was synthesized as previously described (3) and purified by HPLC (4).

GABA, in concentrations of 10^{-9}-10^{-4}M, enhanced (^{125}I)MEL binding by 100-150% in cortical membranes. The GABA agonist, muscimol, also stimulated (^{125}I)MEL binding in brain membranes. This effect was blocked by the GABA receptor antagonist, bicuculline (10^{-5}M), indicating the specific involvement of $GABA_A$ receptors.

Preliminary competition and saturation binding experiments in the absence or presence of GABA (10^{-4}M) indicate that the enhancement of binding is due to a GABA-induced increase in the affinity of high-affinity sites.

(Supported by the Ontario Mental Health Foundation and NSERC, Canada)

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(2) Coloma, F.M. and Niles, L.P. Biochem. Pharmacol. (Submitted).
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(4) Pickering, D.S. and Niles, L.P. Soc. Neurosci. Abstr. Vol. 13 (1987).

415.10 MULTIPLE BINDING SITES FOR 2-(^{125}I)-IODOMELATONIN IN HAMSTER BRAIN. D.S. Pickering* and L.P. Niles (SPON: E. Werstiuk). Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

In earlier binding studies with (^{3}H)melatonin, we observed shallow competition curves and low Hill coefficients which suggested the presence of multiple binding sites although Scatchard analysis indicated a single population of sites (1). Using an improved radioligand for melatonin binding sites, we have now re-examined the saturation binding characteristics of these sites in order to resolve this discrepancy. The radioligand, 2-(^{125}I)-iodomelatonin ((^{125})MEL), was synthesized as described by Vakkuri et al. (2), in 30-60% yields at specific activities ranging from 2000-2500 Ci/mmol. The label was purified by HPLC and nonradioactive 2-iodomelatonin was purified by silica gel column chromatography.

Adult (2-3 months old) male golden hamsters (M. auratus) were decapitated and brain tissues were rapidly dissected on ice. Synaptosomal membranes were prepared as previously reported (3) and used immediately in binding assays or frozen at -70°C before use.

Preliminary experiments using a radioligand range of 0.1-10 nM indicated a single high affinity site with a dissociation constant (kd) of 0.7-0.8 nM and a receptor concentration (Bmax) of about 20 fmol/mg protein. However, as full saturation was not achieved in these assays, we utilized a radioisotopic dilution assay to examine binding in the radioligand range of 0.1-100 nM. Specific binding was defined as that displaced by 10 μm 6-chloromelatonin.

Scatchard analysis of data yielded curvilinear plots which were resolved into two binding components using an iterative nonlinear least squares curve-fitting program. The binding parameters for the high affinity component were:- Kd_1 = 1.3 ± 0.1 nM and $Bmax_1$ = 26.6 ± 5 fmol/mg protein. Corresponding values for the low affinity sites were:- Kd_2 = 64 ± 5.1 nM and $Bmax_2$ = 250 ± 45 fmol/mg protein (n = 3).

The high affinity site is comparable to that (Kd = 3.8 nM) recently reported by Duncan et al. (4) using a similar radioligand in hamster brain. Further characterization of these sites is currently in progress.

(Supported by the Ontario Mental Health Foundation, Canada)

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415.11 MONOCLONAL ANTIBODY AE-2 STIMULATES FETAL BOVINE SERUM ACETYLCHOLINESTERASE HYDROLYSIS OF INDOPHENYL ACETATE. A. D. Wolfe*, C. A. Payne* and S. Bone*. (SPON: G. Mueller) Dept. of Applied Biochemistry, Walter Reed Army Inst. of Res., Washington, D. C., 20307-5100.

Fetal bovine serum acetylcholinesterase (FBS-AChE) hydrolyzes the neutral ester indophenyl acetate (IPA), in addition to acetylcholine (ACh), acetylthiocholine (ATC) and other esters typical of E.C. 3.1.1.7 enzymes. Differential effects of ligands on AChE ester hydrolysis have been reported and appear related to the catalytic function of the ligand binding site. For example, covalent binding of 2-chloro-N-(chloroethyl)-N-methyl-2-phenylethylamine (CMP) to bovine erythrocyte AChE inhibits hydrolysis of ATC but stimulates hydrolysis of IPA (O'Brien, R. D., Biochem. J., 113:713, 1969). This effect has been ascribed to CMP attachment to a nucleophilic enzyme site which either binds or influences the binding of electrophilic substrate substituents. AChE-CMP hydrolysis of IPA was characterized by a 1.5 fold increase in the maximal velocity (V_{max}) without change in the Michaelis-Menton constant (K_m). Recently, the monoclonal antibody AE-2 (Fambrough, D. M., Engel, A. G. and Rosenberry, T. L., Proc. Nat. Acad. Sci. U. S. A., 79:1078, 1982) was reported (Kopec, K. K., Gentry, M. K., Osborne, D. M., Ralston, J. S. and Doctor, B. P., Abstracts, 13th Int. Congr. Biochem., P. 391) to inhibit hydrolysis of ATC by FBS-AChE and the AE-2 binding site was located near the N-terminus between amino acid residues 22-82. We now wish to report that AE-2 also exhibits a differential effect on ATC and IPA hydrolysis with a pattern similar to that of CMP. A 4/1 molar ratio of AE-2 to FBS-AChE active site causes more than 75% inhibition of ATC hydrolysis but the rate of IPA hydrolysis is increased. The V_{max} for FBS-AChE hydrolysis of IPA in the presence of AE-2 was 1.23 (arbitrary units), whereas the V_{max} for IPA hydrolysis in the absence of AE-2 was .062, a ratio of approximately 20. In contrast, the Michaelis-Menton constant, K_m, remained unchanged (7 x 10^{-4} M). Incubation of FBS-AChE with graded concentrations of BW 284 C51, a specific AChE inhibitor, produced similar inhibition curves for hydrolysis of both ATC and IPA. In addition, pralidoxime chloride reversed the influence of AE-2 on both ATC and IPA hydrolysis. The present results, as well as those of O'Brien, indicate that different classes of ligands may bind to, obstruct, or change the conformation of nucleophilic AChE sites with results which depend upon the nature of the substrate.

415.12 ENDOGENOUS URIDINE AS A MODULATOR OF CEREBRAL EXCITATION. T. McCown, M.J. Rosner, R.A. Mueller, J.N. Ghia*, R.D. Hunt* and G.R. Breese. Departments of Surgery, Anesthesiology, Psychiatry and the BRSC, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

CSF from ventriculostomy sites of head injury patients or neonatal subjects undergoing shunt revision was analyzed for xanthine, hypoxanthine, and uridine. As part of clinical management, ventricular CSF is removed to help decrease intracerebral pressure, in addition to numerous pharmacological manipulations of systemic perfusion pressure, mannitol, steroids, and other routine clinical care. Patients scheduled for lumbar subarachnoid blockade for surgical procedures served as controls. CSF was mixed with a solution of EHNA (an adenosine deaminase inhibitor) dipyridamole (to block adenosine uptake into cellular elements) and indomethacin (Gehrke CW, Kuo KC, Davis GE, and Suits RD: J. Chromatog 150:455-476, 1978) and stored at 4°C until analysis within several days. Analysis was via high pressure liquid chromatography (20 mM NH_4PO_4, pH 6.6 with 1% CH_3CN on C_{18} reversed phase column-U.V. detector at 2500A). Values are expressed as nmoles/ml of original CSF. Recovery was 85-90%.

Source	Uridine	Hypoxanthine	Xanthine
Head Injury	0.49**	3.88	5.99*
Adults (7)	±0.23	±0.55	±0.79
Hydrocephalic	2.57	6.45	10.7
Infants (3)	±0.82	±1.95	±3.3
Subarachnoid	4.13	3.31	3.21
Block (32)	±0.34	±0.26	±0.27

All values are Mean ± SEM of individual patient means expressed as nMoles/ml CSF ( ) = No. of patients. * $p < .05$ ** $p < 0.01$ relative to subarachnoid block subjects.

CSF uridine in head injury patients was only 12% that of normal subjects. Xanthine values were also significantly elevated ($p < .05$). Patients with elective shunt revision had uridine present at concentrations only 62% of normal adult lumbar fluid. The uridine concentration was not a function of red cell contamination in the CSF samples. A significant, though weak, positive correlation between xanthine and CSF lactate was also noted.

In rats, we have demonstrated that locally applied uridine (0.1 μmoles) significantly increases the threshold current necessary to initiate a wild running seizure from the inferior collicular cortex. Since uridine is an endogenous compound with anticonvulsant activity similar to that of GABA, and since it is reduced in head injury patients, the deficiency of CSF and possibly CNS uridine may contribute to post-traumatic cerebral excitation. It is presently unknown if the low uridine concentrations reflect decreased transport of the base into brain or increased utilization in brain.

415.13 ALTERATIONS IN BETA-ENDORPHIN LEVELS IN RESPONSE TO STRESS ARE MODULATED BY GONADAL STEROIDS. L.J. Forman and S. Estilow* Department of Medicine, UMDNJ-SOM, Camden, NJ 08103.

Castrated (CAST), and CAST gonadal steroid-replaced male and female rats, were exposed to acute (once for 45 min.) or chronic (45 min. each day for 15 consecutive days) immobilization stress. Immunoreactive beta-endorphin (IR-BE) levels in the plasma, anterior pituitary (AP), neurointermediate lobe of the pituitary (NIL) and the hypothalamus, were determined in these animals.

Acute stress increased plasma levels of IR-BE to the same extent in CAST male rats and CAST male rats replaced with testosterone proprionate (TP). TP potentiated the decrease in the concentration of IR-BE observed in CAST males in response to acute immobilization stress. Acute stress increased the concentration of IR-BE in the NIL of CAST male rats. This effect was abolished by treatment with TP. Exposure to chronic immobilization stress did not affect the concentration of IR-BE in the AP or the NIL of CAST males. By contrast, in rats treated with TP, chronic stress diminished the concentration of IR-BE in the AP and elevated the concentration of IR-BE in the NIL. Hypothalamic IR-BE levels in CAST and CAST TP-replaced animals were not influenced by acute or chronic stress.

Subjection to chronic stress resulted in a similar increase in plasma levels of IR-BE in CAST and CAST estradiol benzoate (EB)-replaced female rats. In CAST female rats, acute stress produced an increase in the concentration of IR-BE in the AP, which was attenuated by the administration of EB. Although IR-BE in the NIL was not influenced by acute stress in CAST females, exposure to acute stress resulted in an elevation in IR-BE levels in the NIL of CAST EB-treated rats. Chronic stress did not affect the concentration of IR-BE in the AP of CAST or CAST EB-replaced animals. Chronic stress did however, increase the concentration of IR-BE in the NIL of CAST females, which was potentiated by EB. The concentration of IR-BE in the hypothalamus of CAST and CAST EB-treated females was not affected by stress.

These data suggest that a) stress induced by immobilization affects IR-BE levels in the AP, NIL and plasma b) in male rats, testosterone modulates IR-BE levels in the AP and the NIL in response to acute and chronic stress and c) in female rats, estrogen influences the effects of acute stress on the AP and NIL, and the effects of chronic stress on the NIL alone. Hypothalamic IR-BE does not appear to be affected by acute or chronic stress, regardless of the gonadal steroid environment.

Supported by a grant from the American Osteopathic Association.

415.14 THE IDENTIFICATION OF CALCIUM BINDING PROTEINS IN RAT CORTEX AND AMMONIUM SULFATE FRACTIONS OF WHOLE RAT BRAIN. G.J. Creed*, W.E. Heydorn, and D.M. Jacobowitz, Laboratory of Clinical Science, NIMH, Bethesda, MD 20892

The essential role of calcium in cellular bioenergetics and synaptic transmission is well established. Identification of the calcium binding proteins involved yields insights into the mechanisms of calcium regulation of these events. Proteins from rat cortex and ammonium sulfate fractions of whole rat brain were separated by two-dimensional gel electrophoresis (2DE). The proteins were electroeluted onto nitrocellulose papers, which were then incubated in $^{45}Ca^{+2}$. The calcium binding proteins (CBP) were detected by autoradiography. Three proteins were consistently detected in rat cortex. The enrichment obtained with sequential 20% ammonium sulfate fractions, resulted in the detection of an additional four proteins. All seven CBP were acidic (pI's between 4.8 and 5.3) and of low molecular weight (M_r's between 12,500 and 27,500). Using the same methodology, but incubating with antisera in place of $^{45}Ca^{+2}$, we have previously reported the identification of two of these CBP as calmodulin and the B subunit of calcineurin. With appropriate antisera and comigration of pure proteins, the identifications of three more CBP have been ascertained. The protein (M_r 26,000, pI 5.0) detected in the 20%, 80%, and 100% fractions is the Vitamin D-dependent calcium binding protein. The protein (M_r 12,500, pI 5.2) detected in the S-100 fraction is parvalbumin. Finally, although it did not bind calcium in this system, the protein (M_r 22,000, pI 4.9) found solely in the S-100 fraction was identified as the S-100 protein. This protein also shows a monomeric form at the gel bottom (M_r 10,500). These results demonstrate that using 2DE, CBP are detectable. With enrichment, CBP normally in low concentration in the cell can be detected and identified.

415.15 **MODULATION OF [^{3}H]SPIROPERIDOL BINDING BY CHOLECYSTOKININ IN RAT STRIATUM.** M.A. Morency, J.S. Kajiura* and R.K. Mishra. Departments of Psychiatry and Neurosciences, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

There is a striking overlap between the topographical distribution of dopamine (DA) and CCK in the mammalian brain. The functional significance of this close relationship between DA and CCK is not well understood. We examined the effects of CCK on brain DA-D_2 receptors in the rat striatum.

Male Sprague-Dawley rats (200-250 gm) were sacrificed by cervical dislocation, the brains removed, and the striata were dissected. Tissue was homogenized (Polytron setting 6, 20 sec) in 50 vol of ice-cold 50 mM Tris-HCl buffer (pH 7.4). The membrane suspension was then centrifuged twice at 50,000 g for 10 min. The final pellet was resuspended in assay buffer containing 50 mM Tris-HCl (pH 7.4), 0.1% ascorbic acid, 120 mM NaCl, 5mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$. The tissue was then preincubated in the presence of 1.0 mg/ml bovine serum albumin, 0.1 mg/ml bacitracin and various concentrations of CCK peptides for time periods ranging from 1 to 60 min at 4°C. The preincubation was stopped by centrifugation (50,000 g for 10 min). The pellet was resuspended in assay buffer and analyzed for [^{3}H]spiroperidol binding. Receptor densities (B_{max}) and affinities (K_d) were determined by Scatchard analysis.

CCK_8 and desulfated CCK_8 dose-dependently modified the binding characteristics of the tritiated antagonist for DA-D_2 receptors in the striatum; a significant decrease in receptor density with a concomitant increase in the affinity of [^{3}H]spiroperidol binding sites was observed. Although these changes were observed with the 1 min preincubation, maximal effects of CCK peptides were achieved with the longer incubations. These results suggest a possible regulatory role for CCK in the striatum.
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415.16 THE HIGH AFFINITY CANNABINOID SITE IN BRAIN: REGULATION BY NUCLEOTIDES, METAL IONS AND ASCORBATE.
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The high affinity site in rat brain labelled by [^{3}H]5'-trimethylammonium delta-8-THC is an integral component of brain membranes that recognizes cannabinoids with inhibitory constants (K_i) in the nanomolar range (Nye et al. J.Pharm.Exp.Ther. 234: 784-791, 1985). To clarify its physiological role we studied the regulation of [^{3}H]TMA binding. The site is potently inhibited by the metal ions La^{3+} and Mn^{2+} with IC_{50}'s of 0.1 and 0.3 micromolar, respectively. Calcium, magnesium, barium, and strontium ions are inhibitory (IC_{50} = 200-500 micromolar), but significantly weaker. Ferrous, cuprous, and mercurous ions stimulate binding 2-3 fold at 1-5 micromolar, whereas ferric ion, the oxidized form, fails to stimulate and is inhibitory (IC_{50} = 100 micromolar). The halide ions F^-, Cl^-, Br^-, and I^- and the alkali metal ions K^+, Na^+, Rb^+ and Cs^+ are very weak inhibitors and have minimal effects at millimolar concentrations.

Ascorbate is also a stimulator of [^{3}H]TMA binding showing a maximal effect of 3-4 fold at 2 micromolar, whereas dehydroascorbate, the oxidized analog, is 10-20 fold weaker. The stimulation by ascorbate and cuprous ions depends upon the presence of oxygen, and does not result in covalent modification of the radioligand [^{3}H]TMA. At non-inhibitory concentrations Mn^{2+} is able to block the stimulation of ascorbate or cuprous ion. Thus ascorbic acid enhances [^{3}H]TMA binding via a mechanism which requires the presence of a metal ion and oxygen, suggesting that the [^{3}H]TMA site is linked to an unidentified enzymatic process.

Guanine and adenine nucleotides regulate the high affinity cannabinoid site. The non-hydrolyzable analogs of ATP and GTP, AMP-pNHp and GMP-pNHp, are inhibitors of [^{3}H]TMA binding at micromolar concentrations. The thiophosphate analogs, however, are potent stimulators of binding at 2 micromolar. Stimulation by ATP-gamma-S produces a large increase in the B_{max} and a reduction in the affinity of [^{3}H]TMA for its site. These effects suggest that the high affinity cannabinoid site may be regulated by an ATP hydrolyzing enzyme such as a protein kinase.

416.1 PRENATAL SEROTONIN SYNTHESIS IN THE BRAIN OF RATS AND FREE PLASMA L-TRYPTOPHAN IN NEWBORN HUMANS MALNOURISHED IN-UTERO. J. Hernández, G. Manjarréz*, G. Chagoya*. Lab. de Neurontogenia, Depto. Fisiología, Biofísica y Neurociencias, Centro de Investigación, I.P.N., México, D. F. 07000.

Plasma L-tryptophan (L-Trp) is known to be the precursor of brain serotonin (5-HT) synthesis through at least two possible mechanisms:a) regulation by unbound plasma L-Trp specifically transported through the BBB, which activates brain tryptophan-5-hydroxylase (T5-H);b) plasma L-Trp competing for transport through BBB with other neutral amino acids. It is known that early malnutrition is a condition that affects brain 5-HT metabolism, free plasma L-Trp, brain L-Trp and 5-HT content being increased postnatally. Among other possible relevant physiological roles of 5-HT, it has been recently suggested its participation in brain differentiation processes.

In the present study we report results concerning 5-HT metabolism in two group of small for date (SFD) human babies (gestational age ranging from 33 to 36 and from 38 to 42 weeks), who suffered intrauterine nutritional restriction. A complementary study in the brain of rat fetuses with two types of intrauterine deprivation, in which brain T5-H activity and 5-HT content were determined on days 17, 19 and 21 of gestation. The same parameters studied prenatally were followed in both species during the immediate postnatal period.

In the SFD babies the results were as follows: a) the free fraction of plasma L-Trp was significantly elevated; b) plasma neutral amino acids were not substantially modified in the 38 to 42 age group; c) the bound fraction of L-Trp and plasma proteins were significantly low, as compared to the same parameters in normally grown age-paires babies. In the fetal brain of intrauterine malnourished rats, L-Trp, activity of T5-H and 5-HT content, were significantly elevated, since day 17, as related to normal litter-mates. These alterations in 5-HT metabolism persisted during the early postnatal period in both species.

We conclude that brain serotonin synthesis is accelerated, not only in the postnatal period but also is the fetal differentiating brain of the rat with intrauterine malnutrition and that the observed elevation of the free fraction of plasma L-Trp in early malnourished SFD human babies suggest an increased transport of this amino acid to the brain with a possible enhancement of serotonin synthesis.

416.2 DISPARTIES IN THE EARLY PUP DEVELOPMENT AND MATERNAL BEHAVIOR RESULTING FROM DIFFERENT TRYPTOPHAN CONTENT OF DIET. M. Sakuma, Dept. of Biol. Sci.,Wayne St. Univ.,Detroit MI 48201, R.I.Peters*, Dept. of Biol. Sci.,Wichita St. Univ.,Wichita KA 67208, and L.M. Hryhorczuk*, Lafayette Clinic, Detroit MI 48207

Three groups of pregnant rats were fed a diet differing only in tryptophan content. Seven dams in group H recieved a diet containing 3% tryptophan; three dams in group M recieved 0.3% tryptophan diet; four dams in group F recieved 0.03% tryptophan diet. Mothers and pups were subjected to behavioral scoring on delivery day and subsequently every the other day till Day 21. Presently, data is reported for delivery day, one week and two weeks after parturition. The development of pup was evaluated with respect totwenty five items. Sixty pups and twelve pups of H group (60/80,12/80) showed flat and cruciform postures differing from those of M (21/31, 2/31) and F (8/52,4/52). Higher frequency of incisor eruption found in H group (29/66) than that of M (3/31) and no eruption was observed in F (0/52) group. Both H and F pups showed slowness in uprighting from spinal position than that of M pups,but F group showed lowest response to either tail hang or scruffing the neck (50% & 56% Of 55 pups), and lowest spontaneous cry than H and M groups. But F pups showed loud crying during tail pinch when compared with M and F groups. The behavior of dams corresponding these pups was evaluated with respect to fifteen items. On delivery day three out of seven in H dams failed to retrieve their pups 39/70), but three M and four F dams retrieved all pups. Four dams of F group showed no self grooming, whereas two of three in M and five of seven in H dams showed no self grooming. Above low frequencies in H pups appeared to be consist through one week after birth period. Two weeks following birth H pups still kept the flat posture, staggering during forward moving, falling from slope, no response found during one hind limb hang, and poor grasp associating less rearing. While two weeks of age in F pups exhibited the frequent sitting position and more rearing than H pups, meaning that their development was swayed toward to the controls by through one week of age period, excepting less growth of incisor height. On Day 7-8 of lactation, dams of all groups exhibited only one significant difference in that the number of retrieved pups appeared to be lower for the F dams than for the others. On Day 15-16 of lactation all dams failed to show the group difference in all items of maternal behaviors. Thus, Day 0, Day 7-8 and Day 15-16 confirmed the previous results concerning induced developmental lag in pups following the manipulation of mother rat's pregnancy by tryptophan diets (Sakuma,1986). Scince nursing behaviors of Day15-16 failed to show group difference, the findings of these days may exclude influences from dams on pup development suggesting the real developmental delay in both F and H groups compared with controls.

416.3 VISUAL ACUITY, ELECTRORETINOGRAMS, AND RETINAL MORPHOLOGY IN INFANT RHESUS MONKEYS DEPRIVED OF DIETARY TAURINE. M. Neuringer, H. Imaki*, J.A. Sturman, R.C. Moretz* & H.M. Wisniewski. Oregon Regional Primate Research Center, Beaverton, OR 97006 and New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

Diets lacking the amino acid taurine lead to progressive photoreceptor degeneration in cats. Human infants and children maintained by taurine-free parenteral feeding show reduced plasma taurine levels and ophthalmoscopic and electroretinographic abnormalities which normalize when taurine is added to the infusate. Human infants fed low-taurine synthetic infant formulas have similarly low plasma taurine levels, but functional consequences have not been identified.

We are examining the effects of dietary taurine deprivation in rhesus monkeys. In a previous study, long-term feeding of a low-taurine formula resulted in reduced cone electroretinogram amplitudes and ultrastructural cone degeneration. The present study is following the course of visual, electroretinographic, and morphological retinal changes in young rhesus infants.

From birth until three months of age, rhesus monkeys were fed a taurine-free, soy protein-based human infant formula or the same formula supplemented with 70 µmoles/100 ml of taurine, the amount in monkey milk. At 8-12 weeks, plasma taurine levels were reduced by 50-65% in the taurine-deficient group, as they are in human infants fed similar formulas. Visual acuity was measured by the preferential looking method at 4, 8, & 12 weeks of age. Taurine-deprived infants had significantly poorer acuity at all ages. Ophthalmoscopic examinations revealed no significant refractive errors; therefore, the acuity impairment was due to retinal rather than optical factors. However, full-field electroretinograms at three months showed no changes in the amplitude or peak latency of rod- or cone-dominated responses. These results suggested that the central retina, which is critical for normal acuity, may be selectively affected by taurine deprivation. Examination of retinas by light and electron microscopy at three months demonstrated swelling and disorientation of cone outer segments, with extensive disorganization and vesiculation of disc membranes. These changes were most pronounced in the foveal region. Rod outer segments were also affected, but the changes were less severe. Abnormalities were also seen in photoreceptor inner segments and terminals and in pigment epithelial cells.

These results provide further evidence that dietary taurine is essential for normal retinal development in primates, and support the recommendation that taurine be added to human infant formulas at the same level found in human milk.

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416.4 ALTERATIONS OF NEURAL DEVELOPMENT AND FUNCTION CAUSED BY NEONATAL NUTRITIONAL DEPRIVATION OR ENHANCEMENT. J.M. Bell, W.L. Whitmore*, K.L. Queen*, L. Orband-Miller* and T.A. Slotkin. Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Neural development and autonomic function were examined in neonatal rats whose nutritional status was manipulated by altering litter size to 5-6 pups (small litter) or 16-17 pups (large litter), as compared to a standard litter size of 10-11 pups. In order to evaluate biochemical mechanisms which operate to buffer the brain from growth retardation, comparisons were made between spared brain regions (cerebellum, cerebral cortrex, midbrain + brainstem), and a tissue which is not spared (heart): evaluations included assessment of ODC and the polyamines, which control macromolecule production during cellular replication and differentiation. Litter size manipulations were associated with shifts in cardiac ODC developmental patterns consistent with subsequent growth impairment in the nutritionally-deprived group whereas overnutrition was associated with a pattern which enhances growth. In contrast, there was a relative lack of nutritional alterations on brain ODC, thus providing an early indication of subsequent sparing of growth. Similar patterns were found for nutritional effects on polyamine levels: there was a predominance of early action on cardiac tissue, and a lack of effect on the most highly spared brain regions. However, the cerebellum, a brain region in which the principal microneurons develop predominantly postnatally (like cardiac muscle cells), displayed alterations of spermidine and spermine levels during the period in which granule cell replication and migration occurred. These data suggested that late-developing structures are more sensitive to nutritional status. To test this hypothesis, we examined peripheral sympathetic development which also occurs after birth. Nutritional deprivation was found to suppress development of sympathetic function, as shown by: (a) persistence of the immature non-neurogenic adrenomedullary catecholamine release in response to hypoxia and (b) a delay in the development of the centrally-mediated baroreflex response of cardiac sympathetics to hypotension. Because sympathetic input is thought to control growth of peripheral organs, the effect of nutritional status on β-receptor mediated cardiac hypertrophy was then examined. Animals raised in large litters showed impairment of the ability of isoproterenol to produce cardiac hypertrophy, suggesting a specific uncoupling of the receptors from growth of peripheral tissues. These results suggest that sparing of growth is not uniform throughout the nervous system, and that effects on neural development can participate in growth retardation and functional impairment in non-neural tissues. (Supported by USPHS HD-09713 and NS-06233).

416.5 EFFECTS OF UNDERNUTRITION ON GRANULAR CELLS OF THE DENTATE GYRUS OF HIPPOCAMPUS IN RATS OF THREE AGE GROUPS. L. Cintra, S. Díaz-Cintra and G. Padua*. Depto. de Fisiología, Instituto de Investigaciones Biomédicas, UNAM, México, D.F. 04510.

Previous studies had shown that the hippocampus may be affected by undernutrition in terms of cell reductions on fields CA1, CA2, CA4 and granular cells of the dentate gyrus. The aim of the present paper was the study of the effects of prenatally and postnatally undernutrition using an 8% caseín diet on the granular cells of the dentate gyrus in rats of 30, 90 and 220 days. Using a Golgi morphometric analysis we studied major and minor axis of the cell body, and the number of primary dendrites and dendritic density by the Sholl method and spine density in a 50 microns length of proximal, medial and terminal segments of principal dendrites, as well as, its linear extent. Our results showed in malnourished animals at 30 and 90 days, a significant reduction in the number of spines and dendritic density, however at 220 days, malnourished rats showed a significant increase of primary dendrites. Age related changes showed in malnourished rats a significant increase in the cell body and primary dendrites between 30 and 90 days. Between 90 and 220 days, control rats showed a significant reduction of major axes and significant increase of spines on the basal segment. Undernourished rats showed a significant reduction of major axes and significant increase of dendrites and total spine number between 90 and 220 days. These data shows a general reduction in the synaptic connections represented by the density of branches and dendritic spines, specially in the segments that receive inputs from the perforant path and aminergic brain stem nuclei.

416.6 THE EFFECTS OF PROTEIN DEPRIVATION ON PYRAMIDAL CELLS OF THE VISUAL CORTEX IN RATS OF 30, 90 AND 220 DAYS. S. Díaz-Cintra, L. Cintra and A. Ortega*. Depto. de Fisiología, Instituto de Investigaciones Biomédicas, UNAM, México, D.F. 04510.

The effects of postnatal undernutrition on pyramidal cells of layer V had been revealed that dendritic and spine density are importantly affected, as well as, the visual and motor deprivation affects the spine density on apical dendrites, where projects inespecific afferents. During the development, the layers V and I, mature on first order, followed by layers, VI, III, IV, and layer II. The cellular population of layer V has a prenatal development with postnatal maturation, however in the case of layer III, these mechanisms ocurrs later and probably are more affected by postnatal malnutrition. The aim of the present study was to use an 8% casein diet prenatally and postnatally in order to establish undernutrition in rats. At the ages of 30, 90 and 220 days their brains were processed with rapid-Golgi method and were applied a morphometric analysis on the cells size, the linear extent of apical dendrites and the number of dendritic spines in a 50 microns length in three segments: basal, medial and terminal. The results shown that undernutrition increased significantly the cell size at 30 days and decreased at 220 days on layer III, however layer V showed a significant reduction of cell size at 90 days, linear extent also was reduced at 90 and 220 days in layer V. The number of dendritic spines was significantly reduced at 30 days on medial segment however showed a significant increase at 220 days on terminal dendrites in layer V. Age related changes showed in control rats a significant reduction in the total spine number between 30 and 90 days on layer III, undernourished animals showed a significant increase in total spine number at 90 days in the same layer. In relation to changes between layers III and V, was observed that control animals had reduced number of spines on basal and terminal segment on apical dendrites of pyramidal cells of layer V at 30 and 220 days. Undernourished rats showed a significant reduction on the three segments at 30 days, and at 90 and 220 days the significant reduction was presented on basal segment, finally in control rats, the linear extent was longer on layer V. These results shown the great plasticity of visual area 17 to react to undernutrition and the age related changes. The reductions of different cellular parameters reveals an anatomical deficit of these area that probably affects the mechanisms of visuo-motor integration in undernourished rats.

416.7 ORAL FACTORS INFLUENCE BRAIN GROWTH IN ARTIFICIALLY REARED RAT PUPS. J. Diaz, E. Moore*, E. Ray* and D. Williams*. Department of Psychology, University of Washington, Seattle, WA 98195.

The procedure of rearing rat pups exclusively by direct gastric infusions of formula via chronic gastric fistulas, while useful in a variety of experimental paradigms, has reliably produced animals with smaller brains and larger visceral organs than their mother reared siblings. Oral factors involved in normal digestion and nutrient absorption has been recently described in the adult rat (Ramirez, 1985). In addition, the recent development of a chronic palate cannula for rat pups (Henning and Blake, 1987) has enabled direct testing of oral involvement in the brain growth of young animals. The purpose of the following study is to examine the possible role of oral factors in early brain development.

Eight day old Long-Evans rats were randomly assigned by weight to one of three conditions: 1) animals artificially reared by intragastric cannulas (AR, n=6); 2) animals artificially reared with intra-oral cannulas (PAL, n=5); and 3) animals that were mother reared (MR, n=7). The intraoral cannulas were placed lateral to the 3rd caudal palate ridge on the edge of the hard palate. After four days all the animals were sacrificed and their brains and visceral organs were removed and weighed.

A Repeated Measures Analysis of Variance indicated that the groups grew at different rates. The daily weight gains of the PAL animals were lower than the other two groups. Post hoc pair-wise comparisons further indicated that despite this poor weight gain, the absolute brain weights of the PAL animals were more like the mother reared animals than were the brain weights of the AR animals. However, the visceral organs of the PAL animals were larger than either mother reared or AR animals.

These data suggest that the brain deficits reliably seen following direct intragastric formula infusion may be due partially to the lack of appropriate oral events or factors. The reduced weight gains in the PAL animals presents a problem in the interpretation of these data. The increased visceral organ weights in the PAL animals raises additional concerns. Regardless of these limitations, the fact remains that infusing a milk formula intraorally results in animals with larger brains compared to animals that were infused with the same formula intragastrically. Whether these results may be mediated by lingual lipase, nerve growth factor release, the actual stimulation of swallowing and its possible initiation of gastric motility, or other factors remains to be determined. (Supported by NSF grant RII 8114919, and a grant from the Graduate School of the Univ. of Washington)

416.8 THE EFFECTS OF INFANT MALNUTRITION ON THE DEVELOPMENT OF EVOKED POTENTIALS. A.D. Kirsch*, George Washington University, Washington, D.C. 20037; A. Barnet, I. Weiss, Children's Hospital National Medical Center, Washington, D.C. 20037: J. Flinn, S. Lydick*, George Mason University, Fairfax, VA 22030.

Cortical evoked potentials (EPs) were recorded from 25 severly malnourished infants at admission to hospital, at discharge, and from age matched controls. The average age of the malnourished infants at admission was 174 days and the average age at discharge was 314 days. Auditory and visual evoked potentials (AEPs and VEPs) were recorded from the scalp at C_3, C_z, C_4 over left, right and central cortex and at O_1, O_2, O_z over left, right and central occipital cortex. We have previously reported an analysis of EPs recorded at C_3 and C_4 at admission. This analysis showed that, for EPs to click and patterned lights, the malnourished infants had significantly smaller EPs, with fewer peaks, than the controls, while AEPs to the child's name showed only a trend toward fewer peaks.

We report here that at discharge there were no significant differences in amplitude or number of peaks for the AEPs to click, and there were no significant differences in amplitude for the VEPs to patterned light, although there were fewer peaks ($p < .05$). However at discharge the EPs to name showed significant differences in amplitude ($p < .01$) and number of peaks ($p < .01$).

A frequency analysis of the VEPs to patterned light and the AEPs to name is consistent with these results. The power in three frequency ranges, 0-4, 4-8, 8-12, Hz has been examined. At admission the malnourished infants had significantly less power in all three frequency bands for the VEPs ($p < .01$). At discharge there was no differences in the 0-4 Hz or 8-12 Hz bands, with a tendency ($p < .1$) for lower power in the 4-8 Hz band. A preliminary analysis of the AEPs to name showed no significant differences at admission, but significant reductions in power at discharge ($p < .01$).

The results show that infant malnutrition affects the development of auditory and visual EPs. The EPs to clicks and patterned light improved during hospitalization. However, either because of malnutrition, or because of hospitalization the AEPs to name showed larger differences at discharge than at admission.